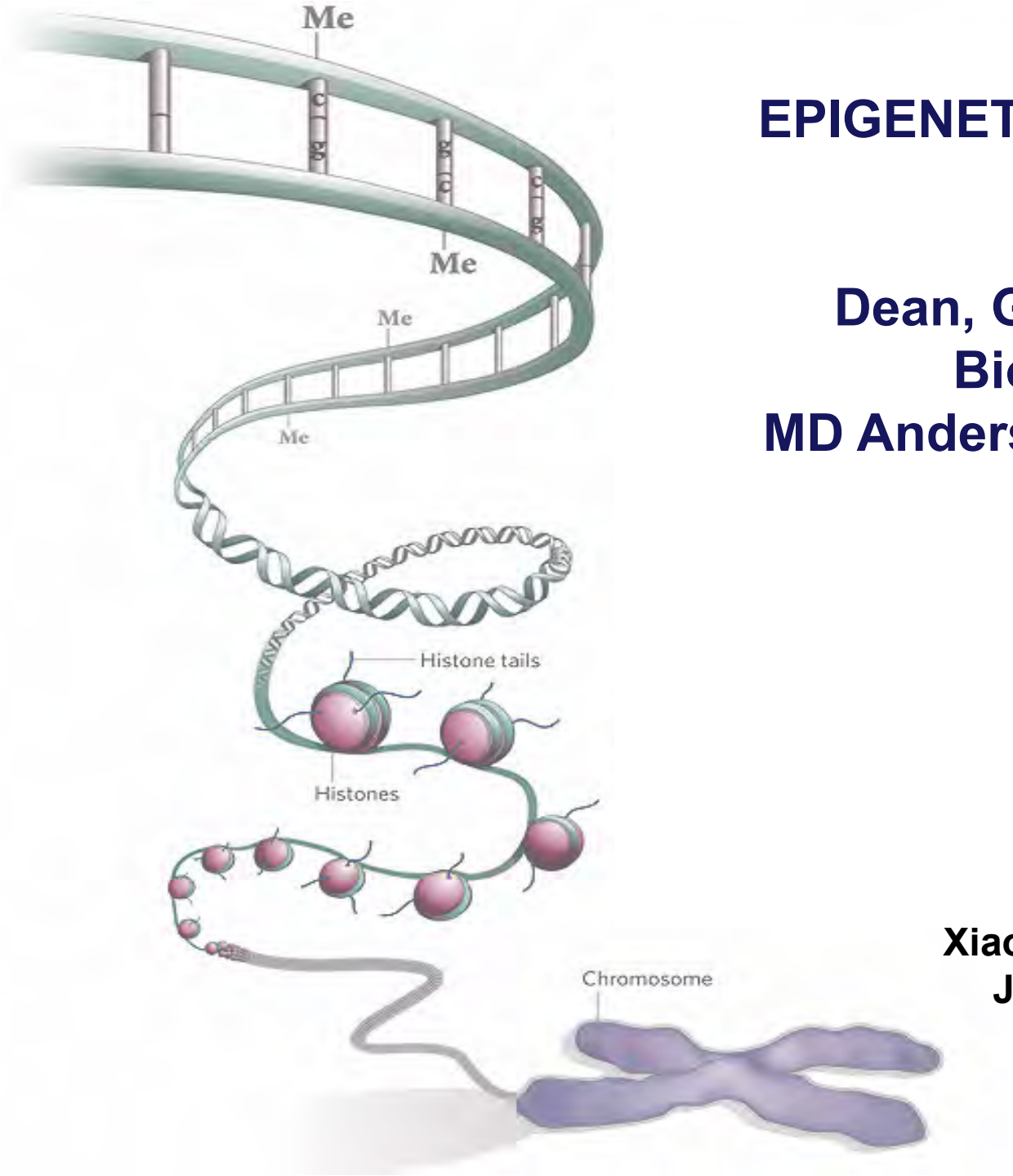
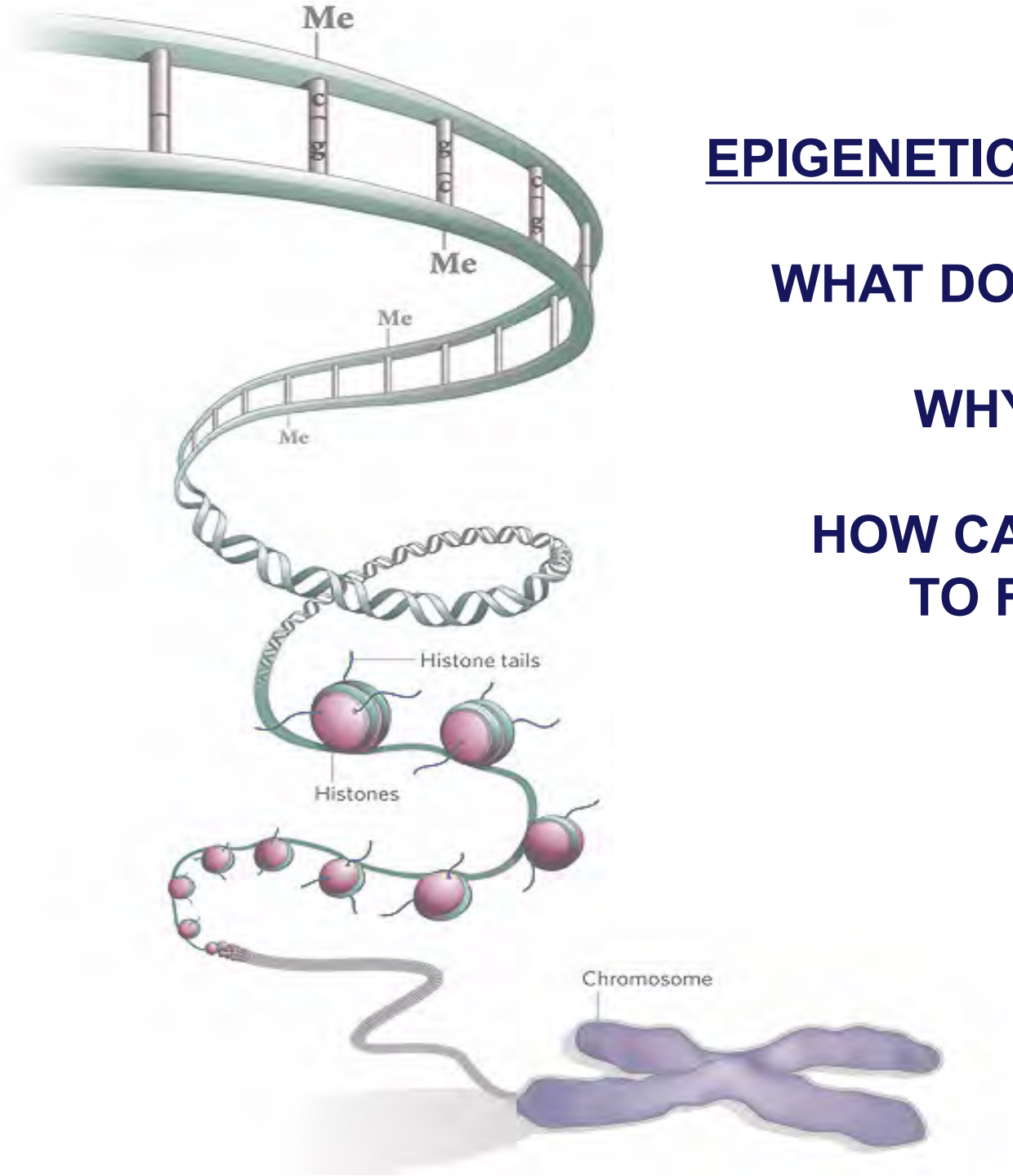


EPIGENETICS AND CANCER

Michelle Barton
Dean, Graduate School of Biomedical Sciences
MD Anderson Cancer Center

slides contributed by Drs. Xiaobing Shi, Jessica Tyler and Joya Chandra, MD Anderson Cancer Center



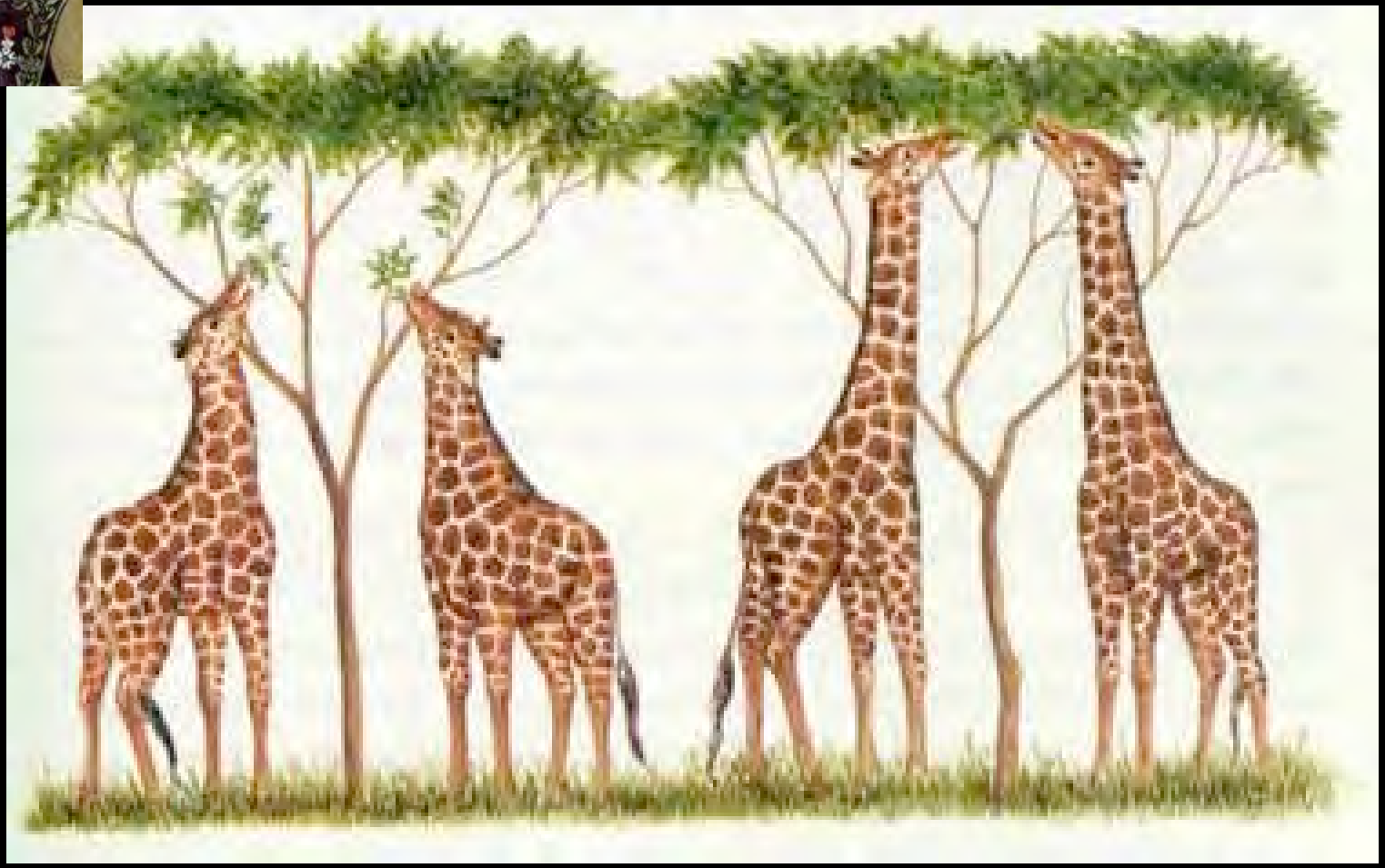
EPIGENETICS AND CANCER

WHAT DOES THIS MEAN?

WHY DO WE CARE?

HOW CAN WE USE THIS TO FIGHT CANCER?

Jean-Baptiste Lamarck – 1802 –
“soft evolution”: The environment, or “complexifying forces”, gives rise to adaptations that are inherited.



ep·i·ge·net·ics

/ˌepəjəˈnediks/

Noun

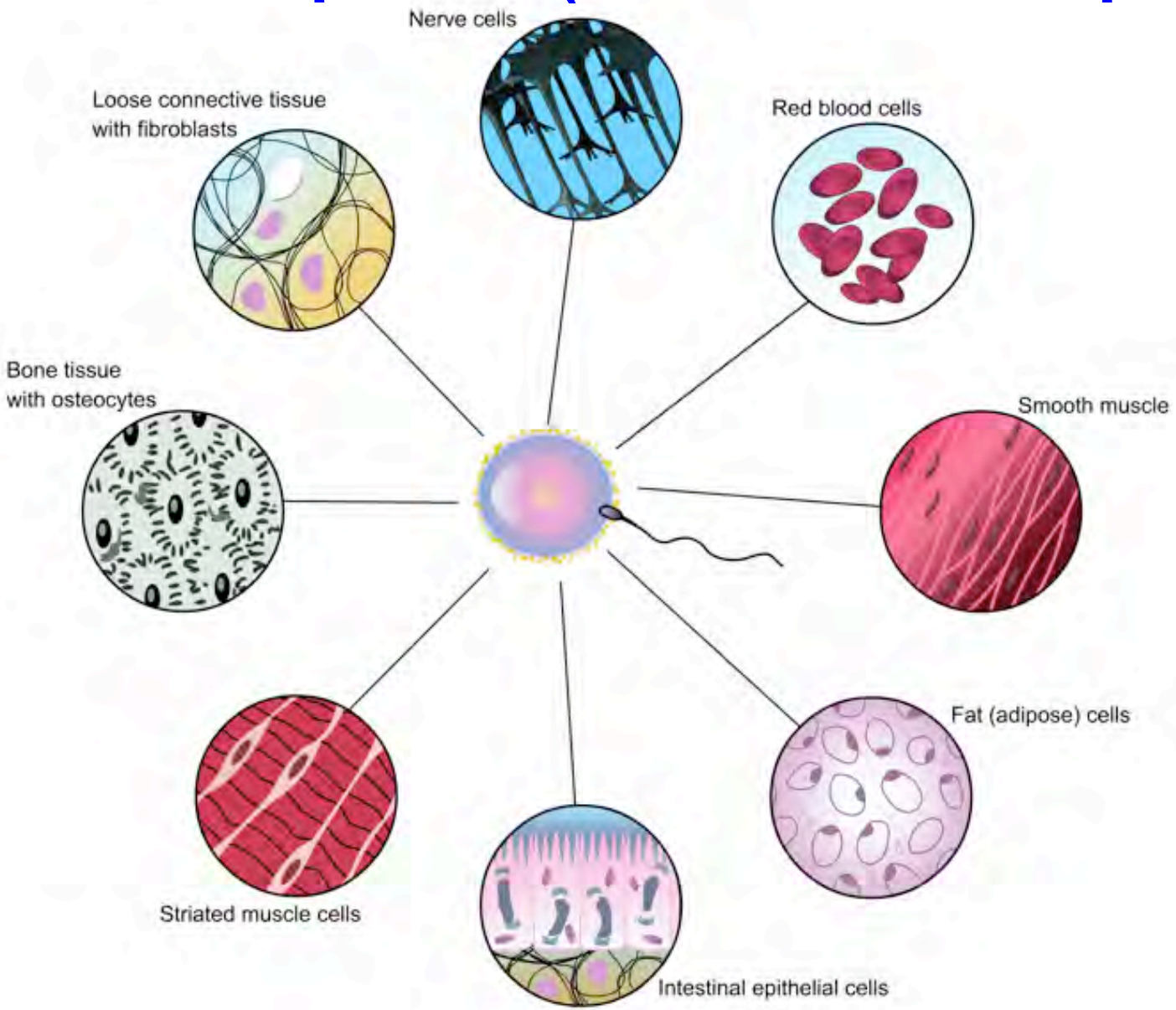
the study of heritable changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.

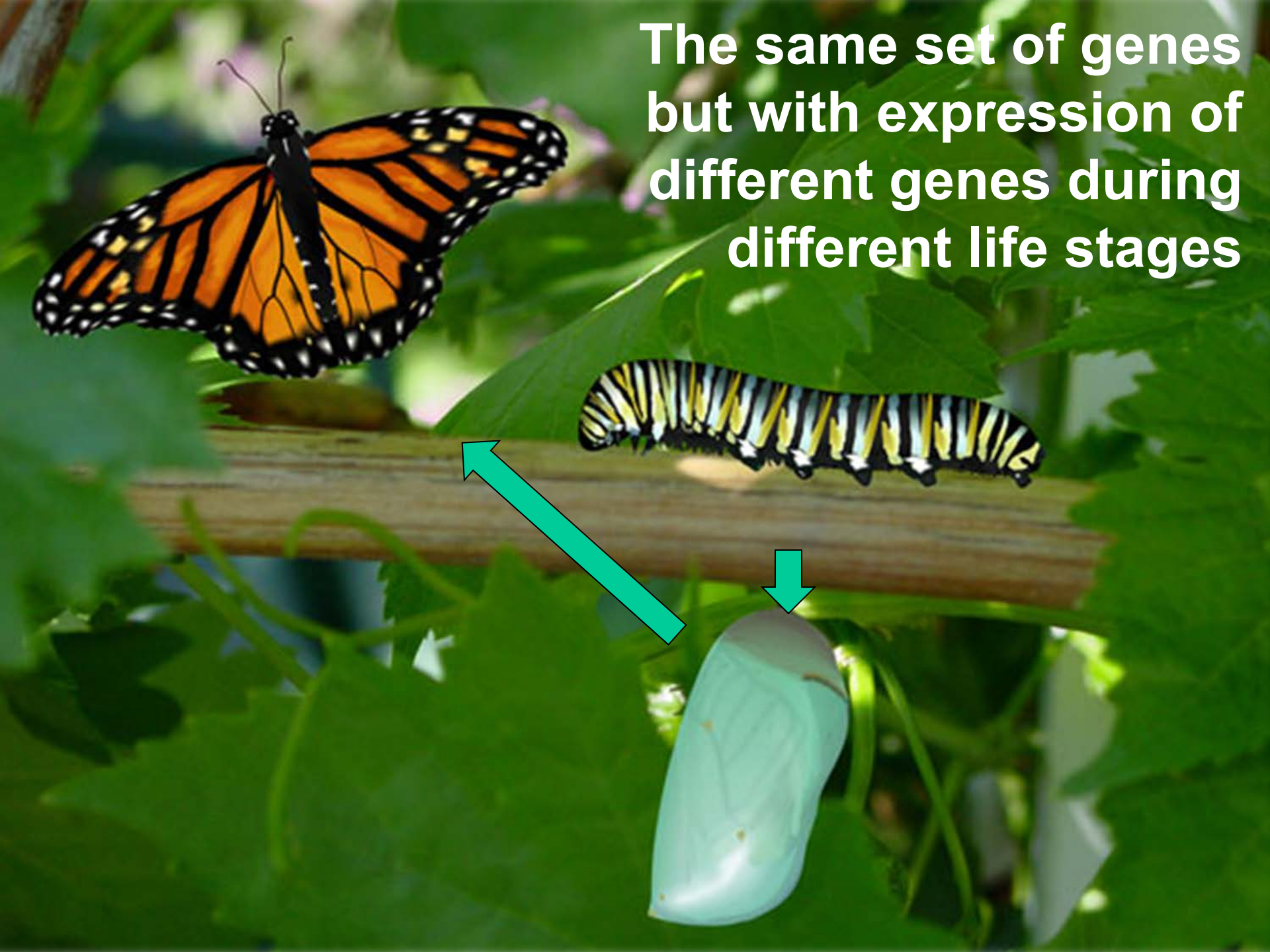
"epigenetics has transformed the way we think about genomes"

The term epigenetics was first coined by Conrad Waddington in the 1940's:

"How genotypes give rise to phenotypes during development"

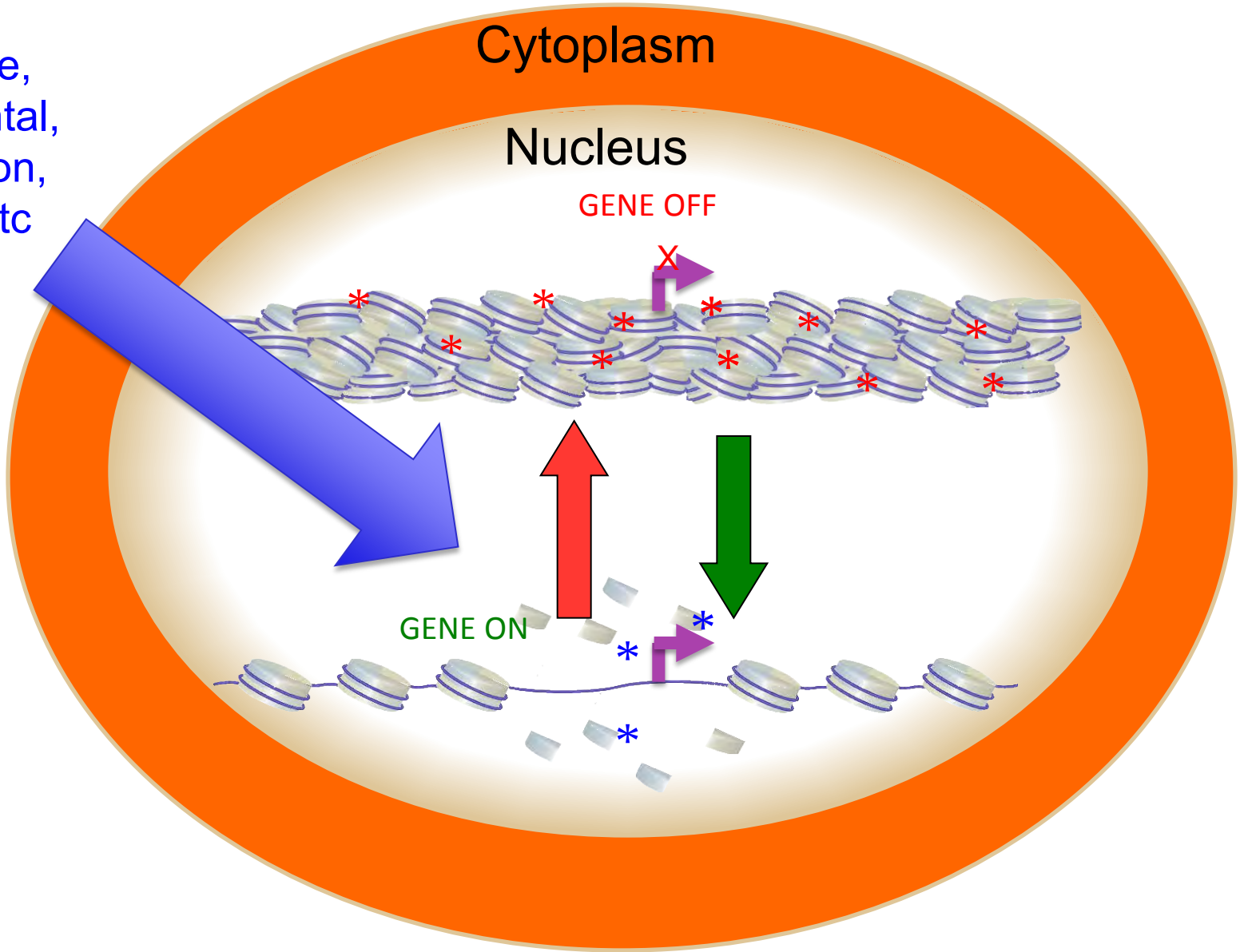
All our cells derive from one cell and have the same DNA sequence (with a few exceptions)



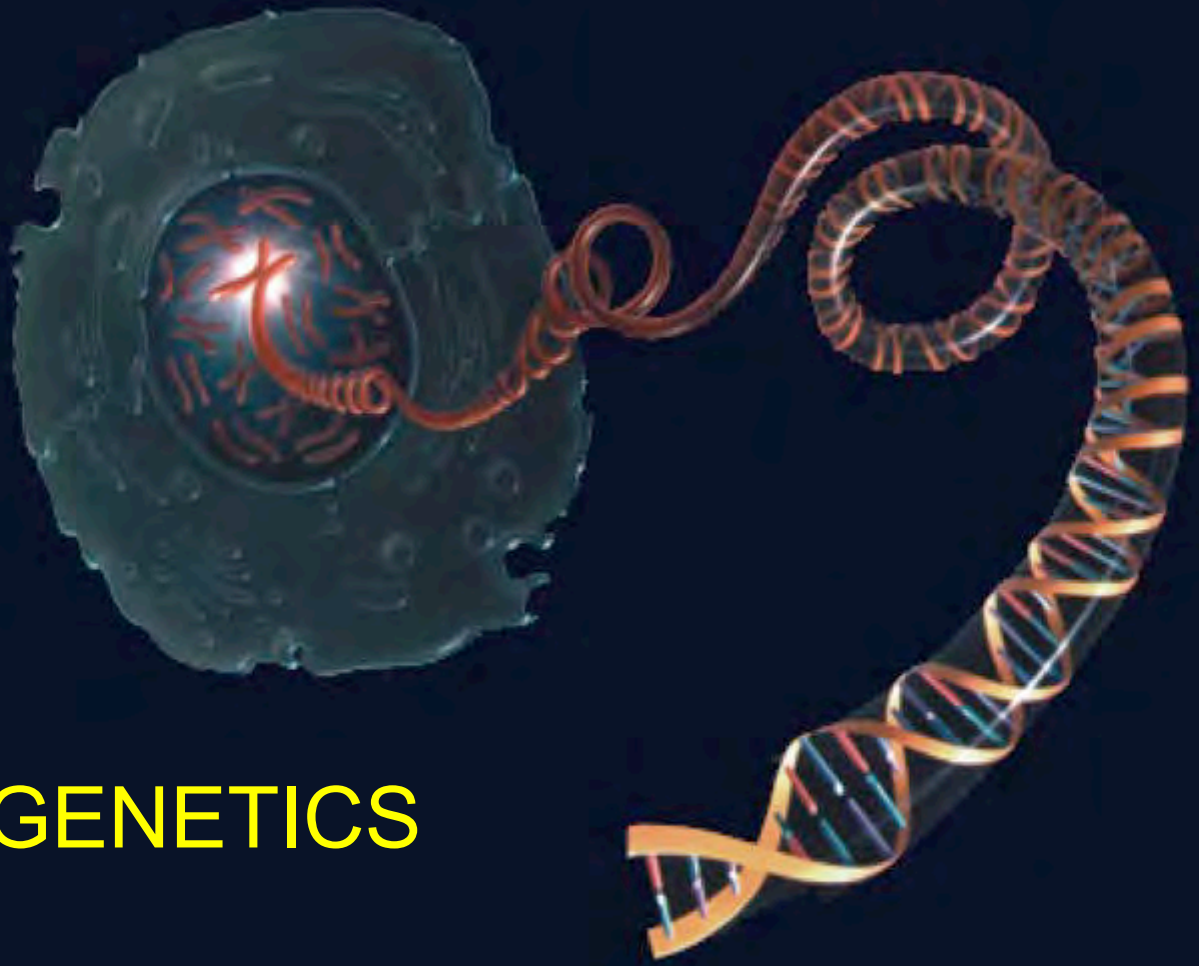

The same set of genes but with expression of different genes during different life stages

Epigenetics allows cells to make different proteins in response to changes in the environment

Temperature, environmental, differentiation, metabolic etc signals



WHAT DOES THIS MEAN?



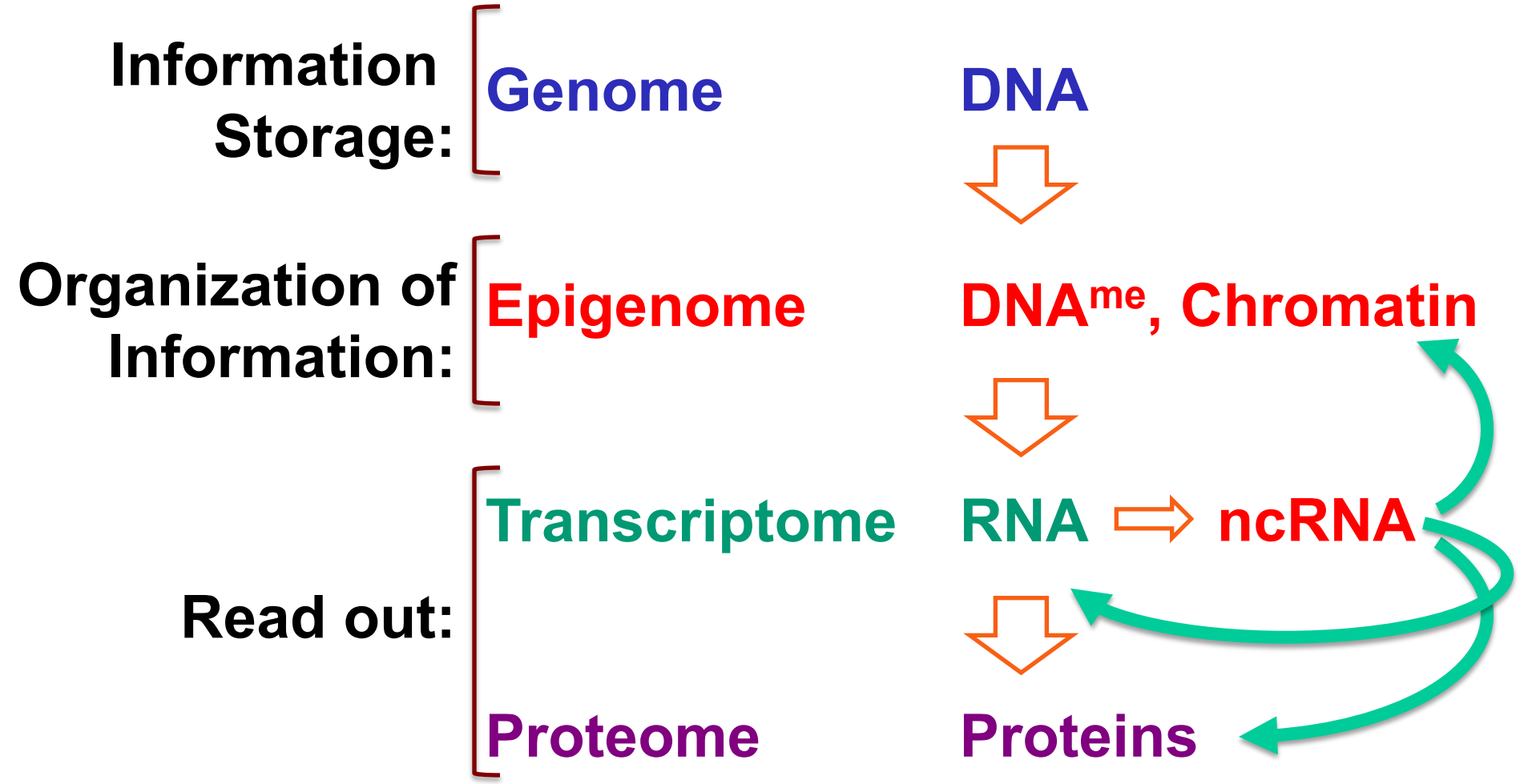
EPIGENETICS

Epigenetic Mechanisms

Epigenetics can be defined as heritable changes in gene expression or phenotypes that do not involve changes in the DNA sequence.

- DNA methylation
- Histone modifications
- Histone variants
- Chromatin remodeling
- Regulatory non-coding RNAs

What is the Epigenome?

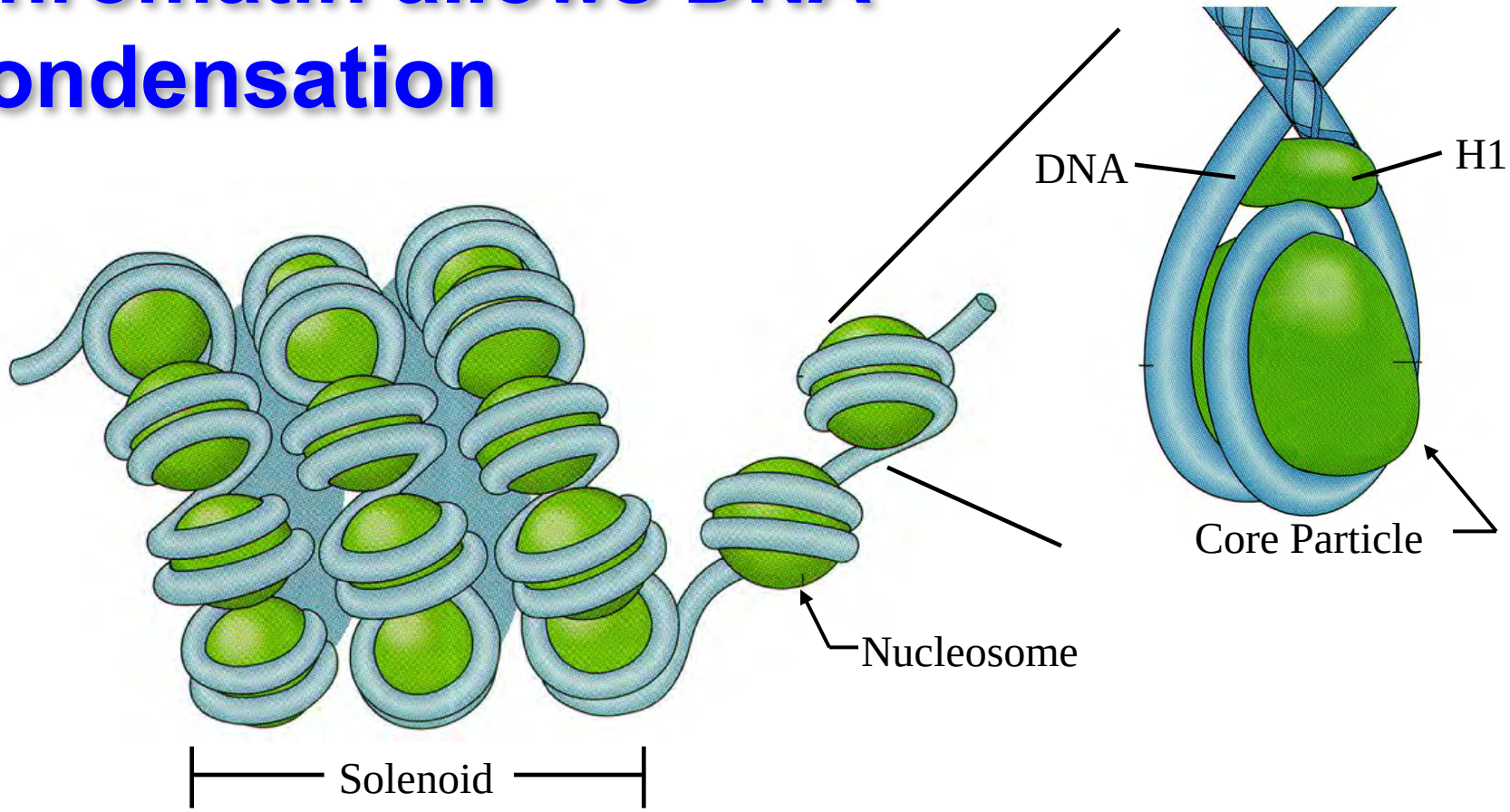


Chromatin structure – Why we need it

- Human genome (in diploid cells) = 6 x 10^9 bp of DNA in each cell
- 6 x 10^9 bp X 0.34 nm/bp = 2.04 x 10^9 nm = 2 m of DNA/cell
- Diameter of nucleus = 5-10 μm
- DNA must be wound tightly to package & protect it, but must still be accessible to allow gene expression and cellular responsiveness

NOTE - DNA length in a human body:
2m x 5x10^{13} cells=10^{11} kilometers
Distance to the sun and back about 300 times

Chromatin allows DNA condensation



- chromatin is composed of nearly equal amounts of DNA and globular histone proteins
- histones provide a physical means of packaging the DNA molecule in a very compact and orderly way, which plays an important role in gene regulation

Histones

- Main packaging proteins
- Small highly basic proteins rich in Lysine (K) and Arginine (R)
- 5 types: H2A, H2B, H3, H4 and H1.
- Histones are highly conserved in eukaryotes in both structure and function

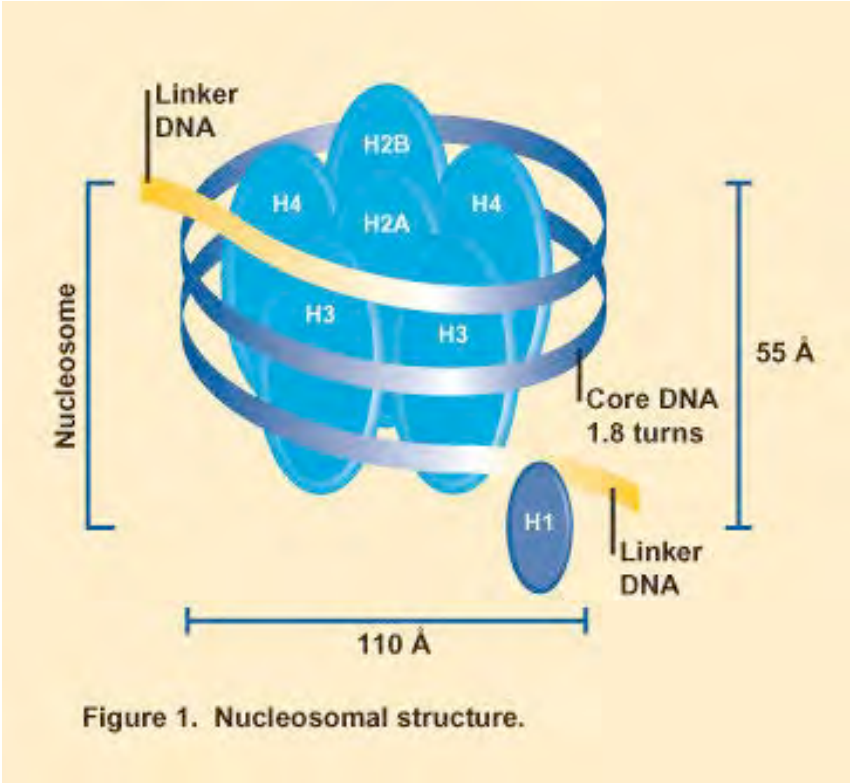
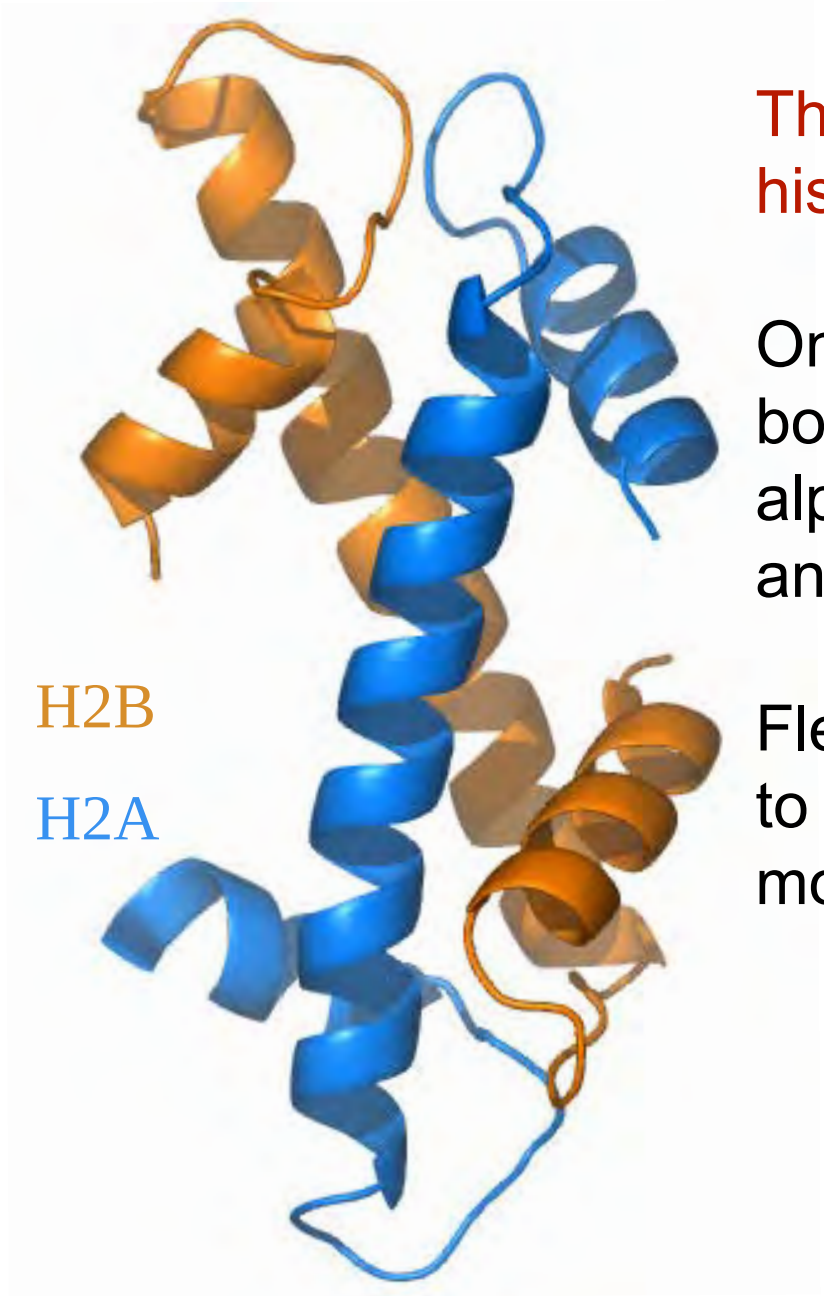


Figure 1. Nucleosomal structure.

Core histones

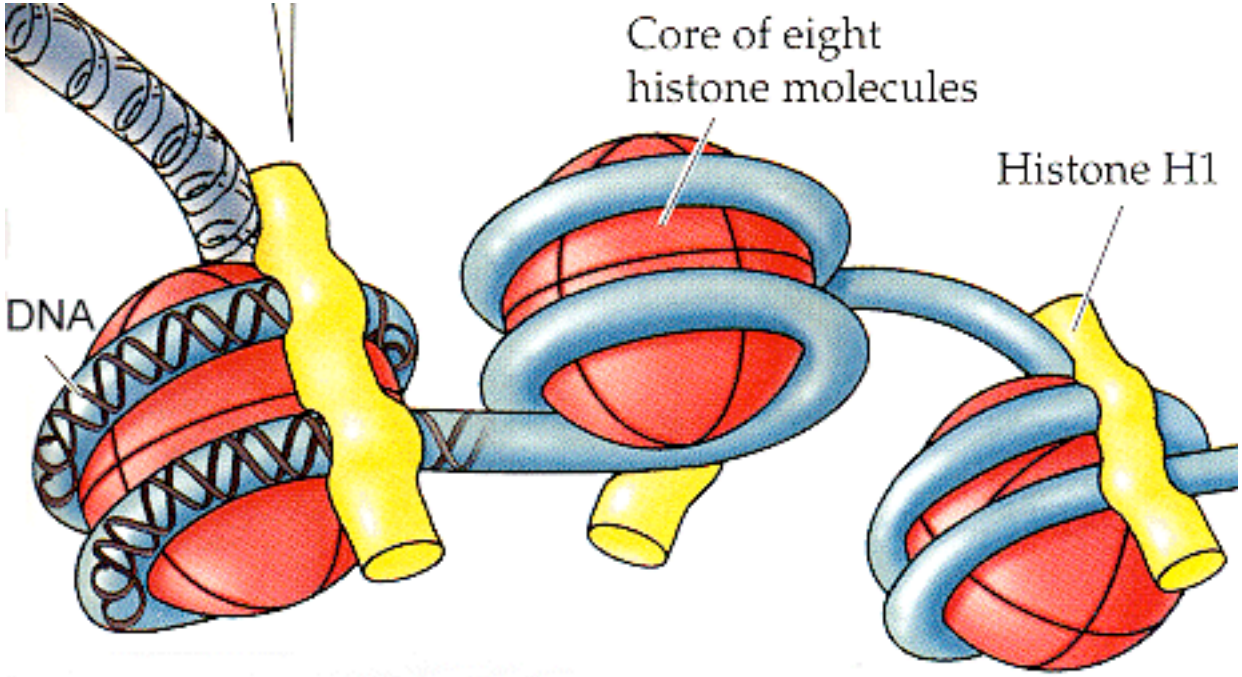


The basic structure of ALL core histones is the “histone fold”:

One long hydrophobic alpha-helix, bordered by two short hydrophobic alpha helices that form pairs (H2A/H2B and H3/H4).

Flexible amino termini that are subject to reversible post-translational modifications (PTMs)

The nucleosome



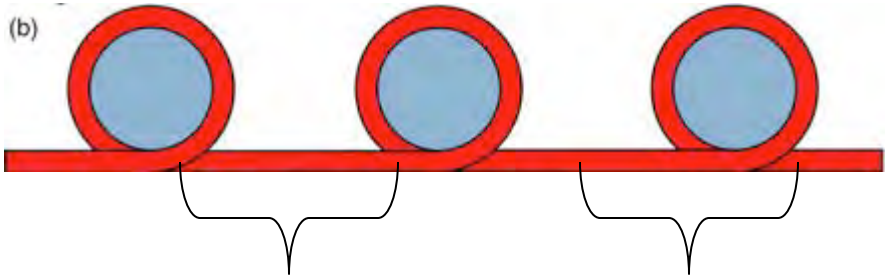
Fundamental repeating unit of chromatin – nucleosome

147 base pairs of DNA wrapped 1.7 times around a HISTONE OCTAMER

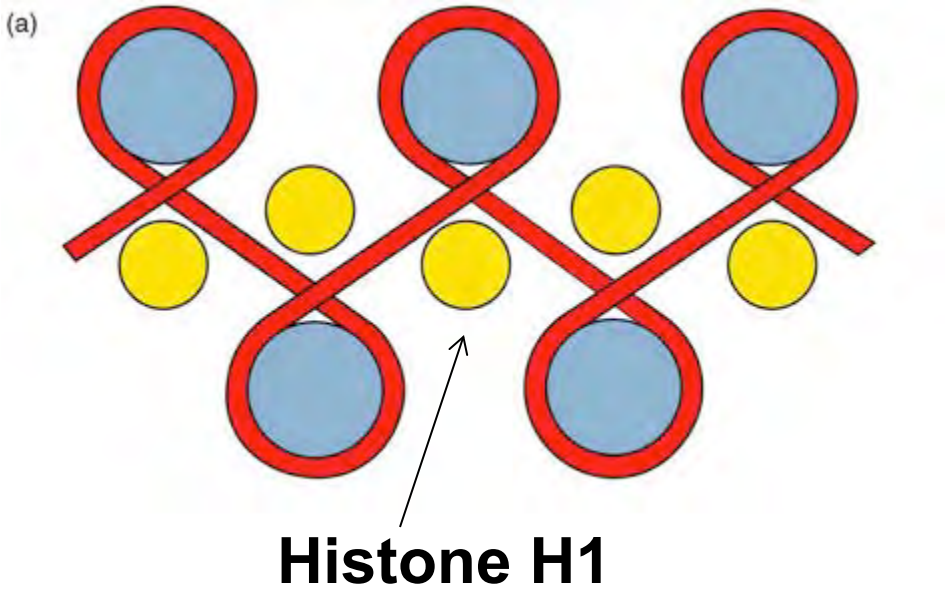
The histone octamer = 2 x H2A/H2B pairs and 1 x H3/H4 tetramer

Association stabilized or compacted by linker histone H1

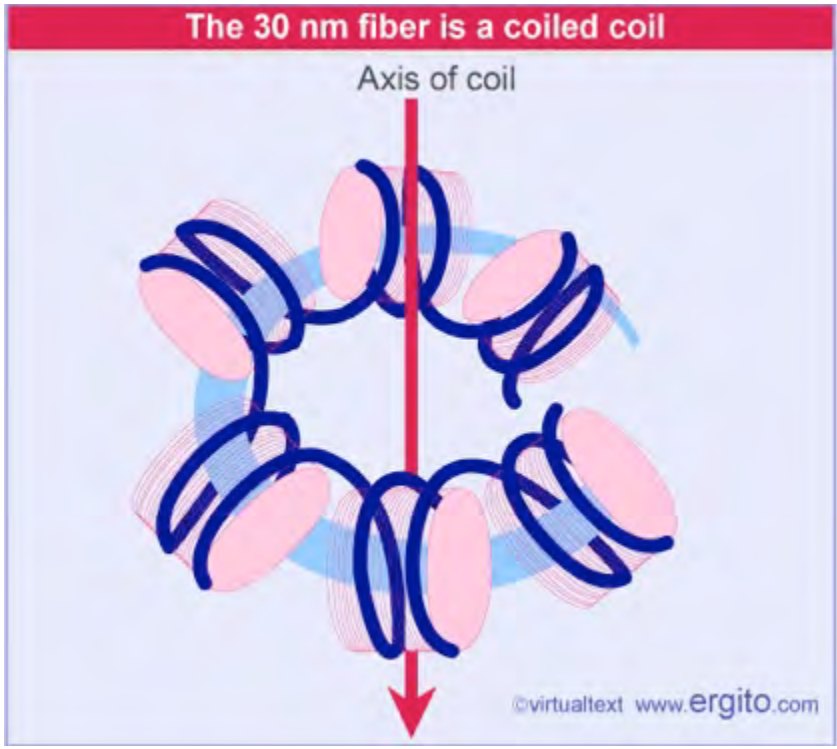
Histone H1 binds to the linker DNA to help convert the 10nm chromatin fiber to a 30nm fiber



Linker DNA (10-90 bp)

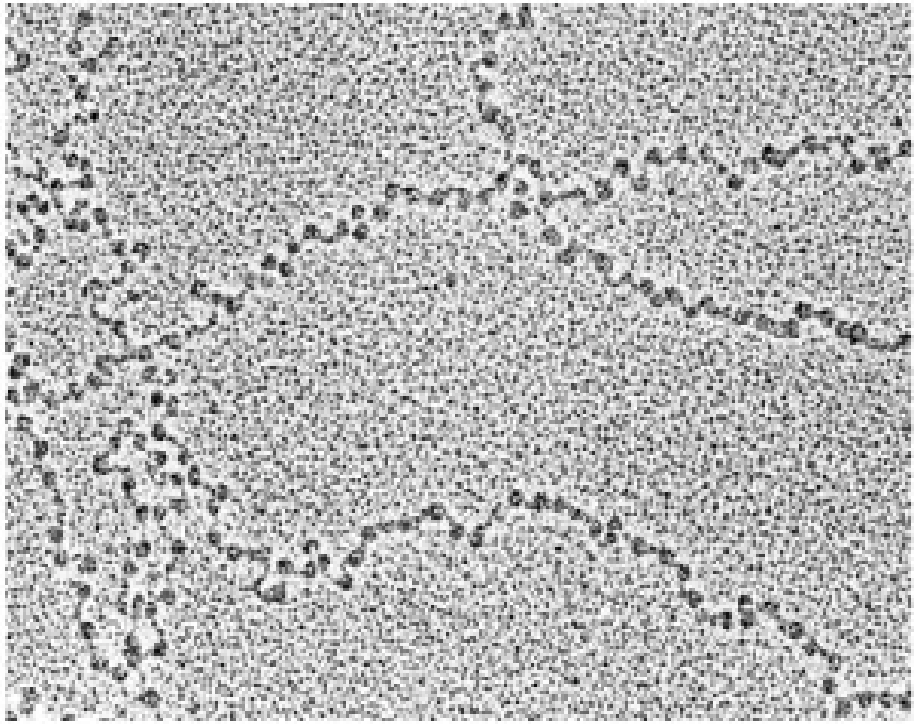


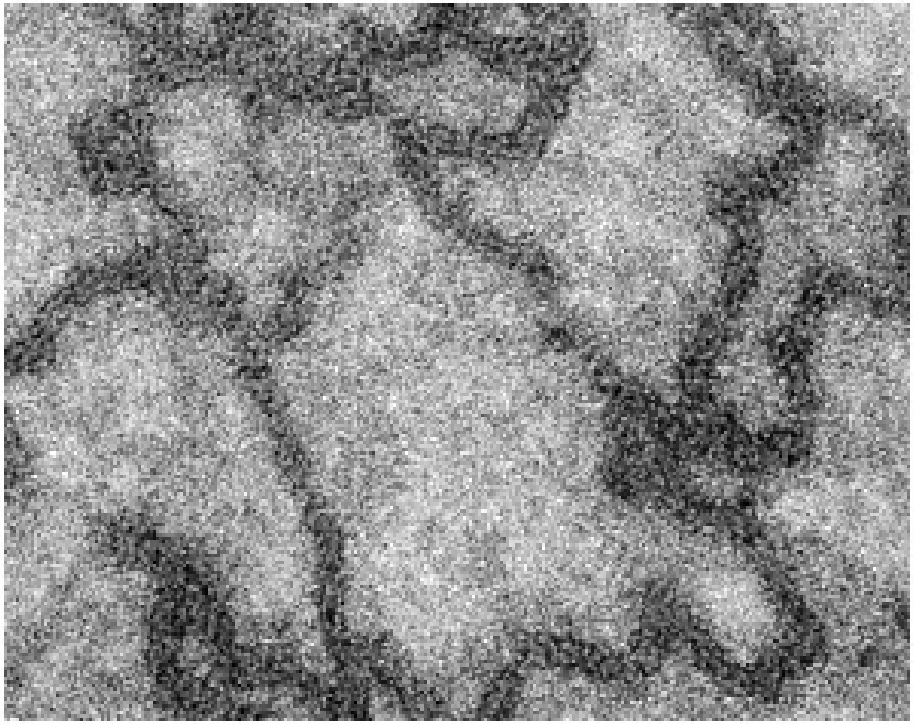
Histone H1



- 30 nm fiber is coil of nucleosomes with 6/turn

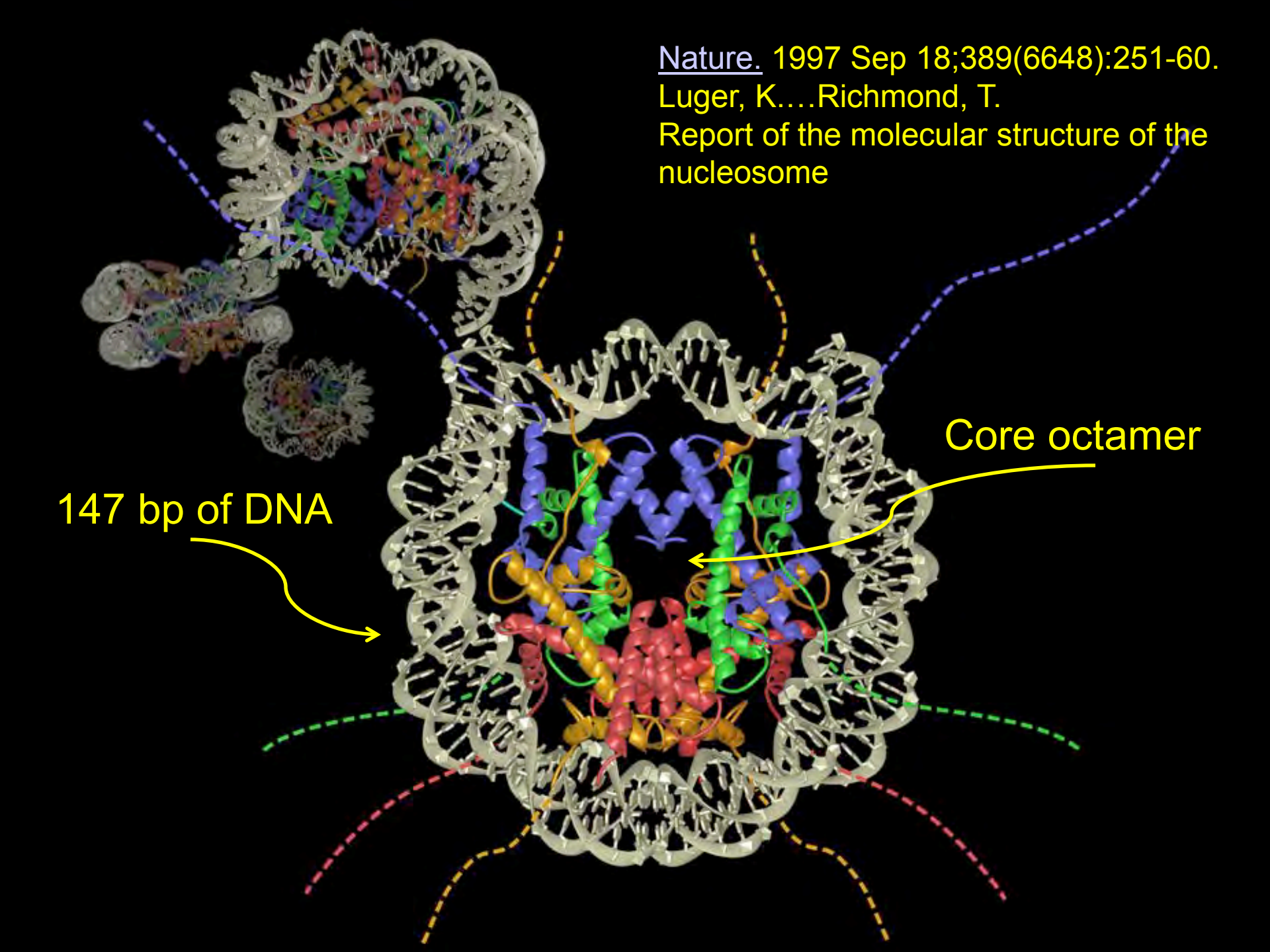
EM images of real chromatin

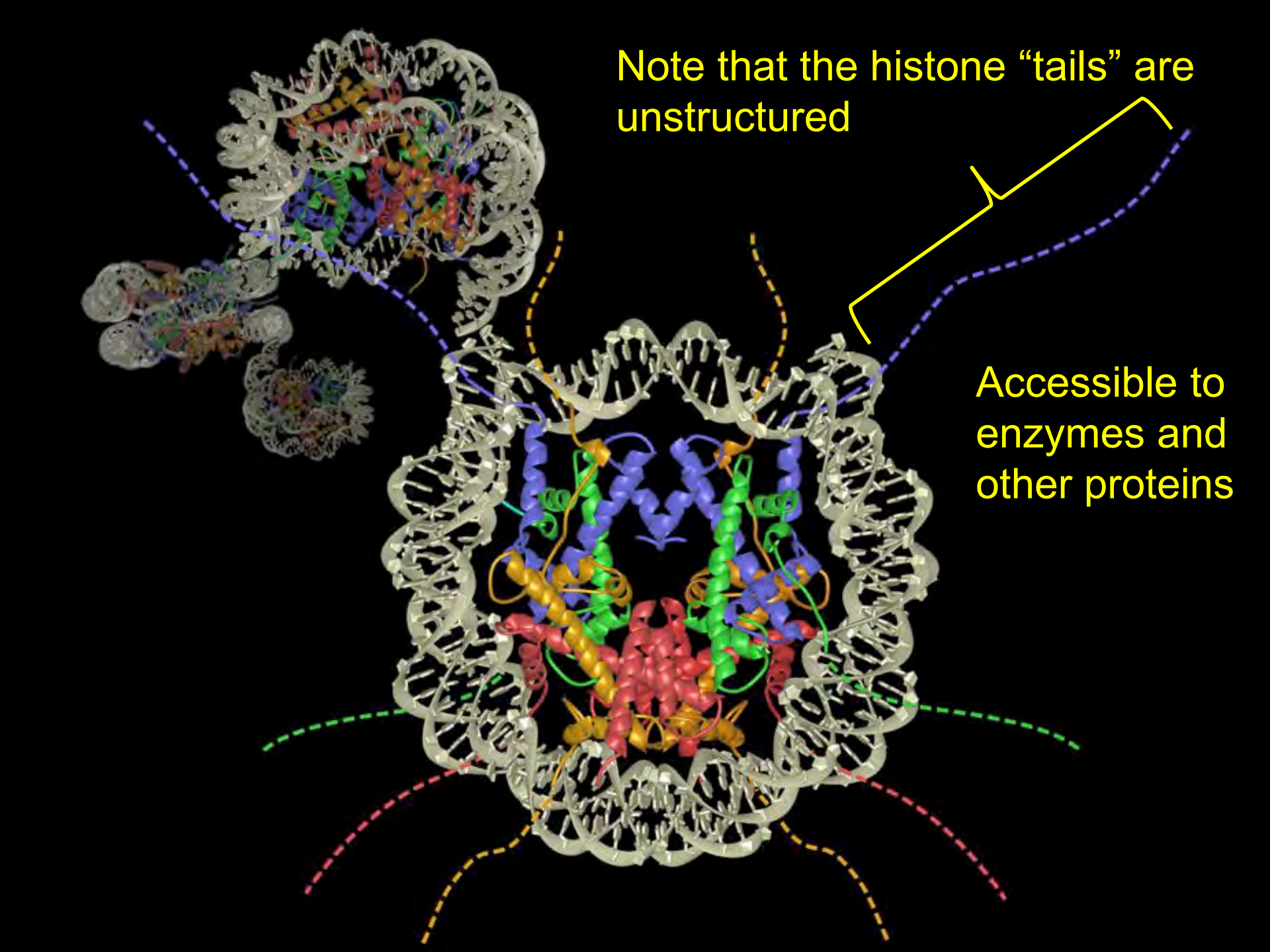




10nm fiber → 30nm fiber

DNA compaction ratio=36


Nature. 1997 Sep 18;389(6648):251-60.
Luger, K.…Richmond, T.
Report of the molecular structure of the nucleosome
Core octamer
147 bp of DNA


Note that the histone “tails” are unstructured
Accessible to enzymes and other proteins

Chromatin structure – Why we regulate it

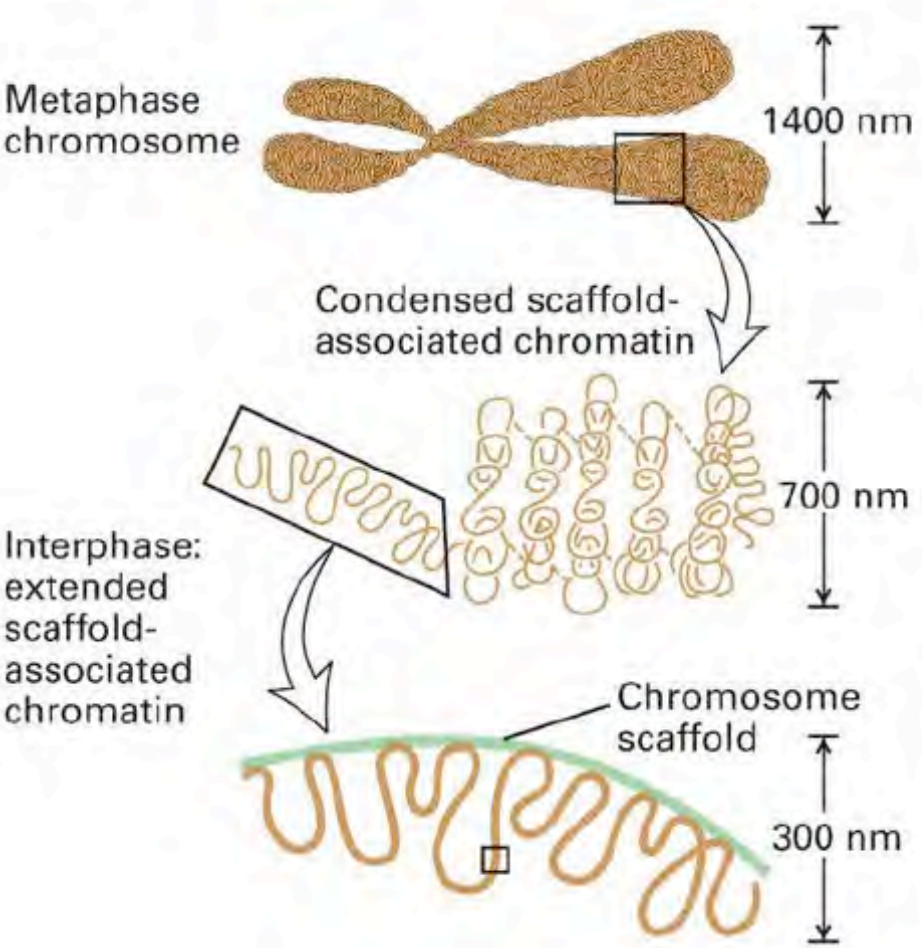
Histones do not bind DNA in a sequence-specific manner but nucleosomes do tend to adopt particular positions on a given DNA sequence

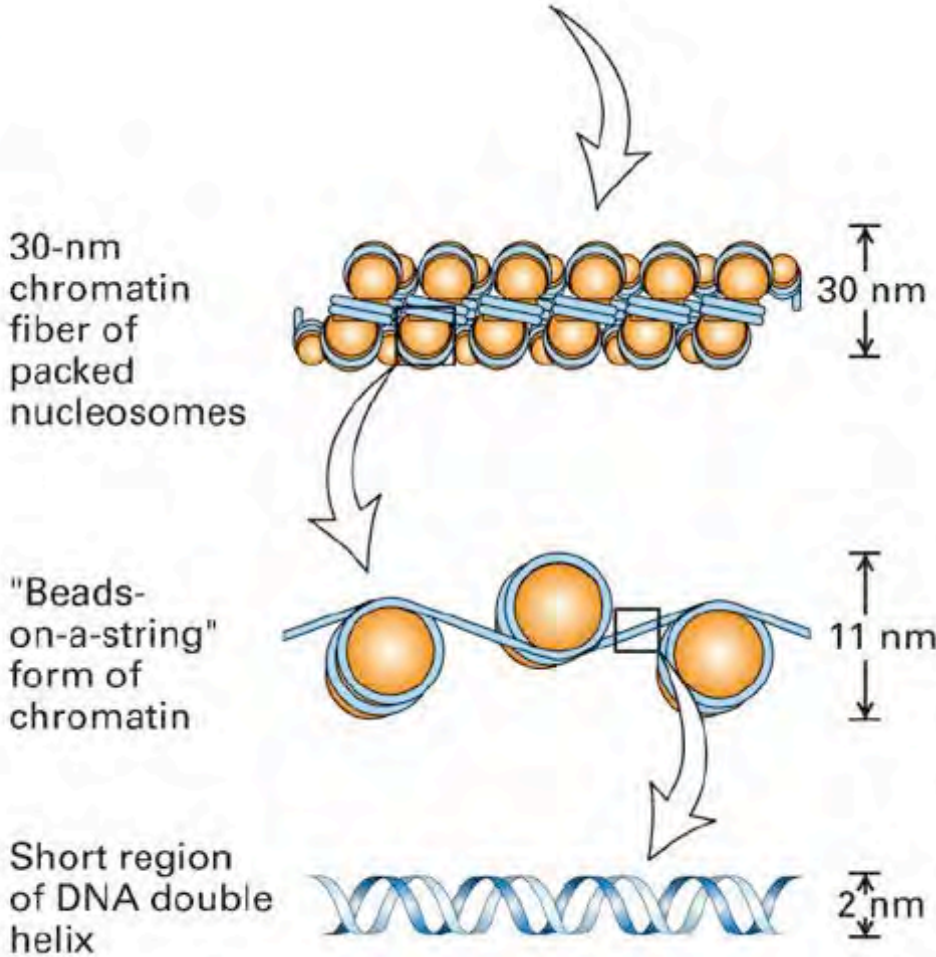
Steric hindrance of DNA (information and regulation) by nucleosomes and DNA-bound protein complexes

"…very important consequences for the activities of the genome (transcription, DNA repair and replication) given that histone octamers limit access to the DNA sequence."

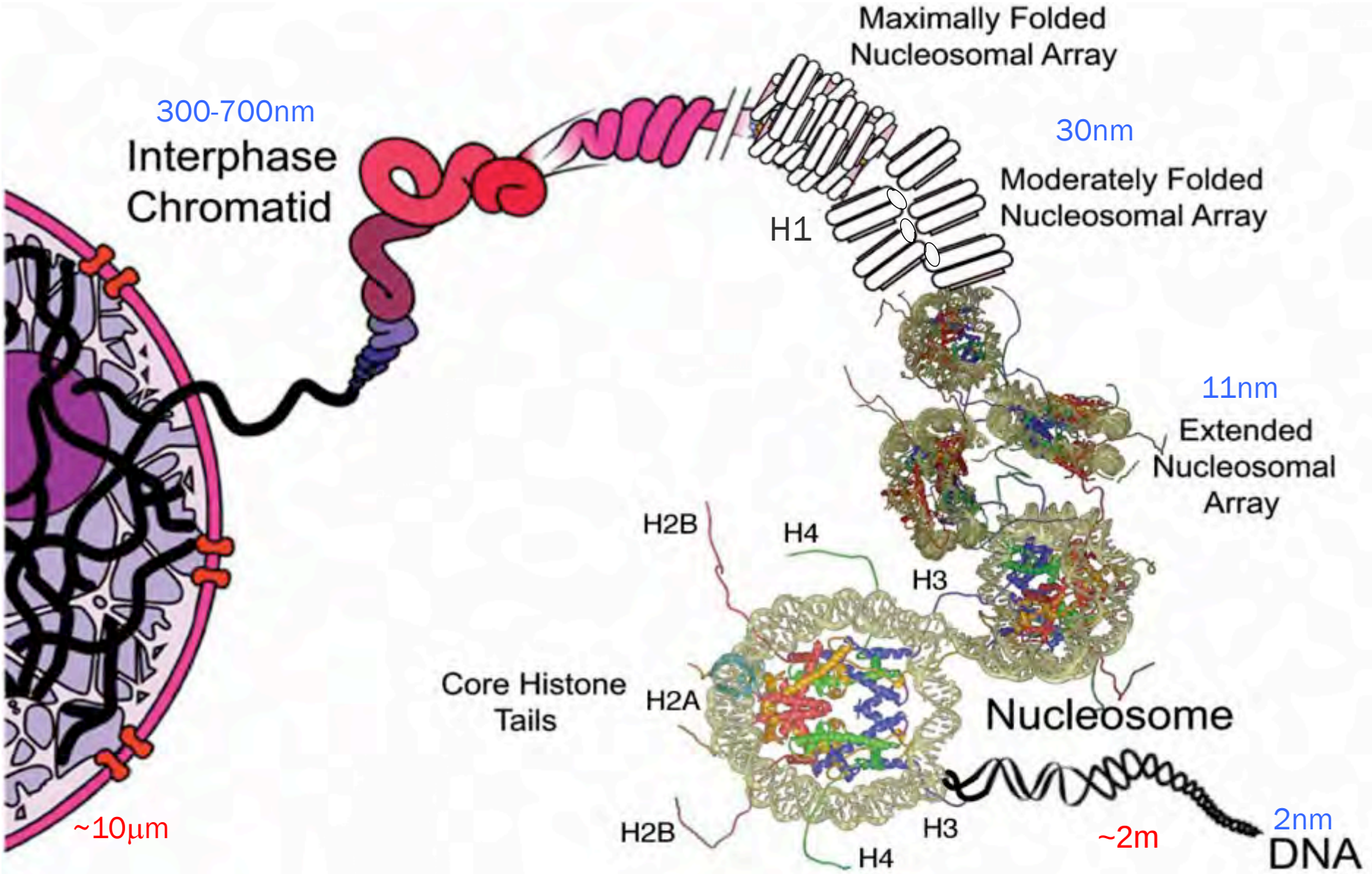
What controls nucleosome positions?
Segal E, Widom J.
Trends Genet. 2009 Aug;25(8):335-43. Epub 2009 Jul 10.

Packaging eukaryotic DNA into chromatin

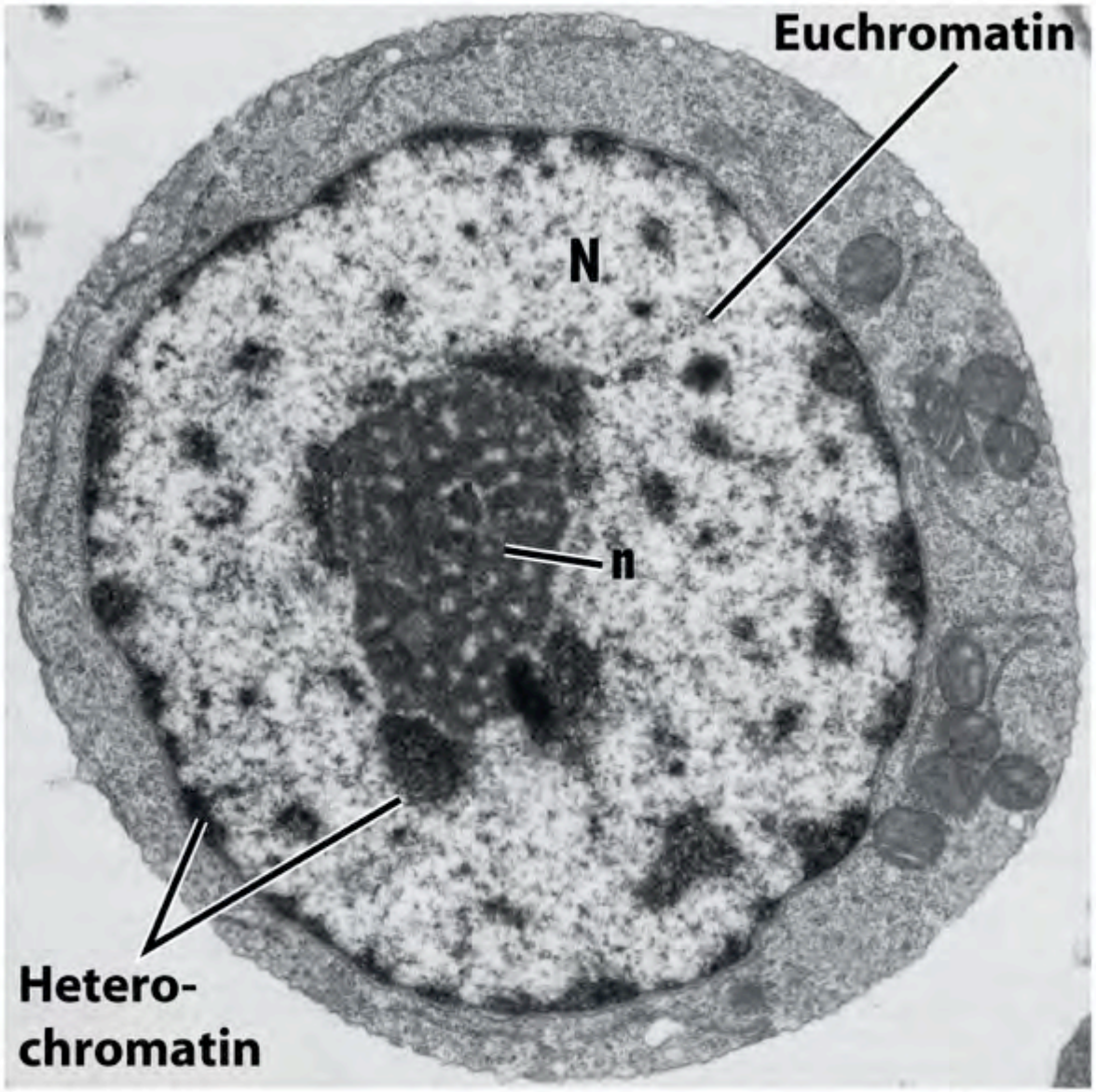




Hierarchical Packaging of the Chromosome



Euchromatin vs Heterochromatin



Euchromatin is where active genes reside. It is a more accessible form of chromatin.

Heterochromatin is repressed because the DNA is inaccessible (found at centromeres, telomeres and some internal chromosome positions).

Characteristics of heterochromatin

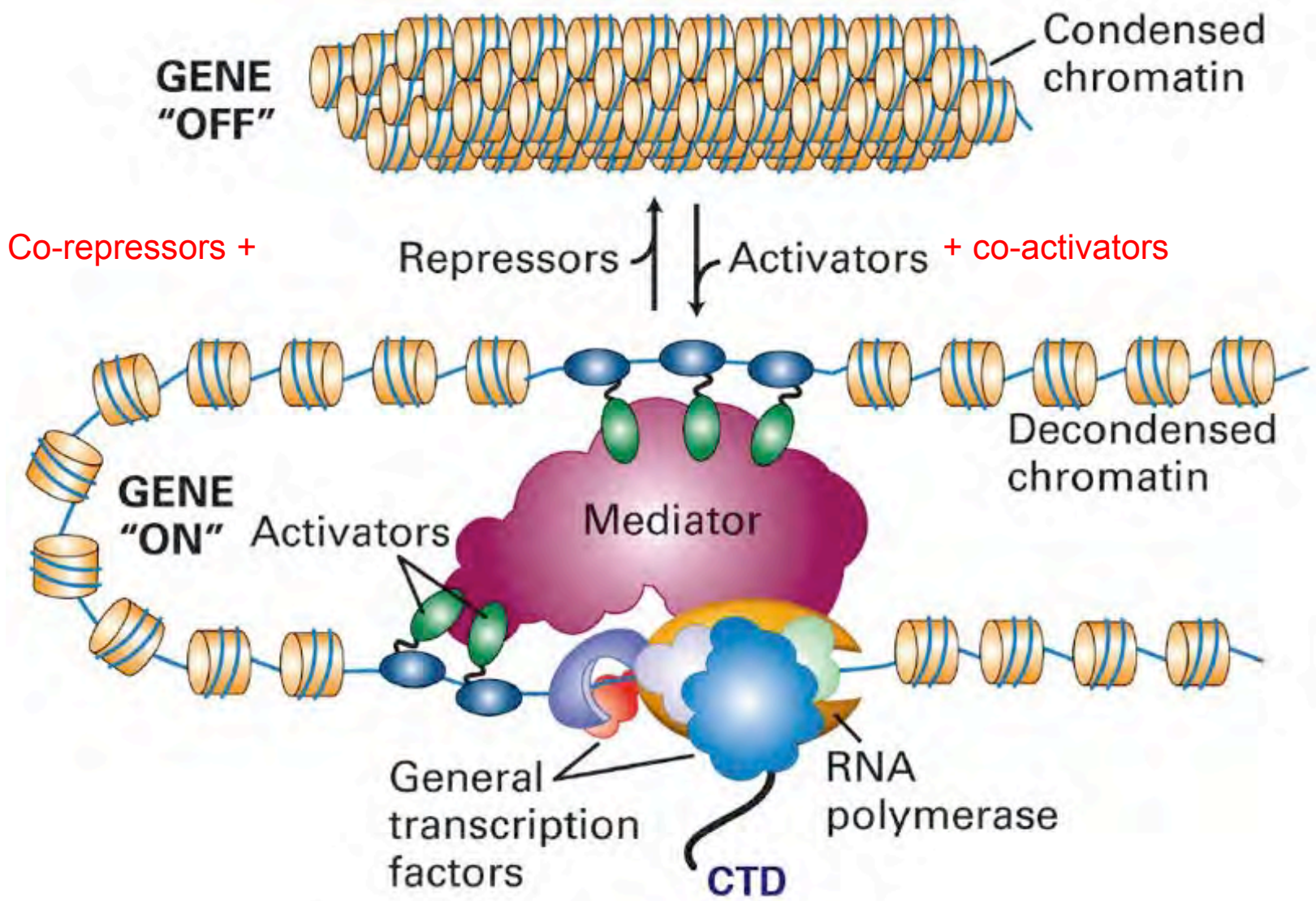
Heterochromatin -
~200nm fibre
histones are hypoacetylated.
histones are methylated on H3 K9
DNA is often methylated
Inaccessible to restriction enzymes and DNAse I digestion
Transcriptionally inactive
Late replicating
Contains tandem repeated sequences

Constitutive Heterochromatin is always heterochromatin and contains satellite DNA. Example - centromeric DNA

Facultative Heterochromatin can change to euchromatin, depending on the cell type or developmental stage.

Example - X-inactivation in males versus females

Take home message:
Chromatin permits or blocks access to the DNA



Why? What? How?

The Scientist
Volume 25 | Issue 3 | Page 32
Date: 2011-03-01

THE BASICS

EPIGENETICS: A PRIMER

There are many ways that epigenetic effects regulate the activation or repression of genes. Here are a few molecular tricks cells use to read off the right genetic program. By Stefan Kubicek

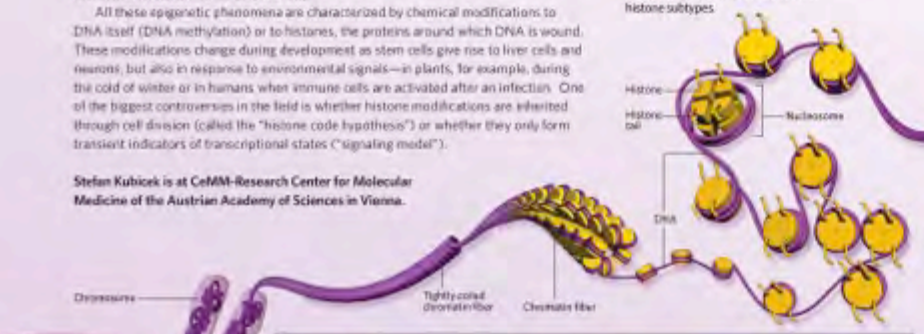
What makes the ~200 cell types in our body remember their identity? What prevents them from becoming cancer cells? Why do we inherit some traits from our father, others from our mother? How do our experiences and environment influence our thinking? Why do plants bloom in spring but not in winter? These important and quite different questions are all addressed by the field of epigenetics, which studies heritable changes in a phenotype arising in the absence of alterations in the DNA sequence. The idea of transgenerational inheritance of acquired characteristics goes back to Lamarck in the early 19th century, but still only correlative evidence exists in humans. In contrast, many cellular epigenetic phenomena are now well understood on the molecular level. In humans, they include the parent-of-origin specific expression of genes (imprinting) and the shutting-down of almost all genes on one of the two X chromosomes in females (X-chromosome inactivation).

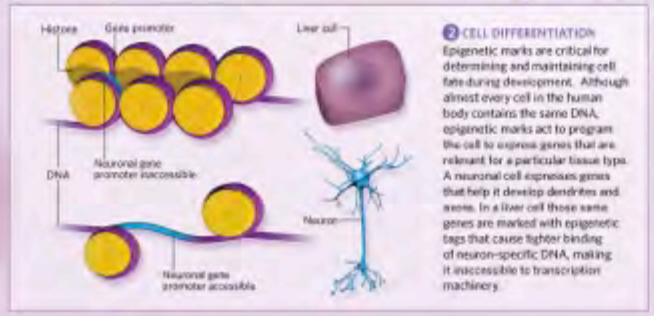
All these epigenetic phenomena are characterized by chemical modifications to DNA itself (DNA methylation) or to histones, the proteins around which DNA is wound. These modifications change during development as stem cells give rise to liver cells and neurons, but also in response to environmental signals—in plants, for example, during the cold of winter or in humans when immune cells are activated after an infection. One of the biggest controversies in the field is whether histone modifications are inherited through cell division (called the "histone code hypothesis") or whether they only form transient indicators of transcriptional states ("signaling model").

Stefan Kubicek is at CeMM-Research Center for Molecular Medicine of the Austrian Academy of Sciences in Vienna.

1 OVERVIEW

Epigenetic events regulate the activities of genes without changing the DNA sequence. Different genes are expressed depending on the methyl-marks attached to DNA itself and by changes in the structure and/or composition of chromatin. The main components of chromatin are histones (in bundles of eight units) around which 146 base pairs of DNA are wound like a thread around a spool, forming a structure called the nucleosome. There are various epigenetic mechanisms that can affect the nucleosome: chemical modification (via molecular additions to histone tails or DNA), a change in its positioning on DNA (via chromatin remodeling proteins), or a variation in histone subtypes.



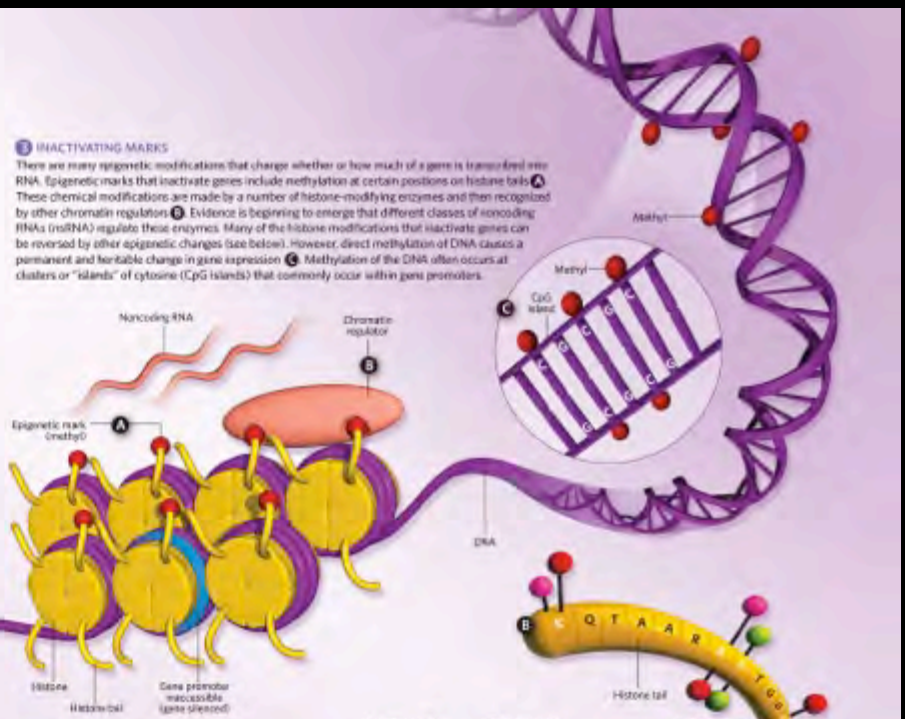


2 CELL DIFFERENTIATION

Epigenetic marks are critical for determining and maintaining cell fate during development. Although almost every cell in the human body contains the same DNA, epigenetic marks act to program the cell to express genes that are relevant for a particular tissue type. A neuronal cell expresses genes that help it develop dendrites and axons. In a liver cell those same genes are marked with epigenetic tags that cause tighter binding of neuron-specific DNA, making it inaccessible to transcription machinery.

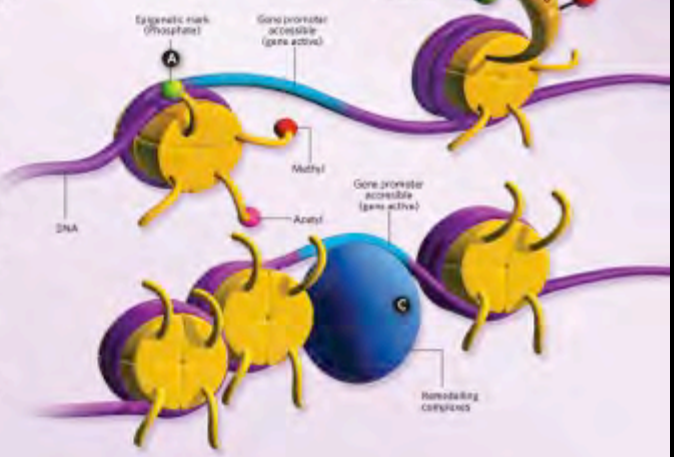
3 INACTIVATING MARKS

There are many epigenetic modifications that change whether or how much of a gene is transcribed into RNA. Epigenetic marks that inactivate genes include methylation at certain positions on histone tails (A). These chemical modifications are made by a number of histone-modifying enzymes and then recognized by other chromatin regulators (B). Evidence is beginning to emerge that different classes of noncoding RNAs (ncRNA) regulate these enzymes. Many of the histone modifications that inactivate genes can be reversed by other epigenetic changes (see below). However, direct methylation of DNA causes a permanent and heritable change in gene expression (C). Methylation of the DNA often occurs at clusters or "islands" of cytosine (CpG islands) that commonly occur within gene promoters.

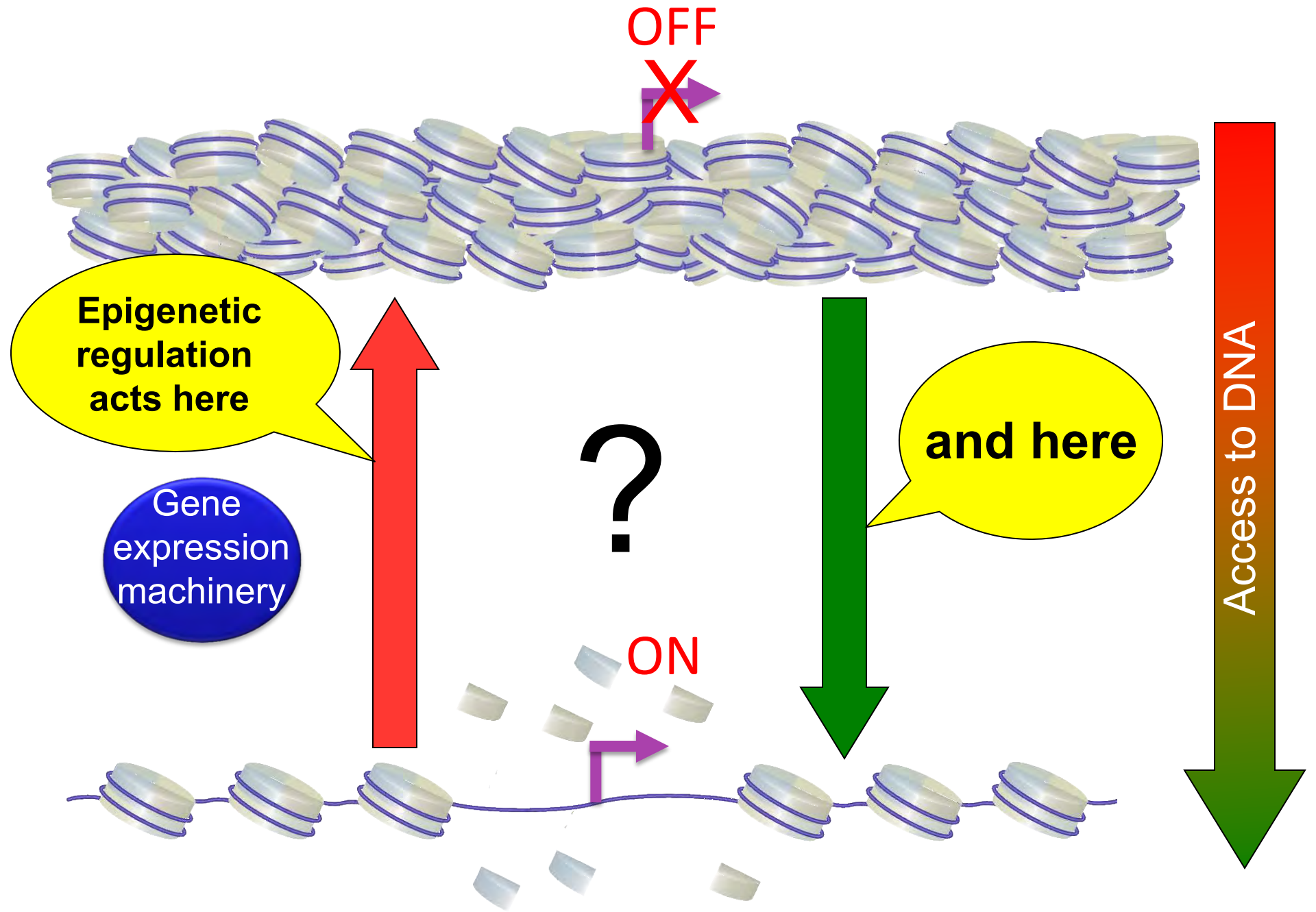


4 ACTIVATING MARKS

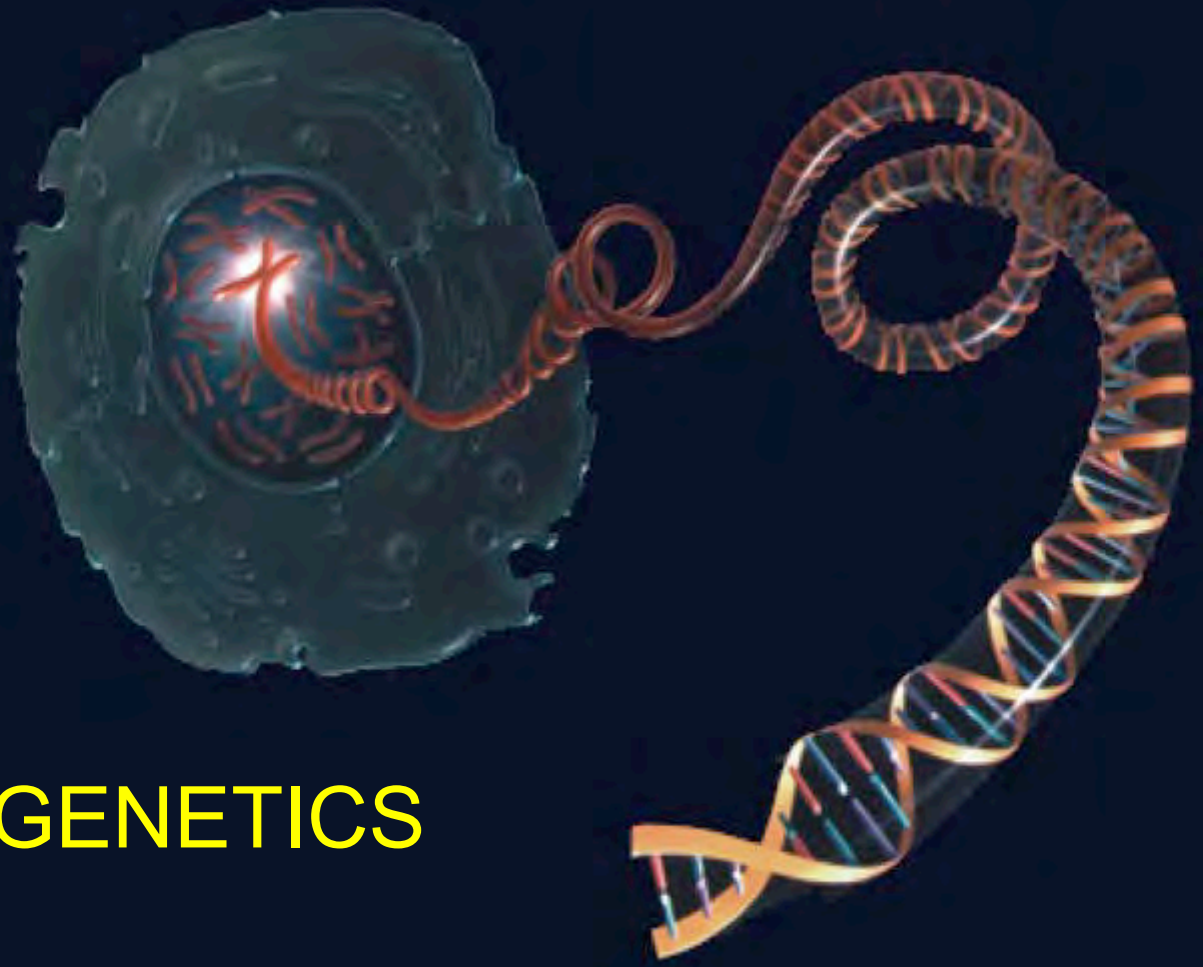
The heritability of DNA methylation, which often occurs in the early stages of development, allows cells to keep irrelevant genes silenced in successive generations of liver or skin cells. However some genes—such as the plant genes that govern winter dormancy and springtime flowering—require silenced genes to be reactivated. Several modifications, including the acetylation, phosphorylation, as well as methylation of certain positions on a histone tail (A), can cause DNA to unwind, releasing the genes that are otherwise inaccessible. These modifications occur mostly at specific positions on the accessible tails of the histones, and subsequently recruit additional activating proteins (B). Histone-remodeling complexes, which slide histones in one direction or another, can also make genes accessible to transcription (C).



How does the cell alter chromatin structure?



WHY DO WE CARE?



EPIGENETICS

TCGA: The Cancer Genome Atlas

The NIH established The Cancer Genome Atlas (TCGA) to generate comprehensive, multi-dimensional maps of the key genomic changes in major types and subtypes of cancer.

Sequencing the genomes of 1000's of tumors/normal tissues

http://ocg.cancer.gov/

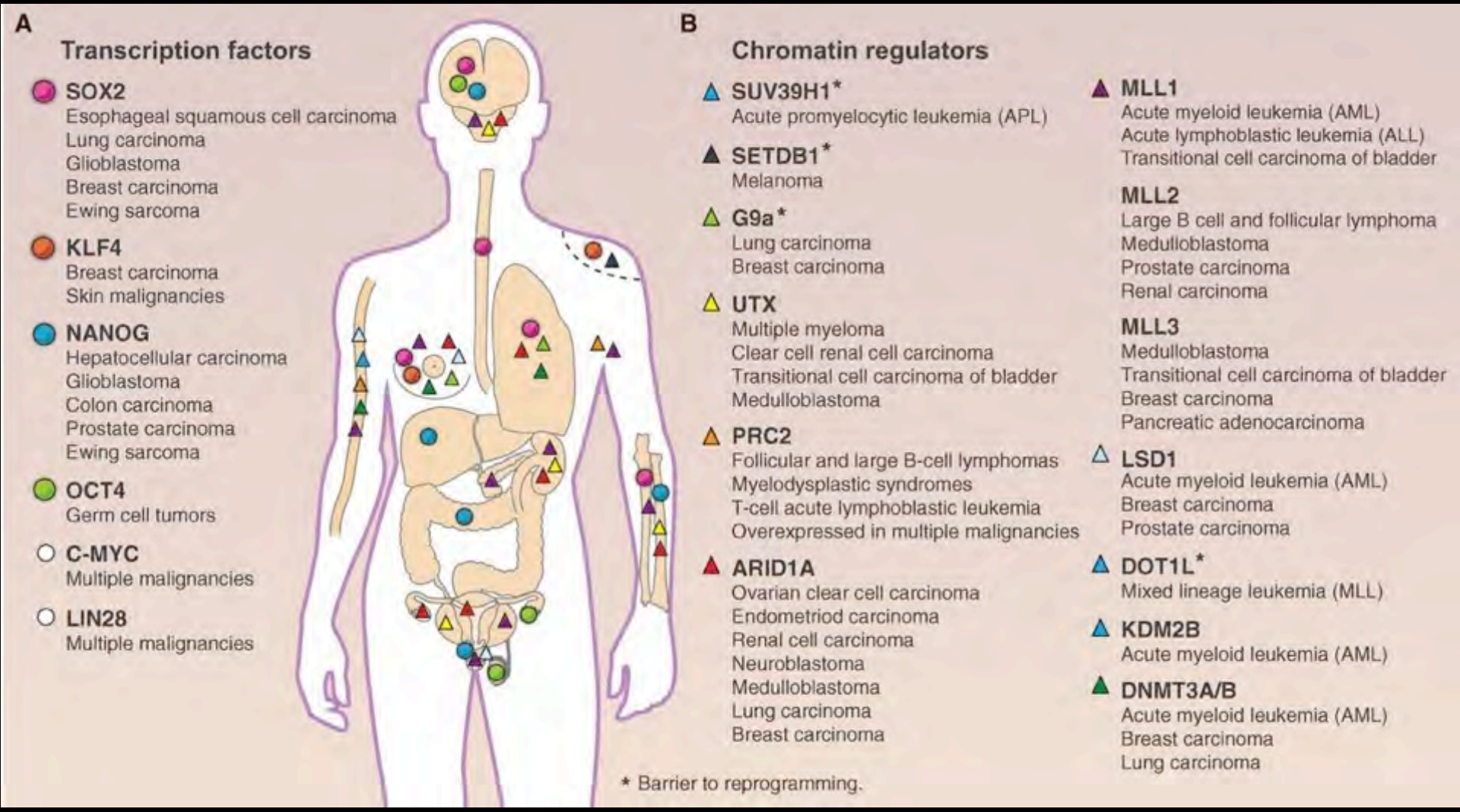
EPIGENETIC MODIFIERS – MAJOR HITS IN GLOBAL UNBIASED ANALYSES: EXPRESSION, MUTATIONS, DYSFUNCTION

Google images

NEW UNDERSTANDING OF CANCER

NEW THERAPEUTIC TARGETS!

EPIGENETICS X STEM CELLS = IMPACT IN CANCER



Epigenetic Reprogramming in Cancer

Mario L. Suvà, Nicolo Riggi, and Bradley E. Bernstein Science 2013

Dynamic regulation of the chromatin structure by the cell enables transcription in eukaryotes to be:

Highly regulated

Highly responsive to environmental stimuli

Epigenetically Inherited

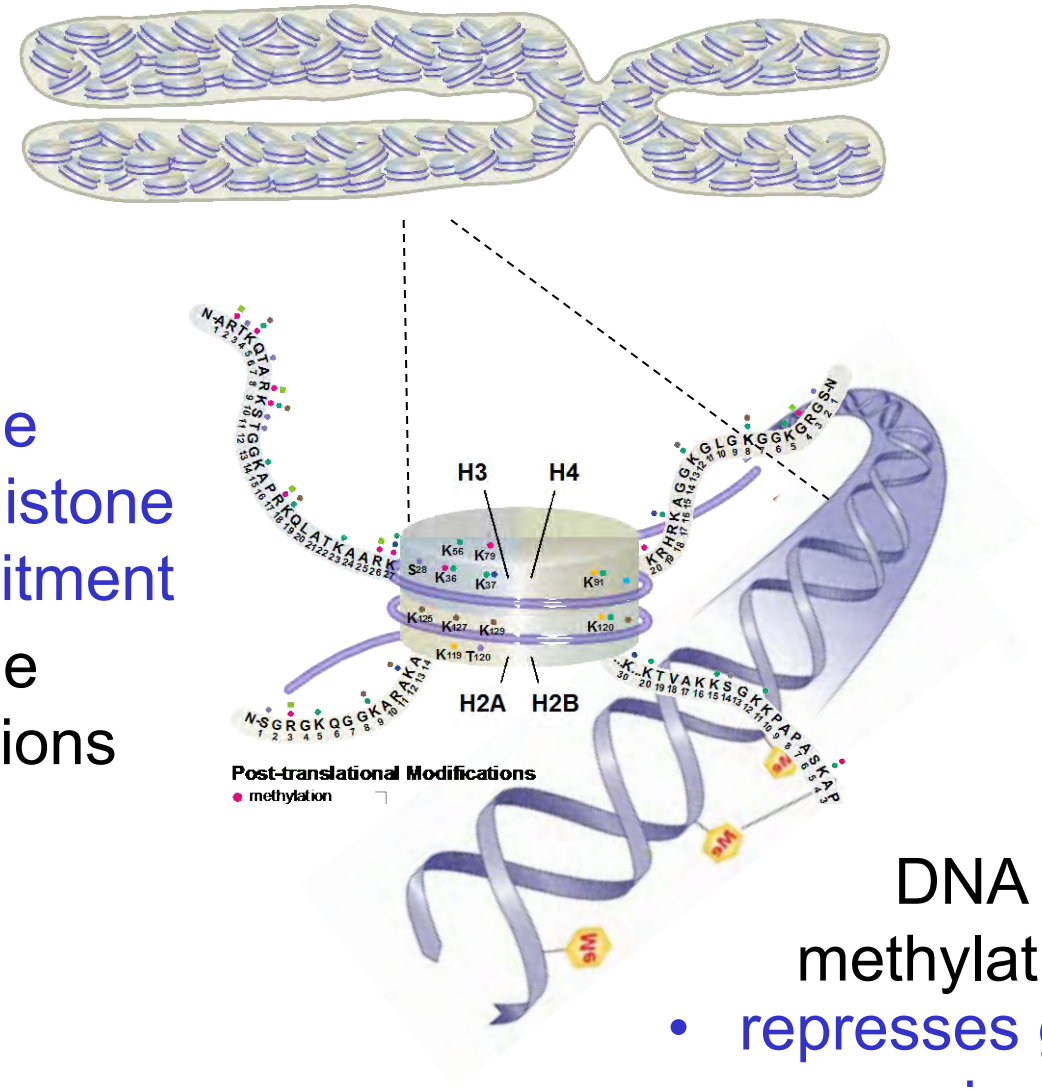
Epigenetic Mechanisms

Epigenetics can be defined as heritable changes in gene expression or phenotypes that do not involve changes in the DNA sequence.

- DNA methylation
- Histone modifications
- Histone variants
- Chromatin remodeling
- Regulatory non-coding RNAs

Epigenetic Code:Two main components

- dynamic and affect the interaction between histone and DNA; allow recruitment of regulators

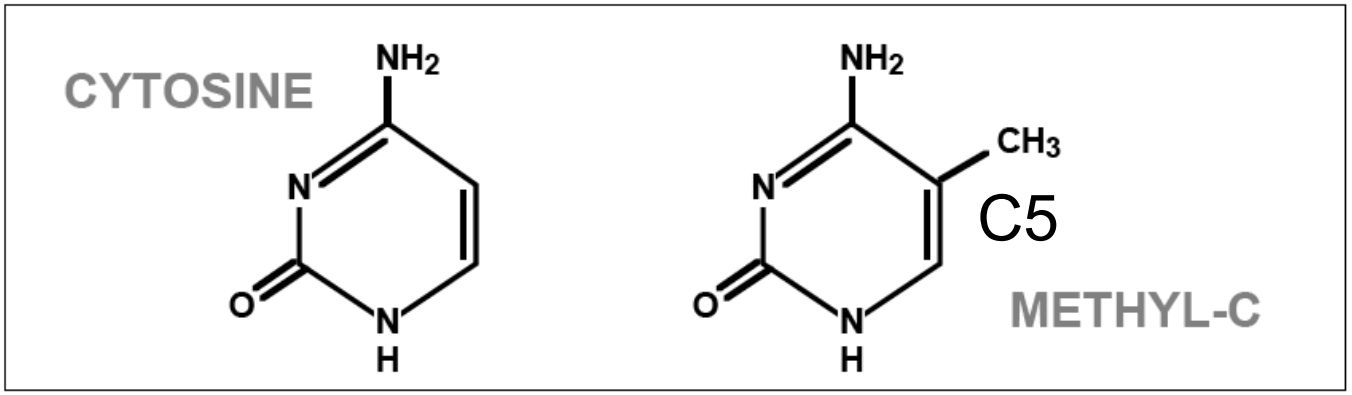


Histone modifications

DNA methylation

- represses gene expression

DNA methylation - locks in the repressed state



Cytosines in CG dinucleotides are subject to methylation at Carbon position 5.

DNA methylation does not alter base pairing but does alter chromatin access and proteins that bind.

CpG Islands:

CpG dinucleotides are under represented in the genome, except in short 1kb stretches called **CpG islands**.

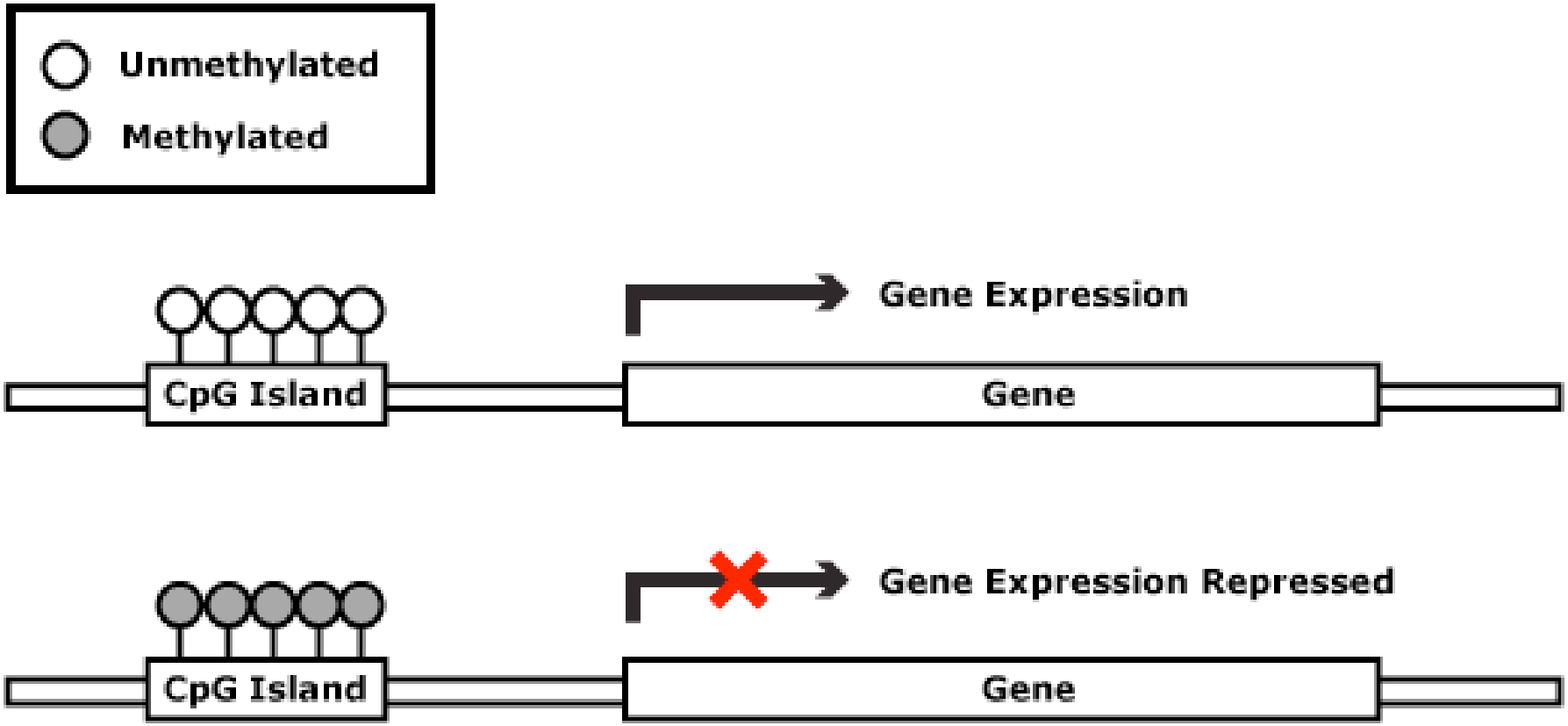
CpG islands usually occur in **promoters** and are **unmethylated** in **normal** cells.

Methylation of CpGs near **tumor suppressor genes** (ex. p53 and p16) causes **silencing** of these genes in tumors.

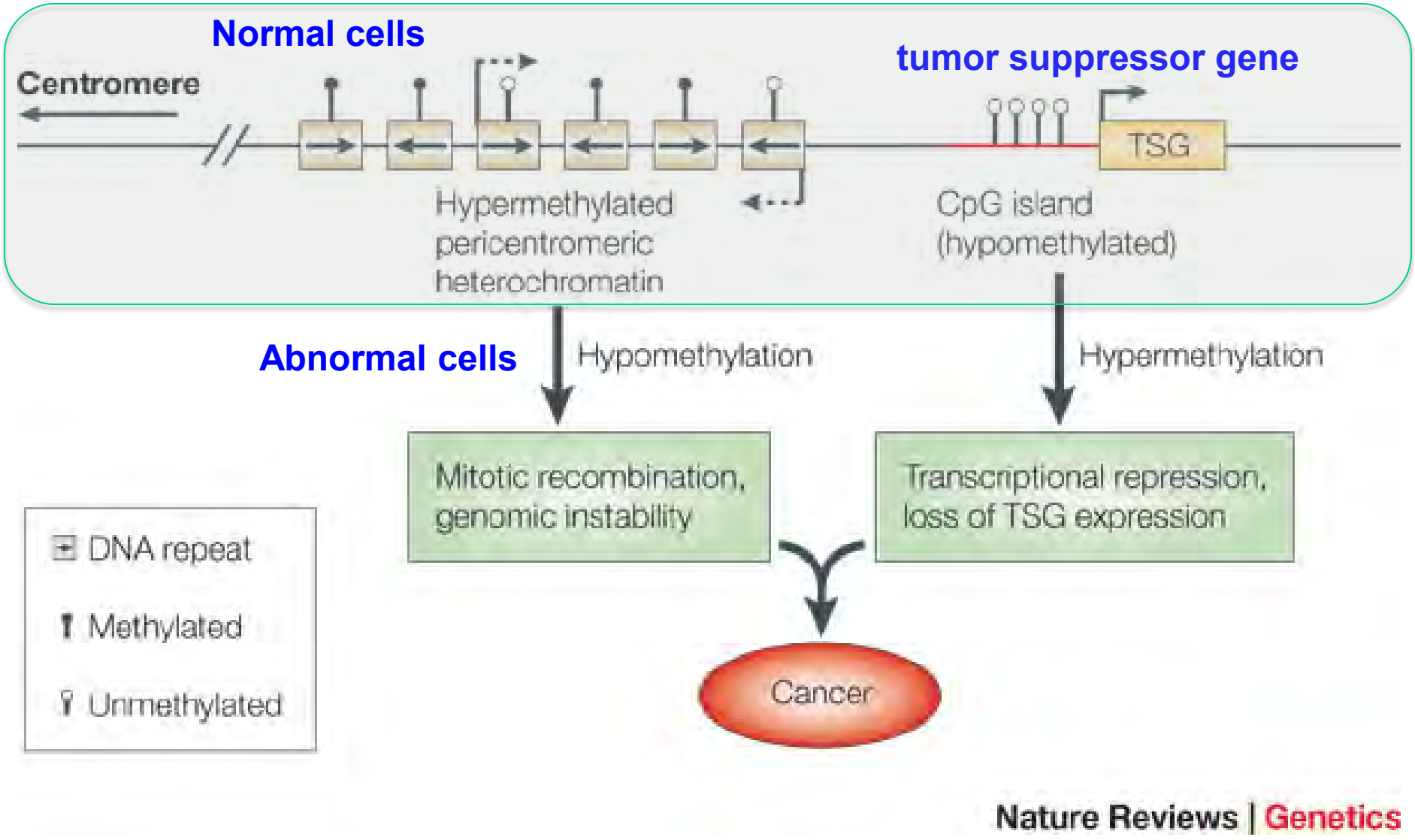
Normal CpG methylation is **regulated tightly** during **development** and is associated with gene silencing, X-inactivation, and allele-specific imprinting.

DNA methylation represses gene expression

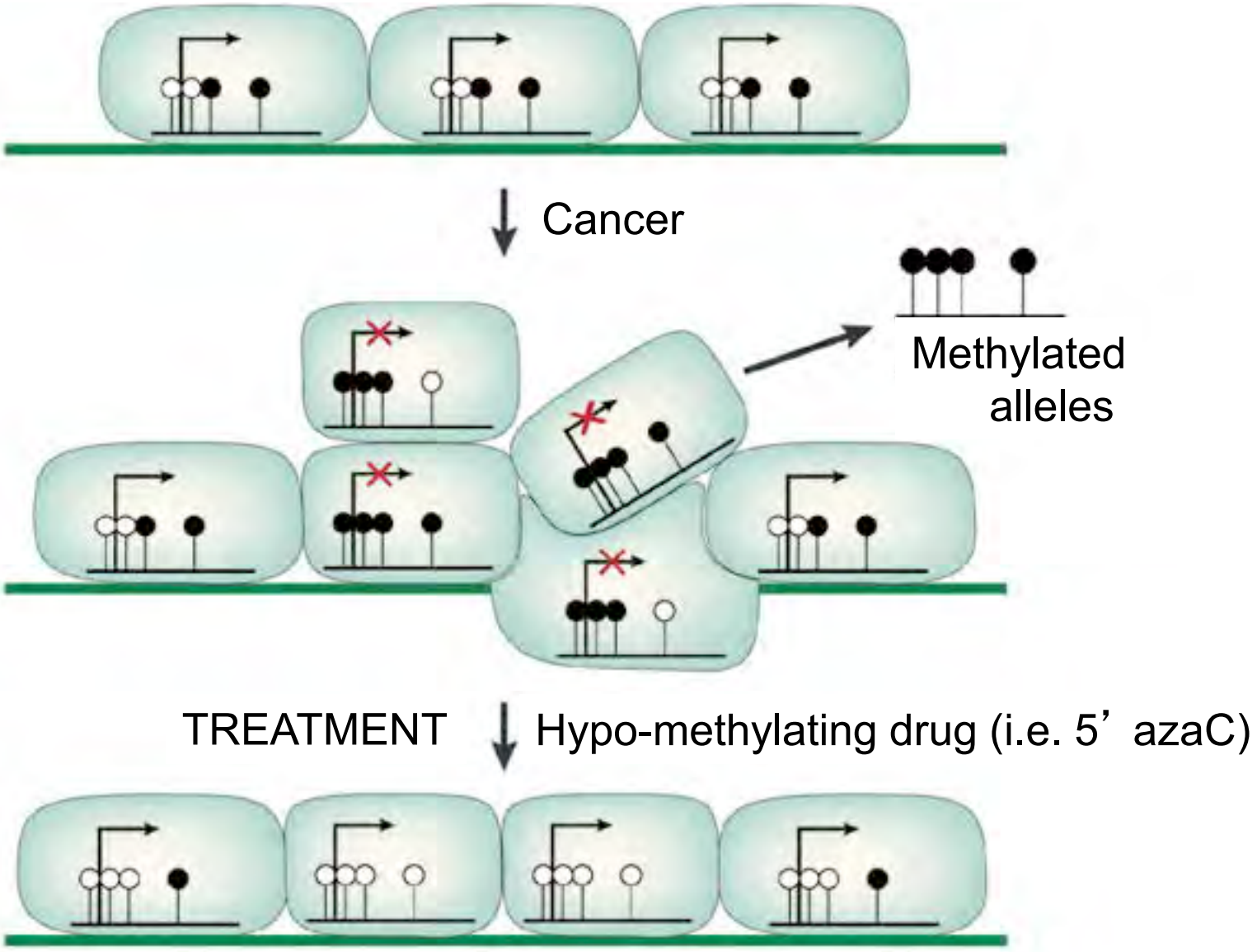
Different cell types have different methylation patterns, contributing to differences in gene expression



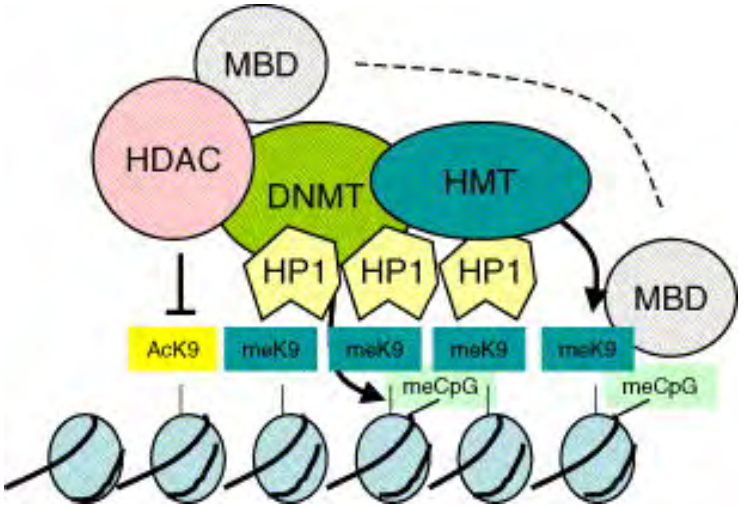
Cancer cells lose normal DNA methylation and gain abnormal DNA methylation



Tumor suppressor genes are turned off by CpG island DNA methylation in cancer



DNA methylation



- DNA methylation provides a platform for several methyl-binding proteins (i.e. MBD1-3) and enzymes that repress chromatin

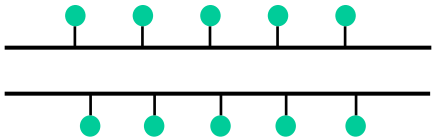
- Mutations in DNMT3A are present in up to 25% of AML patients and impacts prognosis

Two types of DNA methyltransferases:

1. de novo : DNMT3A/3B

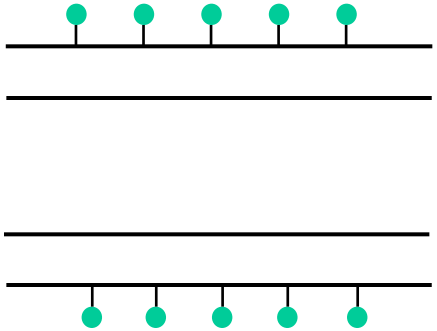
2. Maintenance: DNMT1

Maintenance methyltransferase propagates DNA methylation in somatic cells



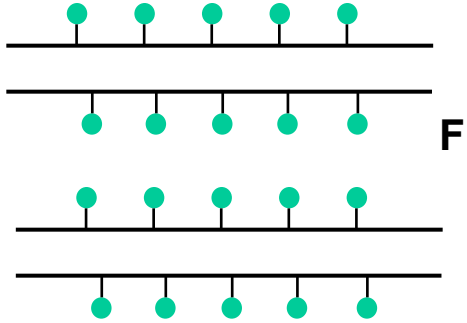
Fully methylated DNA

DNA Replication (semi-conservative)



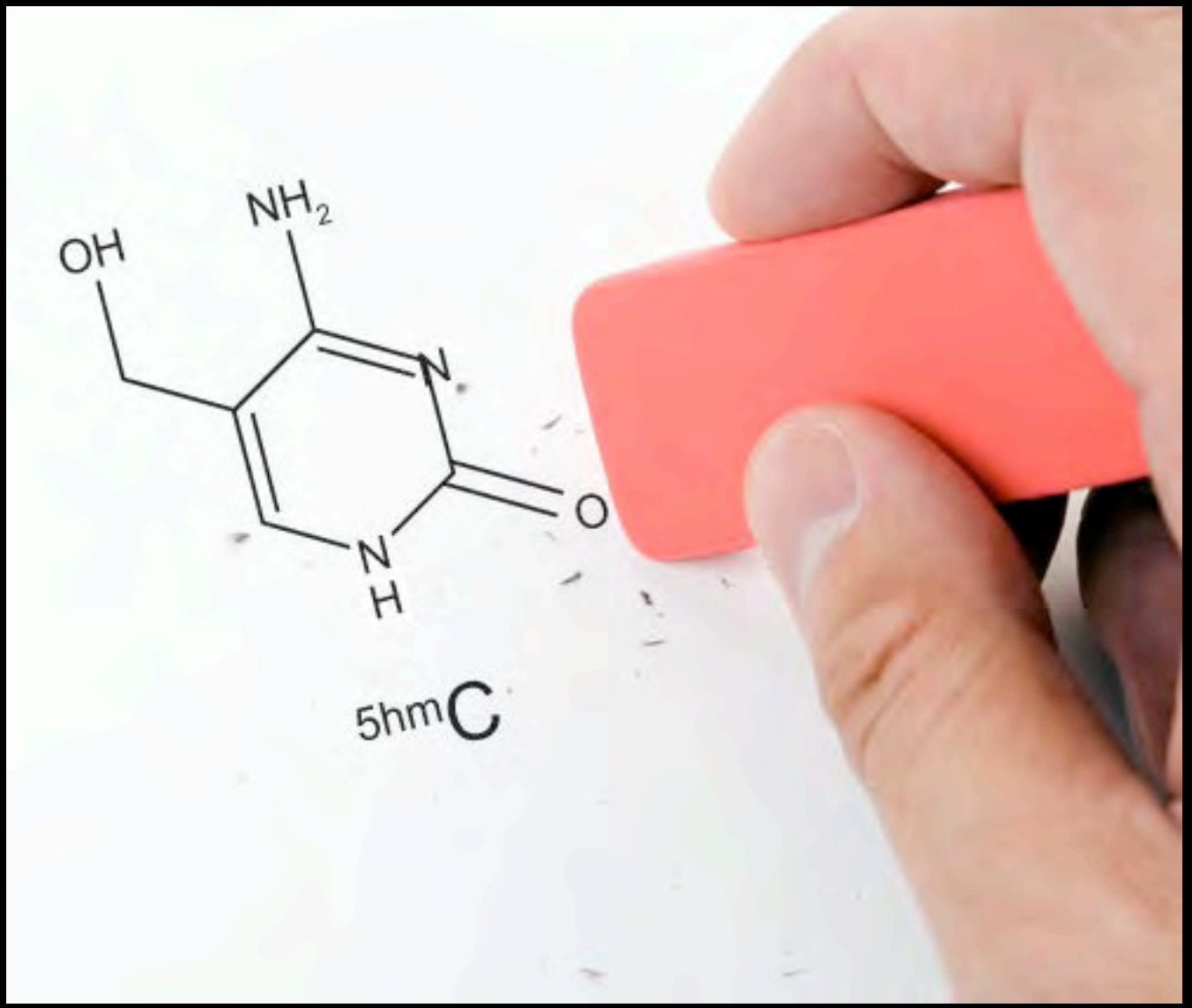
Hemi-methylated DNA

Maintenance DNA Methyltransferase



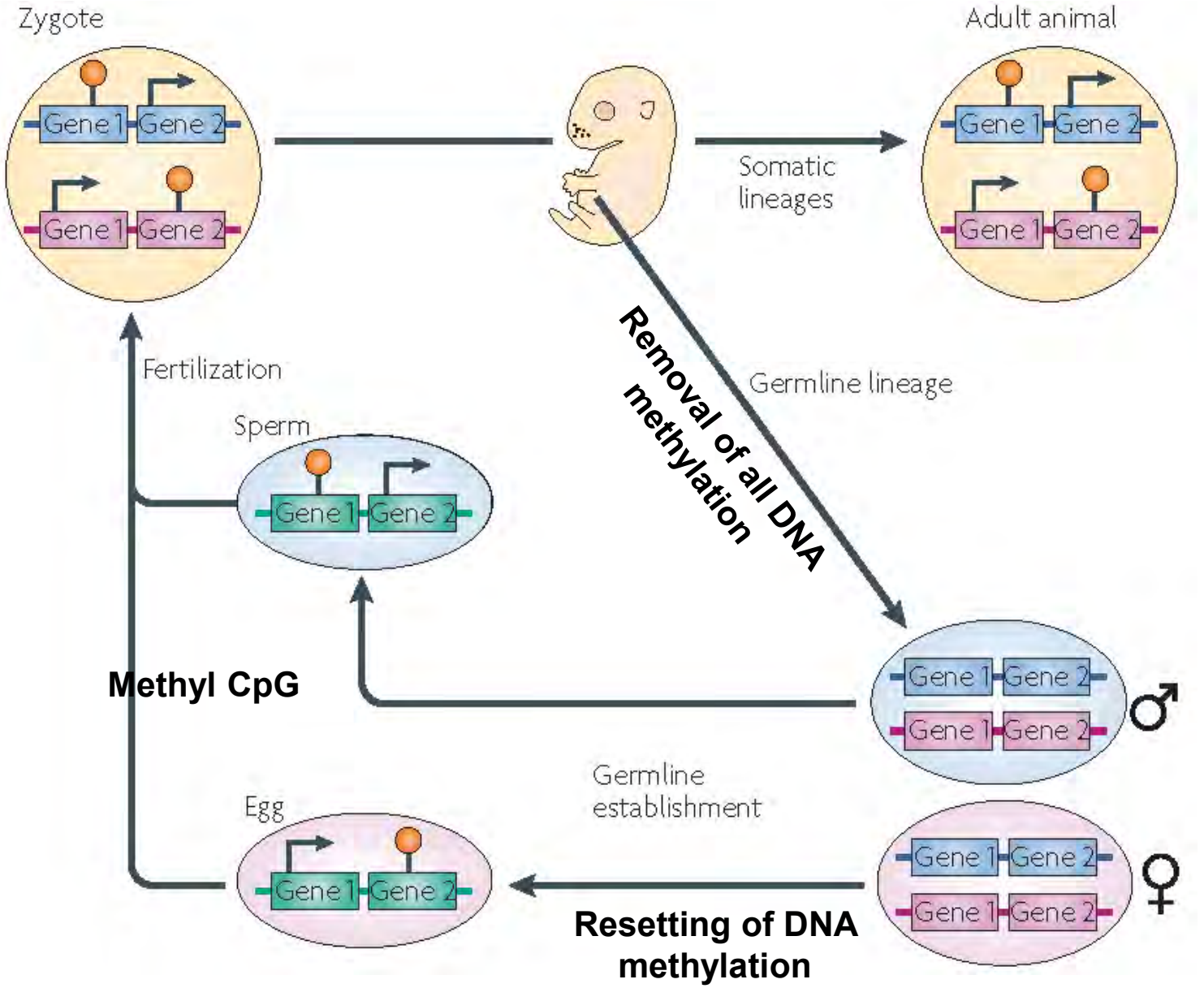
Fully methylated DNA

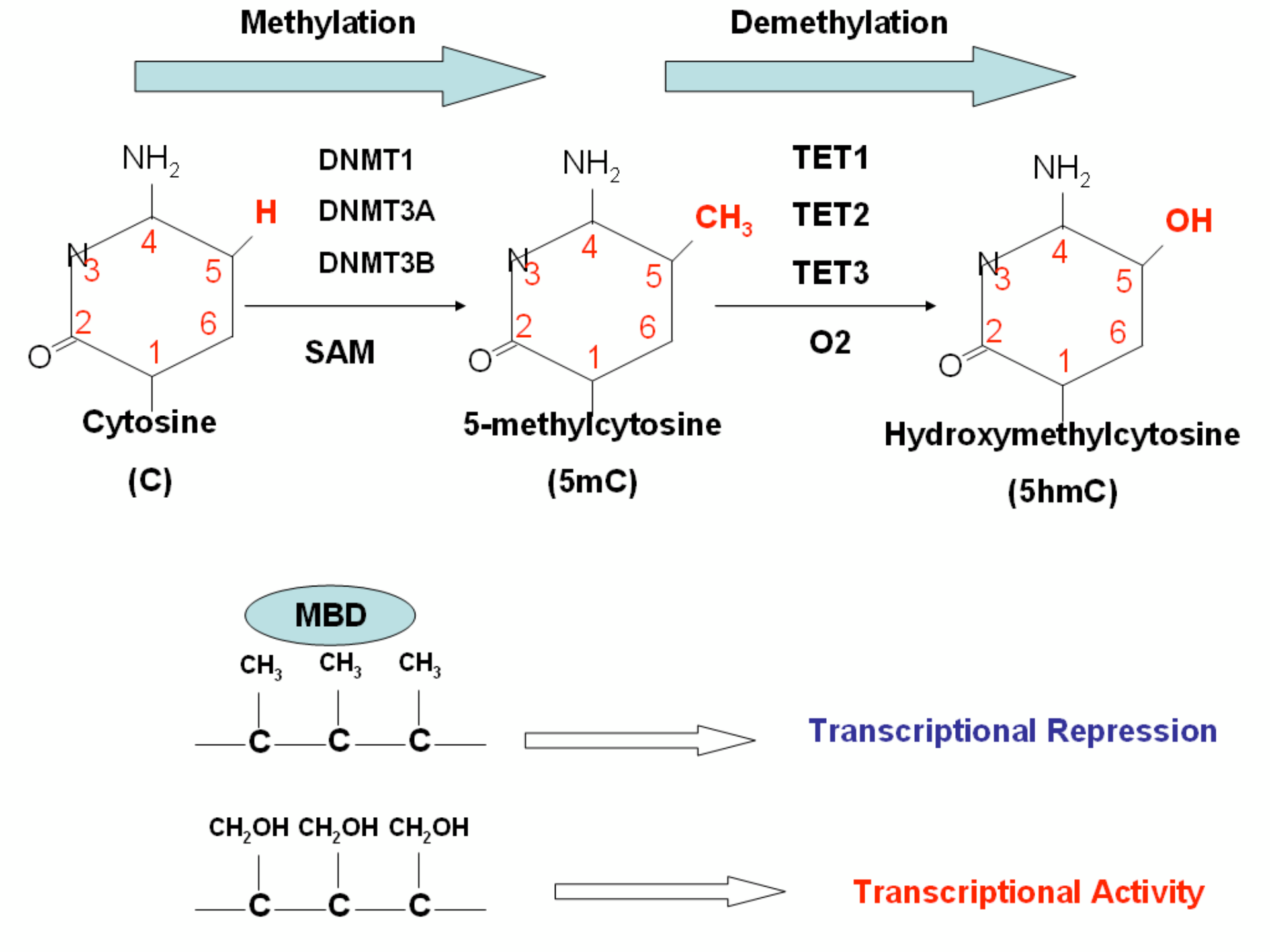
IS DNA METHYLATION REVERSIBLE?



De Novo DNA methylation and demethylation occur during development

allele specific imprinting




Methylation
Demethylation
NH2
H
DNMT1
DNMT3A
DNMT3B
SAM
Cytosine
(C)
CH3
TET1
TET2
TET3
O2
5-methylcytosine
(5mC)
OH
Hydroxymethylcytosine
(5hmC)
MBD
CH3 CH3 CH3
C C C
Transcriptional Repression
CH2OH CH2OH CH2OH
C C C
Transcriptional Activity

DNA Methylation is dynamic

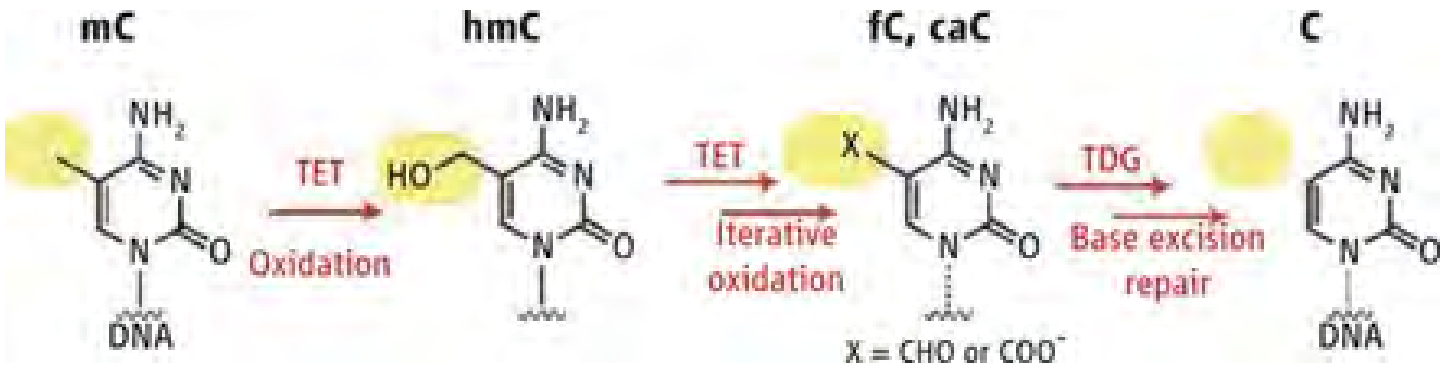
Biological significance of oxidation derivatives uncertain
Act as intermediates in DNA methylation
Affect binding of MBD proteins

5-methylcytosine (5mC)

5-hydroxymethylcytosine (5hmC)

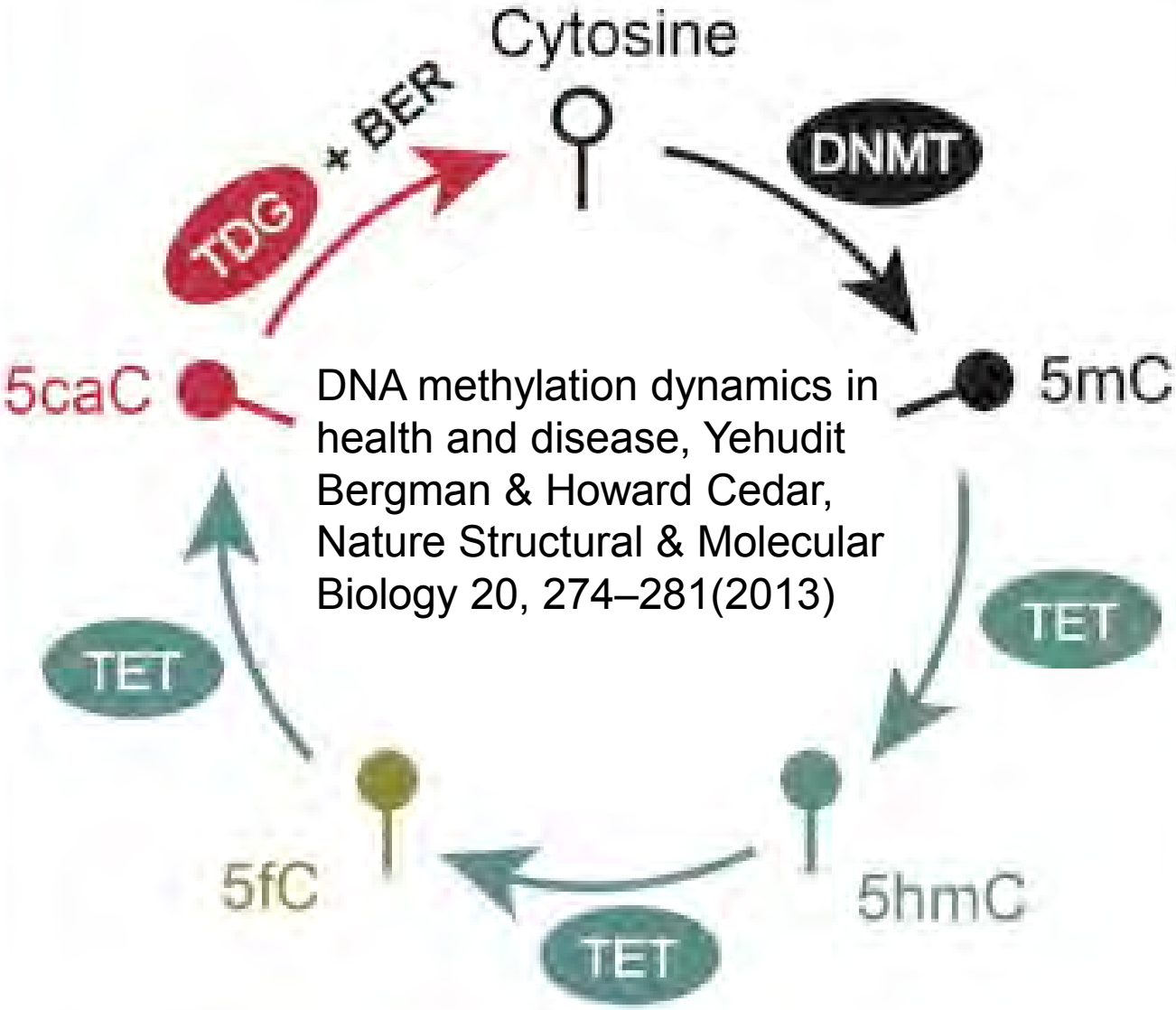
5-formylcytosine (5fC)

5-carboxylcytosine (5caC)



TET2 deficient mice get chronic myelomonocytic leukemia (CMML). TET2 mutations seen in AML and CMML patients. Most are loss of function mutations

TET/TDG-dependent active DNA demethylation

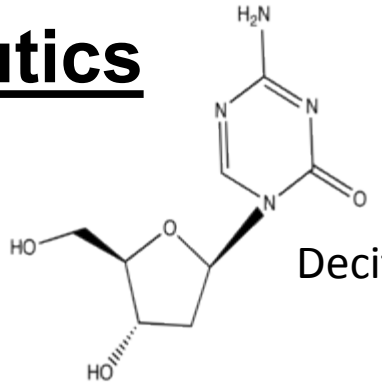


DNA methylation dynamics in health and disease, Yehudit Bergman & Howard Cedar, Nature Structural & Molecular Biology 20, 274–281(2013)

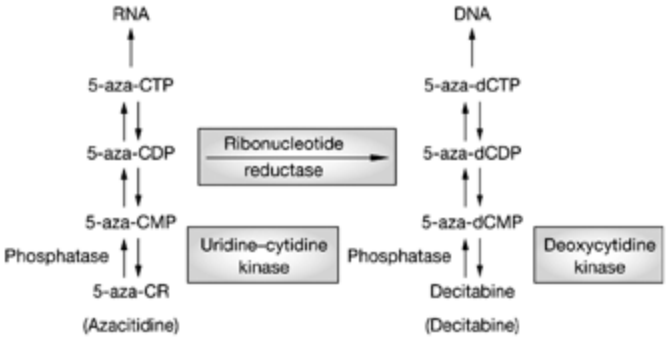
Hypomethylating agents as therapeutics

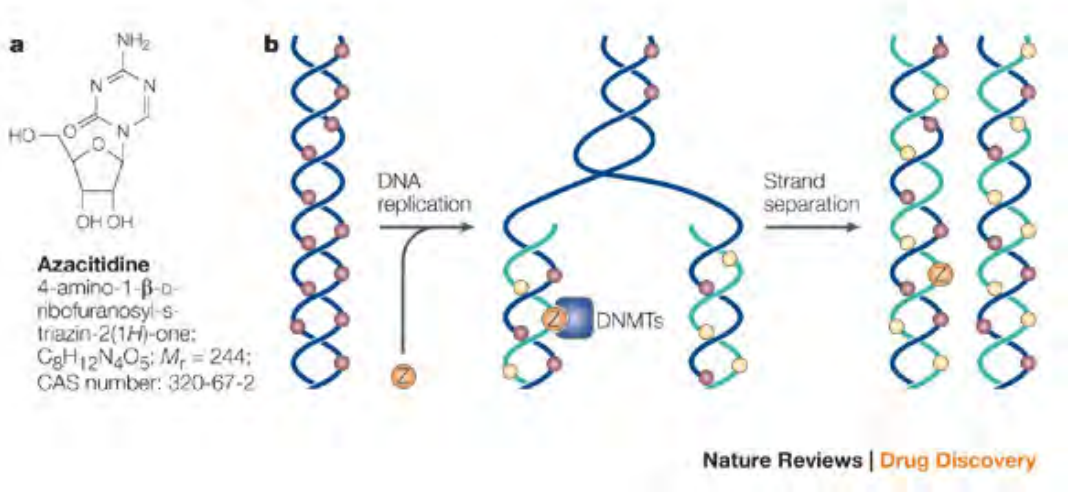
Azacitidine and decitabine are FDA approved for myelodysplastic syndrome

Improves quality of life and extends survival time of MDS patients



Decitabine





[Azacitidine](#)
Jean-Pierre J. Issa, Hagop M. Kantarjian & Peter Kirkpatrick
Nature Reviews Drug Discovery 4, 275-276 (April 2005)

a | Azacitidine. b | A family of DNA methyltransferases (DNMTs) catalyse the methylation of the 5 position of the cytosine ring. After intracellular conversion to 5-aza-2'-deoxycytidine (decitabine), azacitidine (Z) is incorporated in place of cytidine into DNA, where it acts as a direct and irreversible inhibitor of DNMTs. Cells then divide in the absence of DNMTs, which results in progressive DNA hypomethylation and reactivation of previously silenced genes[1, 3, 4]. Azacitidine also incorporates into RNA, but very little is known about the effects of this. Pink circles, methylated CpG; yellow circles, unmethylated CpG.

Epigenetic Diseases Involving DNMTs or meDNA binding proteins:

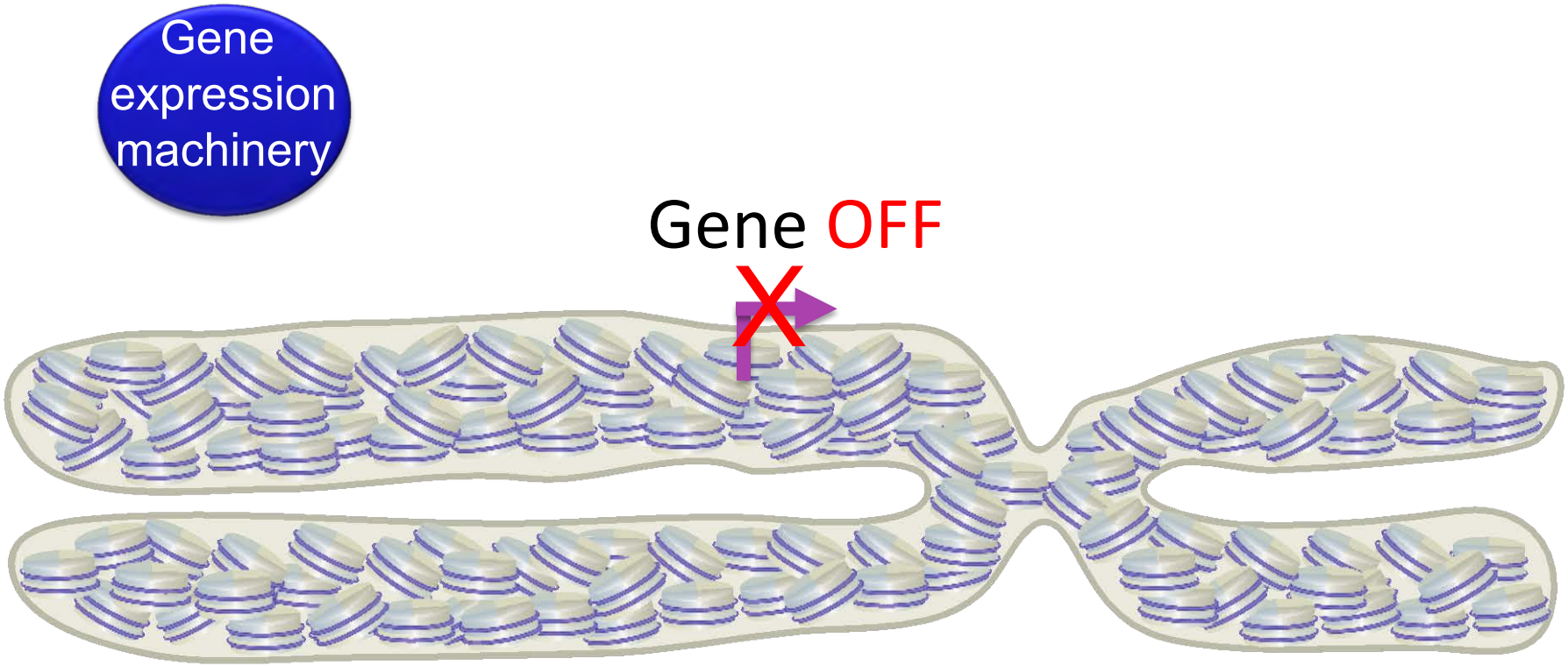
Developmental

- Fragile X syndrome (*FMR1*)
- Rett syndrome (*MECP2*)
- ICF syndrome (*DNMT3B*)
- ATRX syndrome (*ATRX*)
- Imprinting disorders (Beckwith-Wiedemann etc.)
- Alpha Thalassemia

Acquired

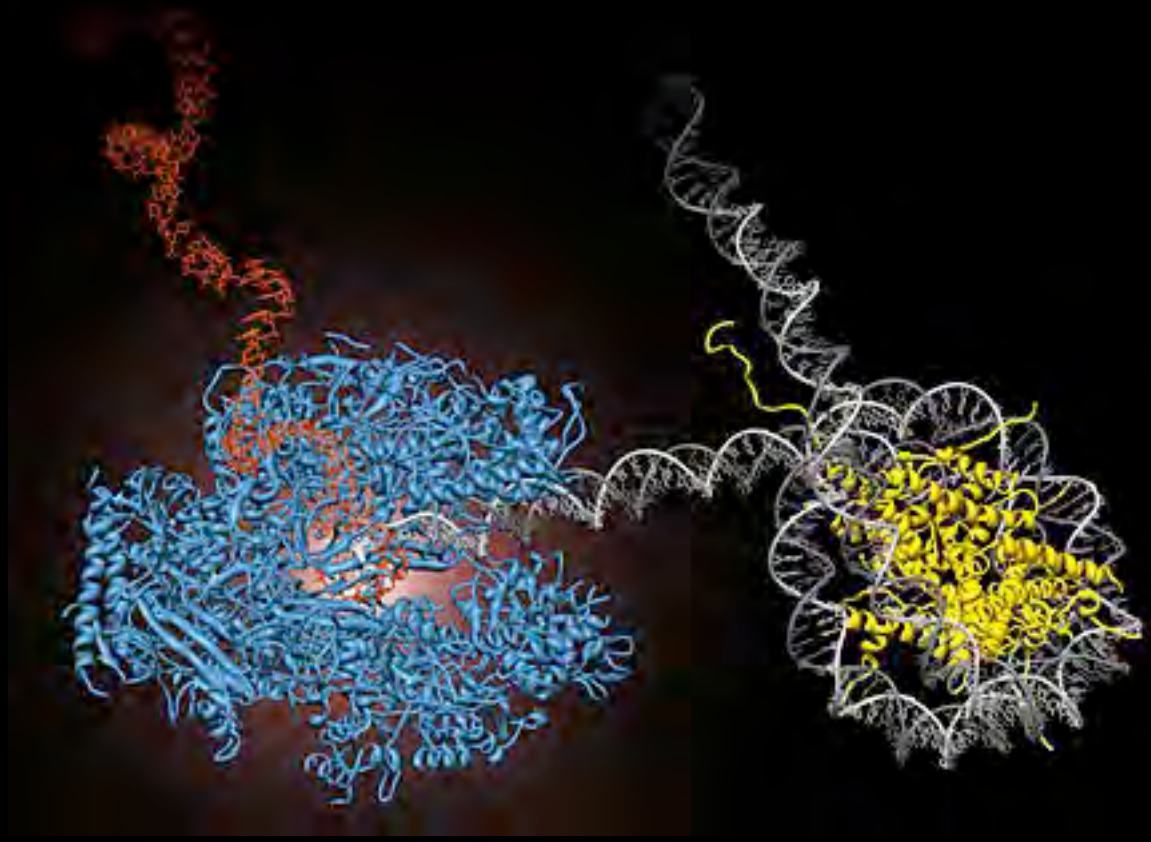
- Cancer
- Age-related diseases

REPRESSIVE CHROMATIN



How can gene expression occur when the DNA is buried in chromatin?

A RNA POLYMERASE II – NUCLEOSOME FACE-OFF

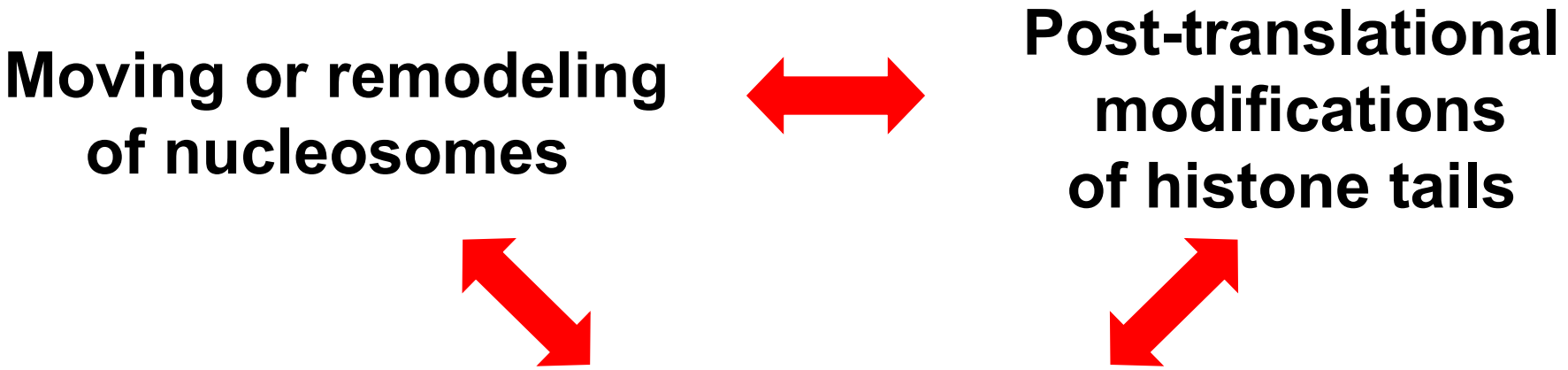


Q. How does the cell move nucleosomes blocking access to DNA sequences in chromatin?

A. Via the use and recruitment of:

1. ATP-dependent chromatin remodelers
2. Histone chaperones to remove histones from the DNA
3. Histone modifying enzymes
4. Histone exchange for more “transcriptionally favorable” histone variants.

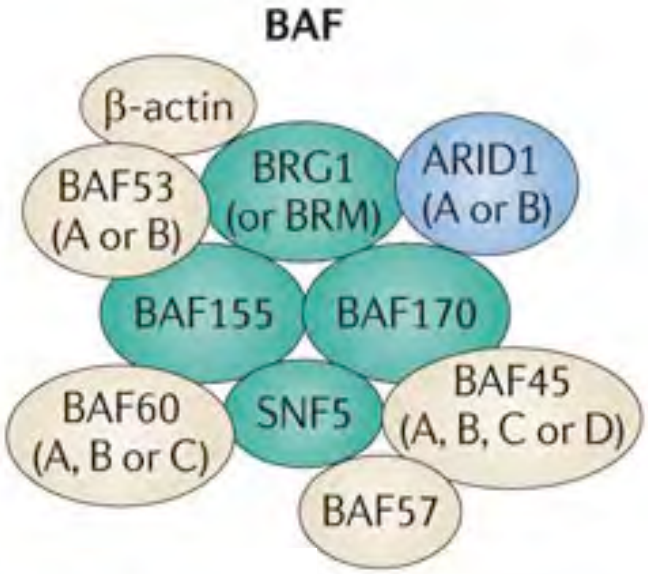
Mechanisms that mediate change in chromatin structure work together in a concerted manner

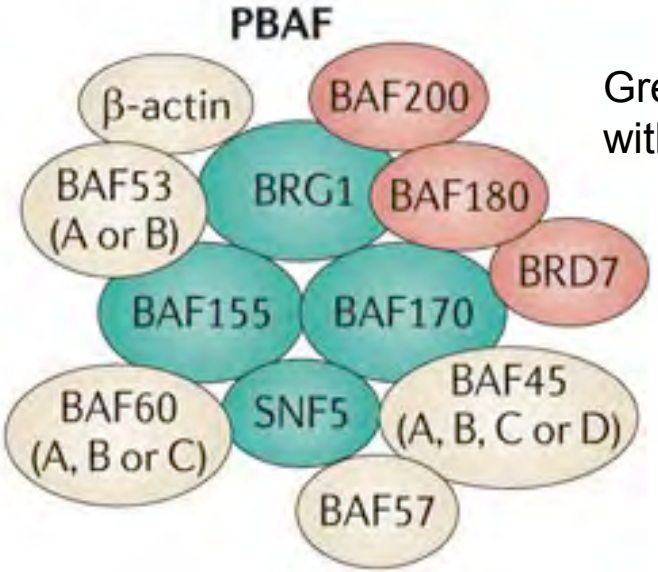


ATP-Dependent Chromatin Remodelers

SWI/SNF complexes

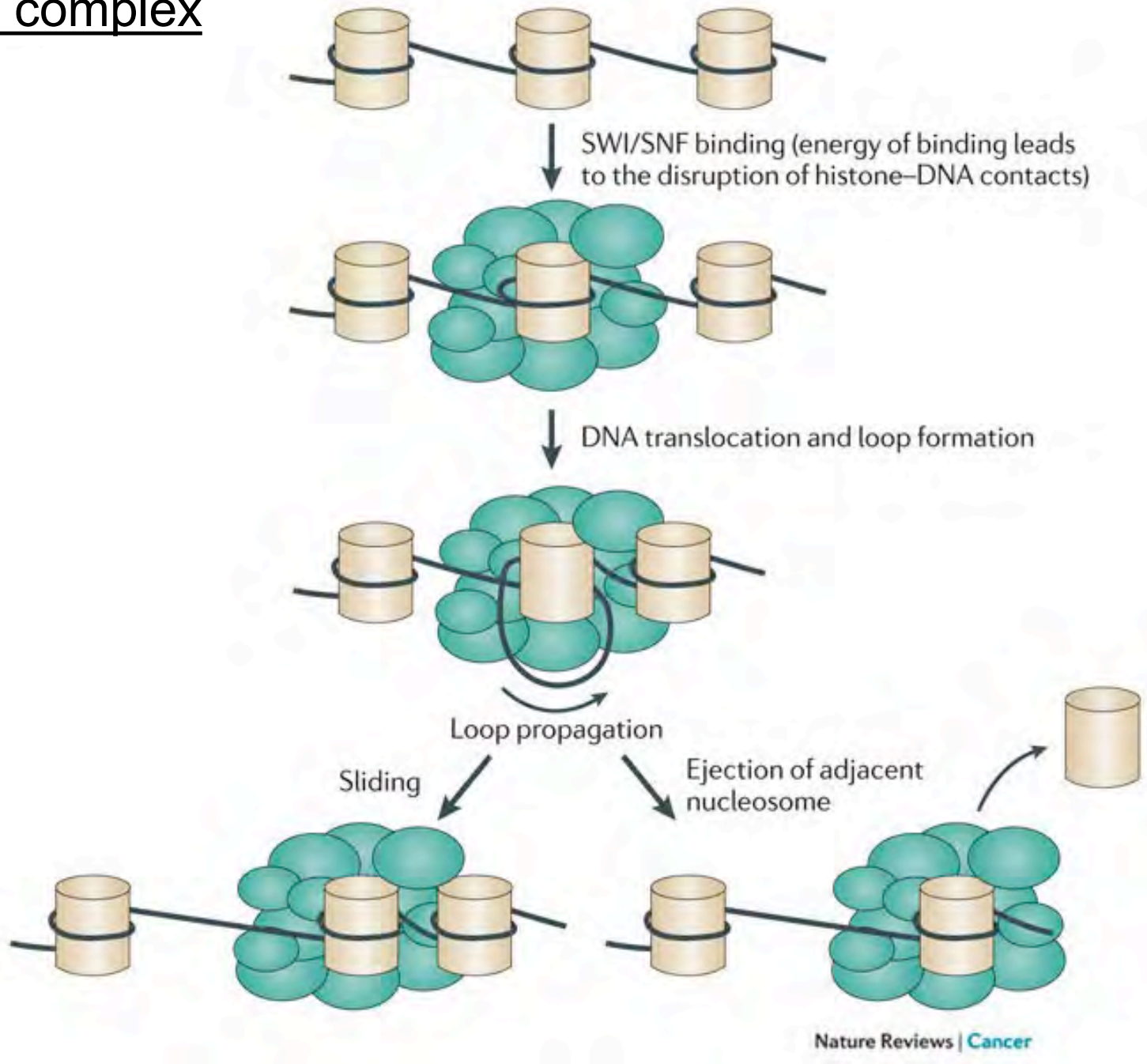
- Chromatin remodelers
- alters DNA protein contacts
- changes how DNA is looped around histones
- remodels nucleosome core particles





Green = major subunits with ATPase activity

SWI/SNF complex




wikiHow


wikiHow


wikiHow


wikiHow


wikiHow


wikiHow


wikiHow

Mutations in SWI/SNF subunits in cancer (I)

SWI/SNF subunit	Associated cancers (mutation frequency)	Primary tumours or cell lines	Haploinsufficiency or homozygous inactivation	Types of mutations	Refs
SNF5	Rhabdoid tumours (98%)	Primary tumours and cell lines	Homozygous inactivation	Homozygous deletion, nonsense, missense and frameshift mutations	30–33
	Familial schwannomatosis (30–40%)	Primary tumours	Homozygous inactivation	Truncating mutations	34,39, 137–139
	Small-cell hepatoblastomas (36 %; 4 of 11)*†	Primary tumours	Homozygous inactivation	Translocations and homozygous deletion of 22q11.2	35
	Extraskeletal myxoid chondrosarcomas (8%; 2 of 24)*	Primary tumours	Homozygous inactivation	Frameshift and homozygous deletion	36
	Undifferentiated sarcomas (29%; 5 of 17)*	Primary tumours	Haploinsuffiency and homozygous inactivation	Homozygous deletion and intragenic mutation	37
	Epitheliod sarcomas (55%; 6 of 11)*‡	Primary tumours	Homozygous inactivation	Homozygous deletion	38
	Meningiomas (<3%; 4 of 126). Frequency may be higher in familial meningiomas*	Primary tumours	Homozygous inactivation	Missense mutations with loss of the second allele	39, 140,141
	Poorly differentiated chordomas (3 of 4)*‡	Primary tumours	Homozygous inactivation	Loss of 22q11.2	40
BAF180	Renal cell carcinoma (41%; 92 of 227)	Primary tumours and cell lines	Homozygous inactivation	Truncating mutations (34%; 88 of 257), nonsense, missense and frameshift mutations	50
	Breast cancer	Cell lines	Homozygous inactivation	Truncating mutations	51

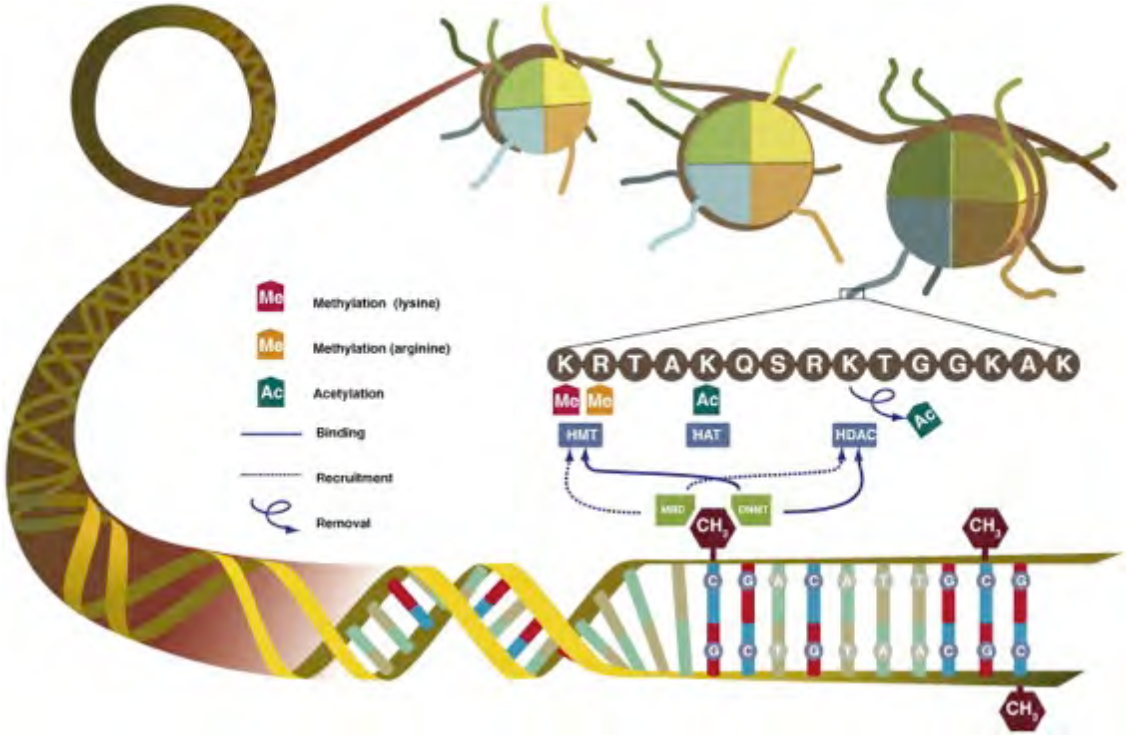
Mutations in SWI/SNF subunits in cancer (II)

ARID1A	Ovarian clear cell carcinoma (50%)	Primary tumours and cell lines	Haploinsufficiency and homozygous inactivation	Truncating mutations	57,58
	Endometriod carcinoma (35%; 10 of 33)	Primary tumours and cell lines	Haploinsufficiency and homozygous inactivation	Truncating mutations	57,58
	Renal cell carcinoma	Primary tumours	Homozygous inactivation and haploinsufficiency	Homozygous deletions and heterozygous missense mutations	50
	Medulloblastoma (1 of 88)	Primary tumours	Not determined	Truncating mutations	59
	Lung cancer	Cell line	Homozygous inactivation	Intergenic deletion	60
	Breast	Primary tumour	Not determined	Genomic rearrangement	60
BRG1	Non-small-cell lung cancer (35%; 13 of 37 cell lines)	Cell lines	Homozygous inactivation	Homozygous truncating mutations and missense mutations	67
	Lung cancer (frequency unclear)	Primary tumours	Homozygous inactivation and haploinsufficiency	Missense, insertion and nonsense mutations	65, 66,70,72
	Medulloblastoma (3%; 3 of 88)	Primary tumours	Not determined	Missense mutations	59
	Pancreatic, breast and prostate	Cell lines	Homozygous inactivation and haploinsufficiency	Truncating mutations and missense mutations	71
	Rhabdoid tumours	Primary tumours	Homozygous inactivation	Truncating mutations	73
BRD7	Breast cancer[‡]	Primary tumours	Not determined	Genomic loss on chromosome arm 16q. Reduced expression in 20% of primary tumours	82

ARID1A, AT-rich interactive domain-containing protein 1A (also known as BAF250A and SMARCF1); BRD7, bromodomain-containing 7; BRG1, BRM/SWI2-related gene 1 (also known as SMARCA4). *These cancers might represent rhabdoid tumours with an atypical histological appearance. ‡These cancers carry large multi-gene deletions rather than SNF5- or BRD7-specific mutations.

DNA and histone modifications alter gene expression

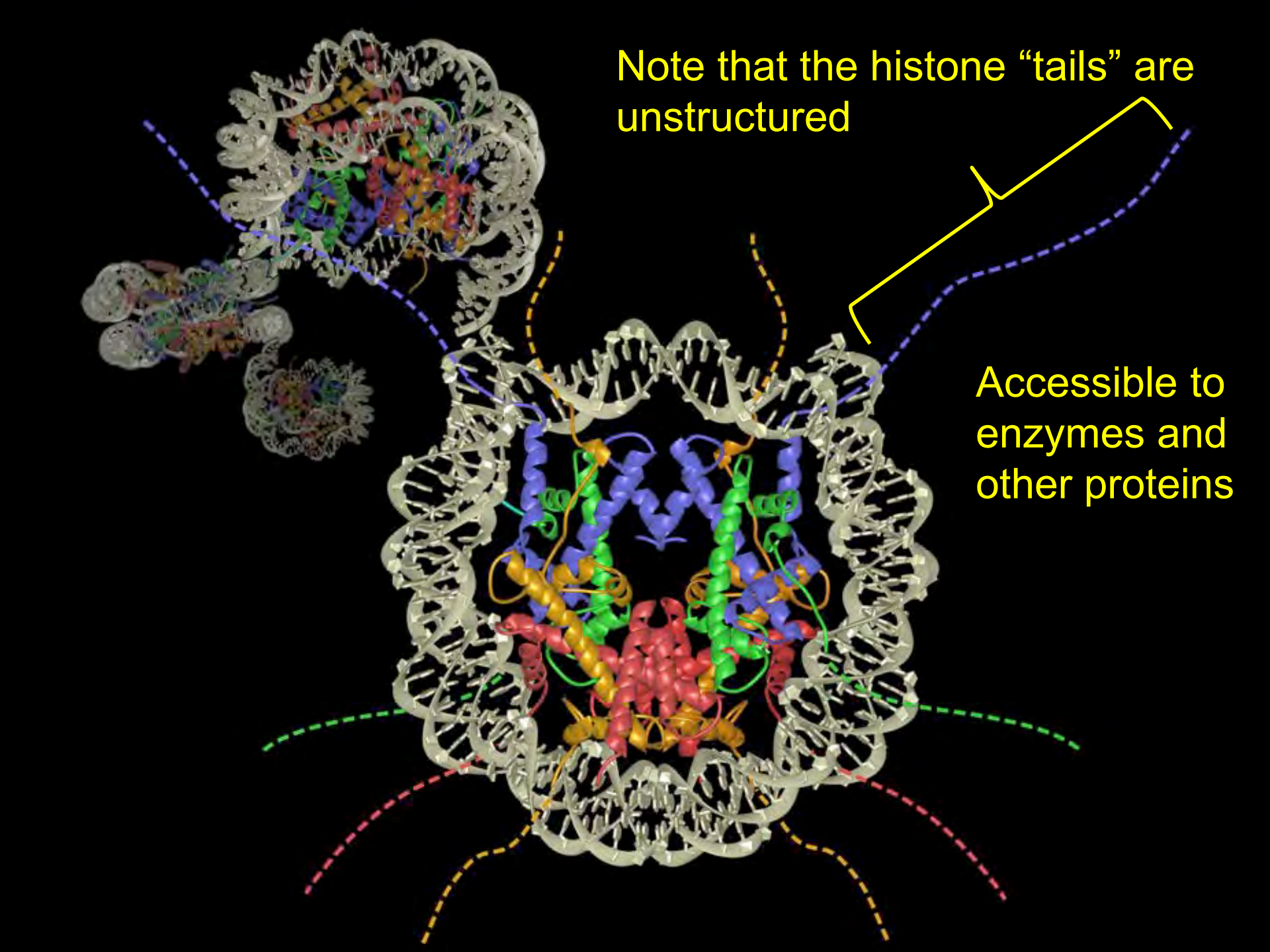
- At least 4 different DNA modifications
- 16 classes of histone modifications



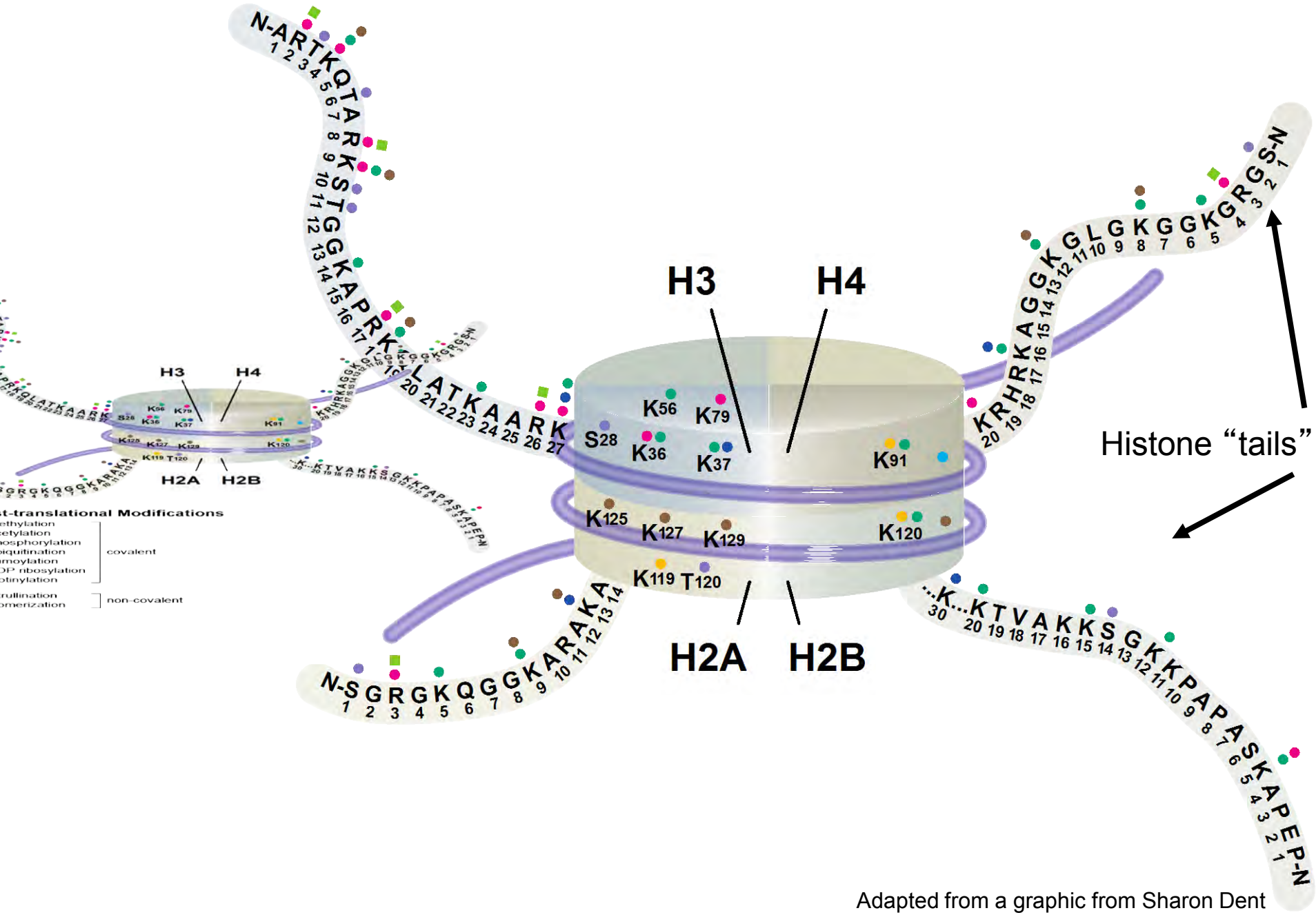
Biochimica et Biophysica Acta (BBA) - Reviews on Cancer Volume 1785, Issue 2, April 2008, Pages 133–155
Genetics and epigenetics of renal cell cancer
Marcella M.L. Baldewijns[a], et al

Histone Modifications

- Reversible post-translational modifications
- Most on N-terminal unstructured tails
- Enzymes impart/remove histone modifications.
- Protein “Reader” domains bind the modified histones
- Crosstalk of histone modifications
- Coordination with nuclear architecture or gene activity (histone code)
- Work in concert with other epigenetic mechanisms


Note that the histone “tails” are unstructured
Accessible to enzymes and other proteins

Reversible Post-translational Modifications of Histones



Adapted from a graphic from Sharon Dent

Different Classes of Modifications on Histones

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.

Kouzarides *Cell* 2007

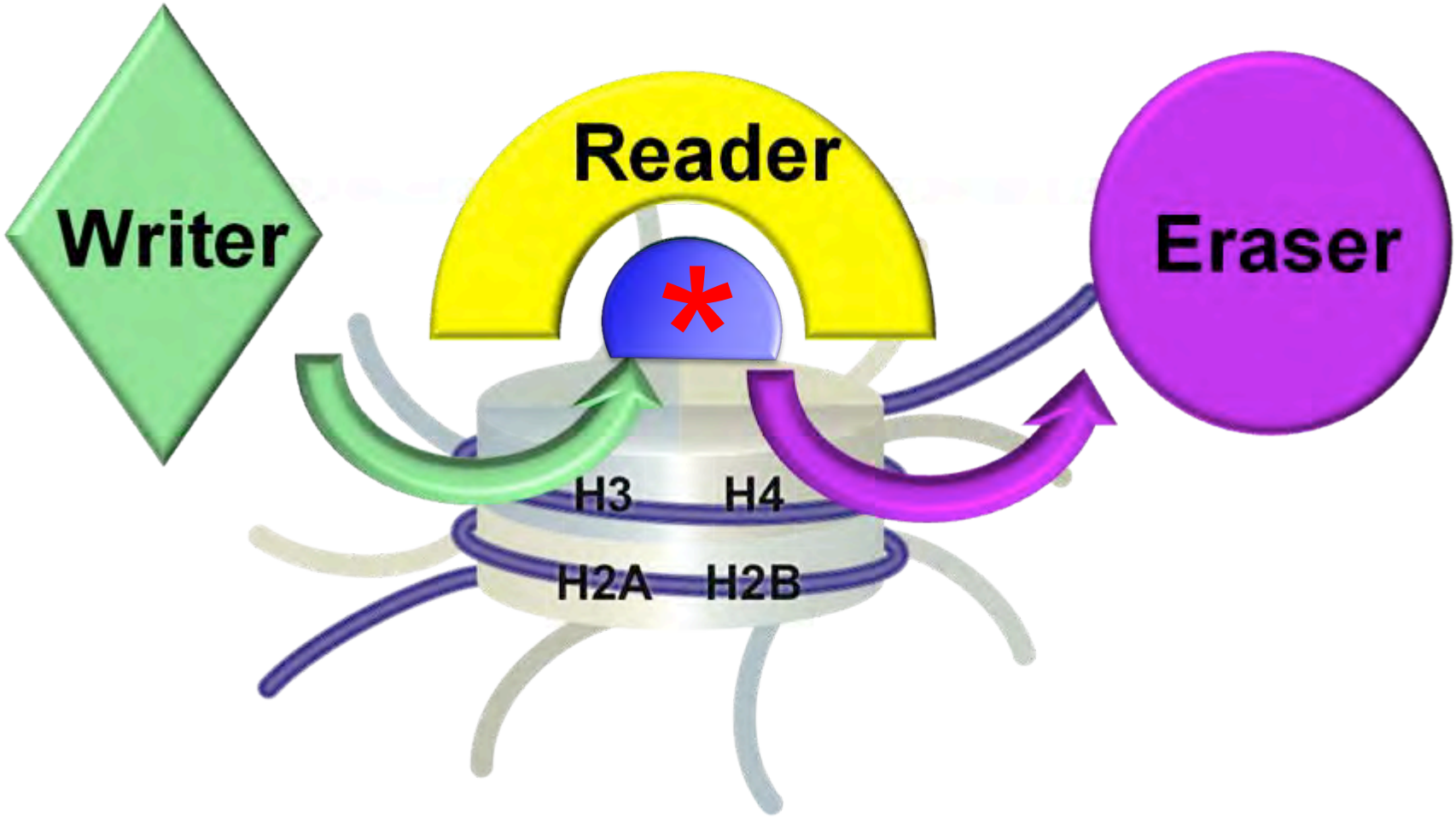
Histone Code Hypothesis

1. Multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions.

2. The modification marks on the histone tails should provide binding sites for effector proteins (readers) that mediate downstream functions.

Strahl and Allis, *Nature* 2000
Jenuwein and Allis, Science 2001

Histone Post-Translational Modifications: Modulate Protein Interactions



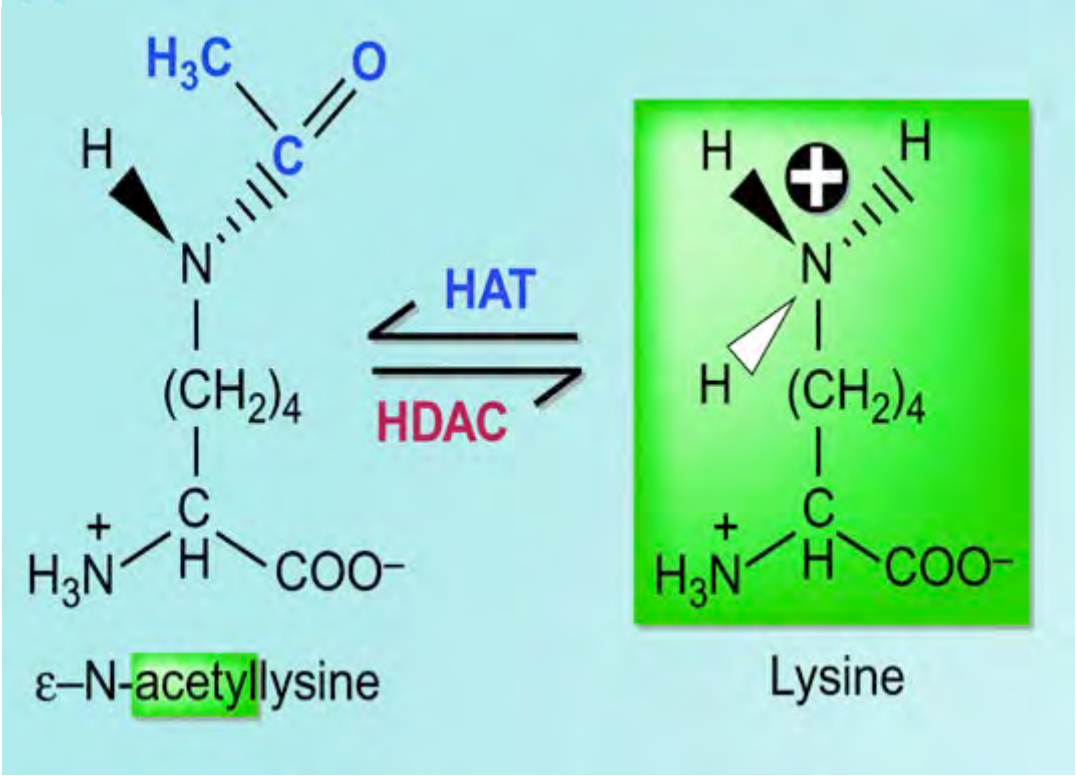
Writers, erasers and readers

Epigenetic writers: proteins that add chemical modifications to DNA or histones

Epigenetic erasers: proteins that remove chemical modifications to DNA or histones

Epigenetic readers: proteins that interact with chromatin modifications via specific domains. This interactions initiates processes like transcription activation, repression or DNA repair, in response to upstream signaling cascades

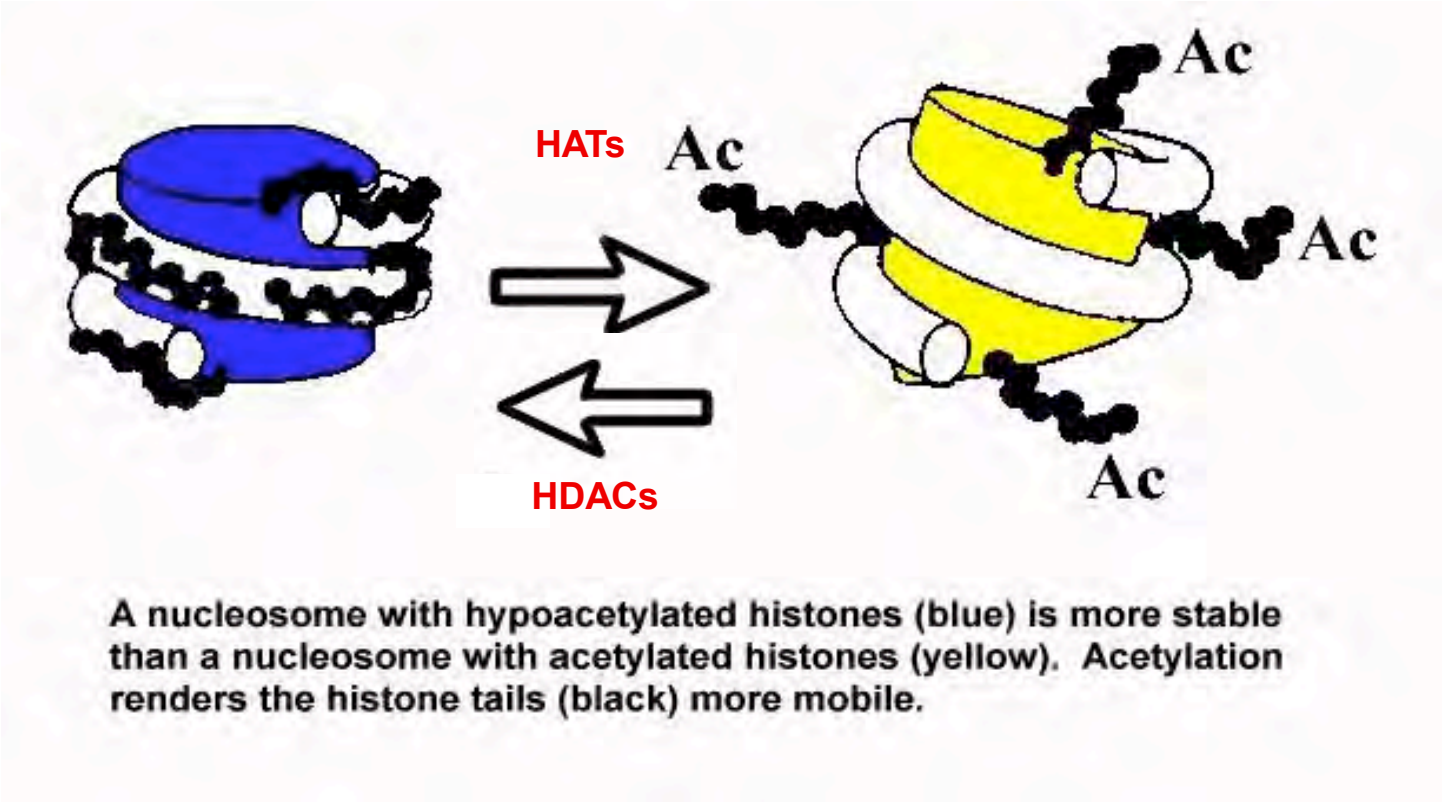
Lysine Acetylation



WRITER → HAT/KAT: Histone/Lysine acetyltransferase

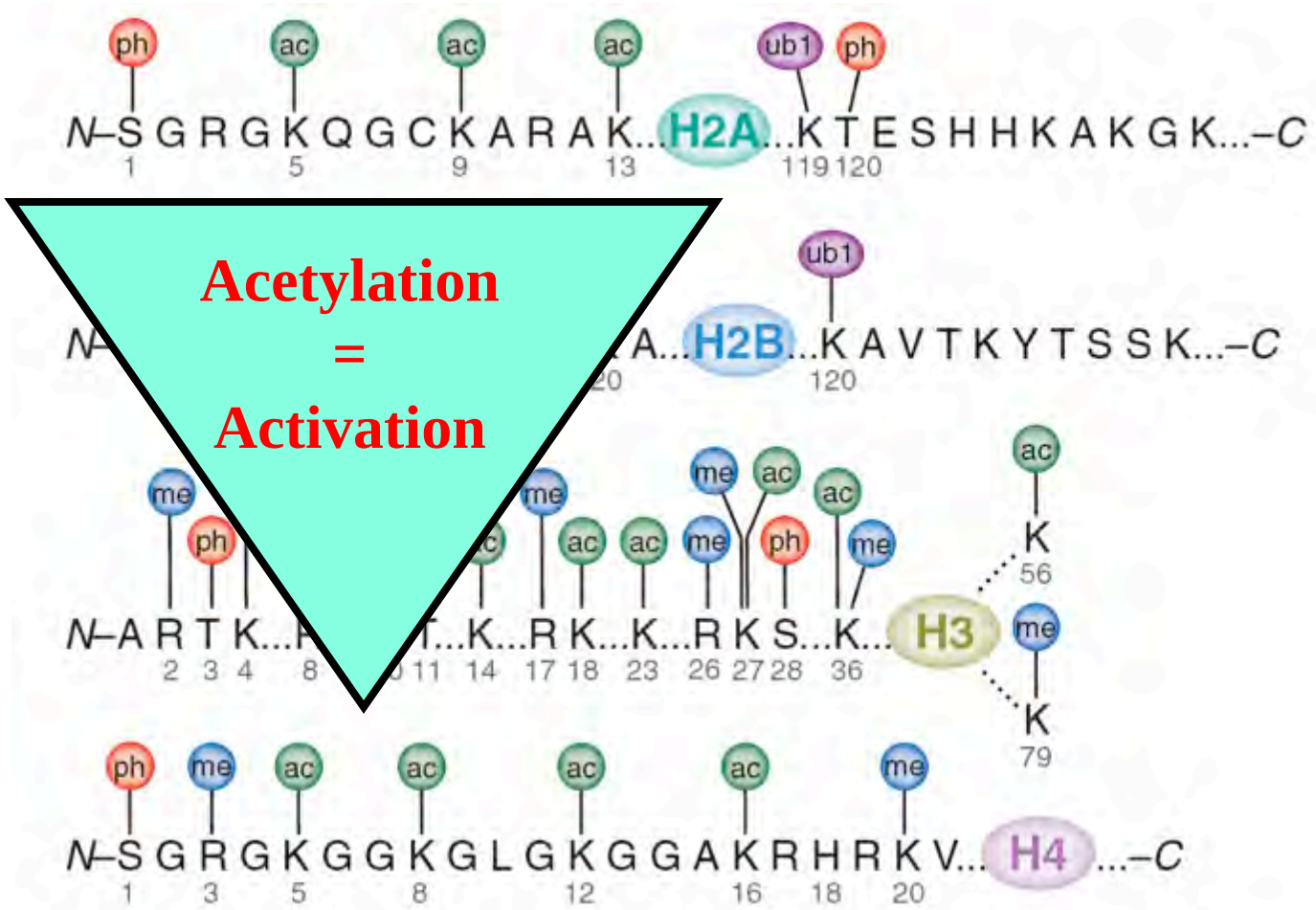
ERASER →HDAC: Histone deacetylase

Acetylation neutralizes the negative charge on lysine



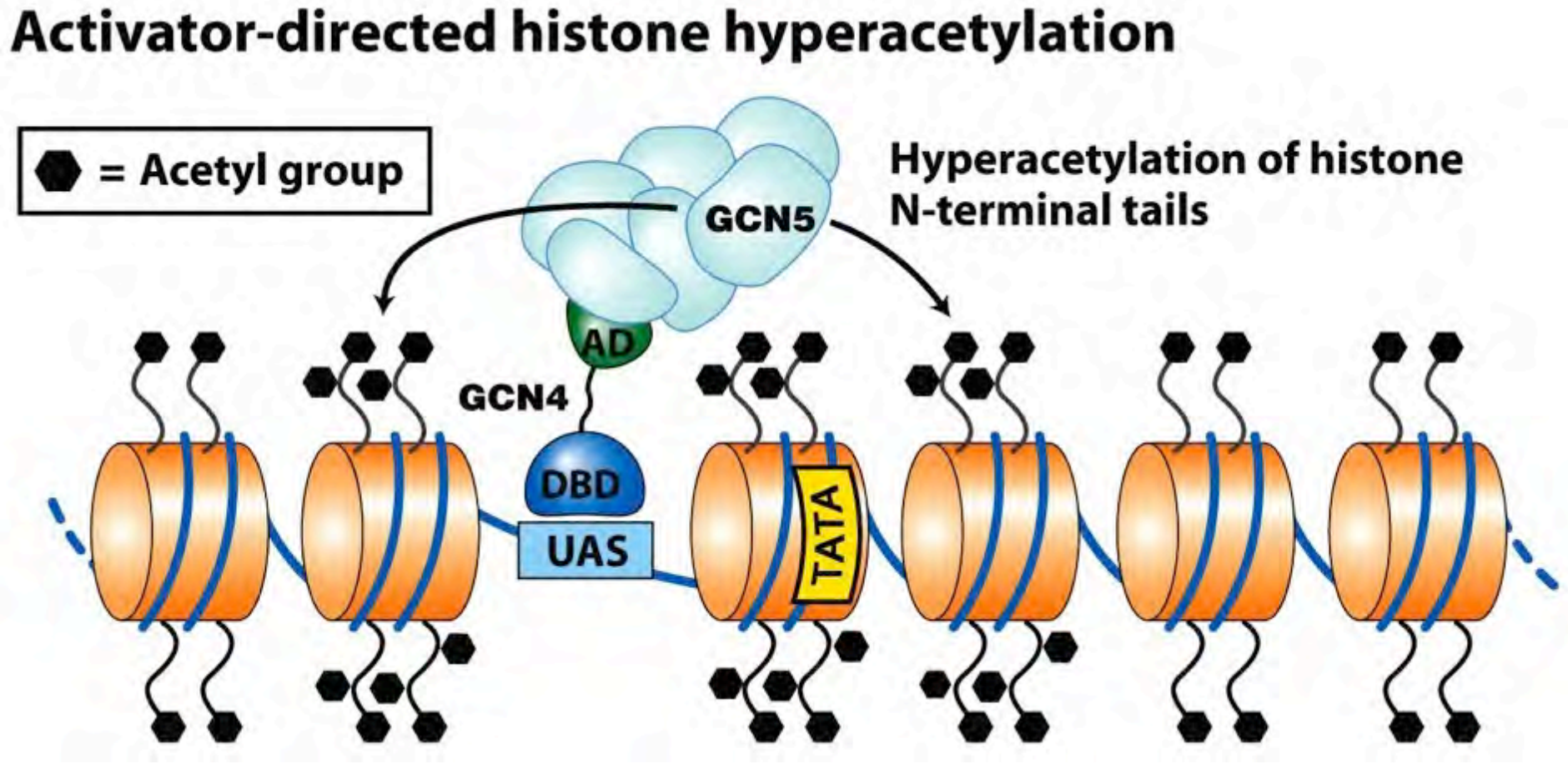
A nucleosome with hypoacetylated histones (blue) is more stable than a nucleosome with acetylated histones (yellow). Acetylation renders the histone tails (black) more mobile.

Histone Acetylation Is An Active Mark



Shilatifard *Nature SMB* 2007

Activators recruit histone acetyl transferases (HATs) examples: GCN5, CBP, p300



HATs are in multisubunit complexes

Table 1a | **Classes and substrates of histone acetyltransferases***

HAT complexes of the GNAT family											
SAGA (*Sc*)	**SLIK** (*Sc*)	**ADA** (*Sc*)	**HAT-A2** (*Sc*)	**SAGA** (*Dm*)	**ATAC** (*Dm*)	**PCAF** (*Hs*)	**STAGA** (*Hs*)	**TFTC** (*Hs*)	**HATB** (*Sc*)	**Elongator** (*Sc*)	**Hpa2** (*Sc*)
Catalytic subunit											
Gcn5	Gcn5	Gcn5	Gcn5	GCN5	GCN5	PCAF	GCN5L	GCN5L	Hat1	Elp3	Hpa2
Histones modified											
H2B/ H3/H4	H2B/ H3/H4	H3	H3	H3	H3/H4	H3/H4	H3/H4	H3/H4	H2A/H4	H3	H3/H4
Associated complex subunits											
Tra1	Tra1			TRA1		PAF400	TRRAP	TRRAP	Hat2	Elp1	Hpa2
Spt7	Spt7‡			SPT7			STAF65γ		Hif1	Elp2	
Spt8										Elp4	
Spt3	Spt3			SPT3		SPT3	SPT3	SPT3		Elp5	
Spt20	Spt20									Elp6	
Ada1	Ada1			ADA1			STAF42				
Ada2	Ada2	Ada2	Ada2	ADA2B	ADA2A	ADA2					
Ada3	Ada3	Ada3	Ada3	ADA3	ADA3	ADA3	STAF54	ADA3			
Sgf29	Sgf29	Sgf29	Sgf29	SGF29							
Sgf73	Sgf73						SCA7	SCA7			
Ubp8	Ubp8					TAF5L	TAF5L	TAF5L			
Sgf11	Sgf11					TAF6L	TAF6L	TAF6L			
Taf5	Taf5			TAF5		TAF9	TAF9	TAF9			
Taf6	Taf6			TAF6		TAF10	TAF10	TAF10			
Taf9	Taf9			TAF9		TAF12	TAF12	TAF12			
Taf10	Taf10			TAF10B				TAF2			
Taf12	Taf12			TAF12			STAF36	TAF4			
	Rtg2						STAF46	TAF5			
Chd1	Chd1							TAF6			
		Ahc1		WDA	ATAC1						
		Ahc2			HCF1						

Lee and Workman *Nat Rev MCB* 2007

Mutations of HATs and Cancer

Acetyltransferases

Enzyme	Mutation	Tumor
KAT3A (CBP)*	T, N, F, M	AML, ALL, DLBCL, B-NHL, TCC
KAT3B (p300)*	T, N, F, M	AML, ALL, DLBCL, TCC, Colorectal, Breast, Pancreatic
KAT6A (MOZ)+	T	AML, MDS
KAT6B (MORF)+	T	AML, Uterine leiomyoma

AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; B-NHL, B-cell non-Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma; and TCC, transitional cell carcinoma of the urinary bladder. Mutation types are as follows: M, missense; F, frameshift; N, nonsense; S, splice site mutation; T, translocation; and D, deletion.

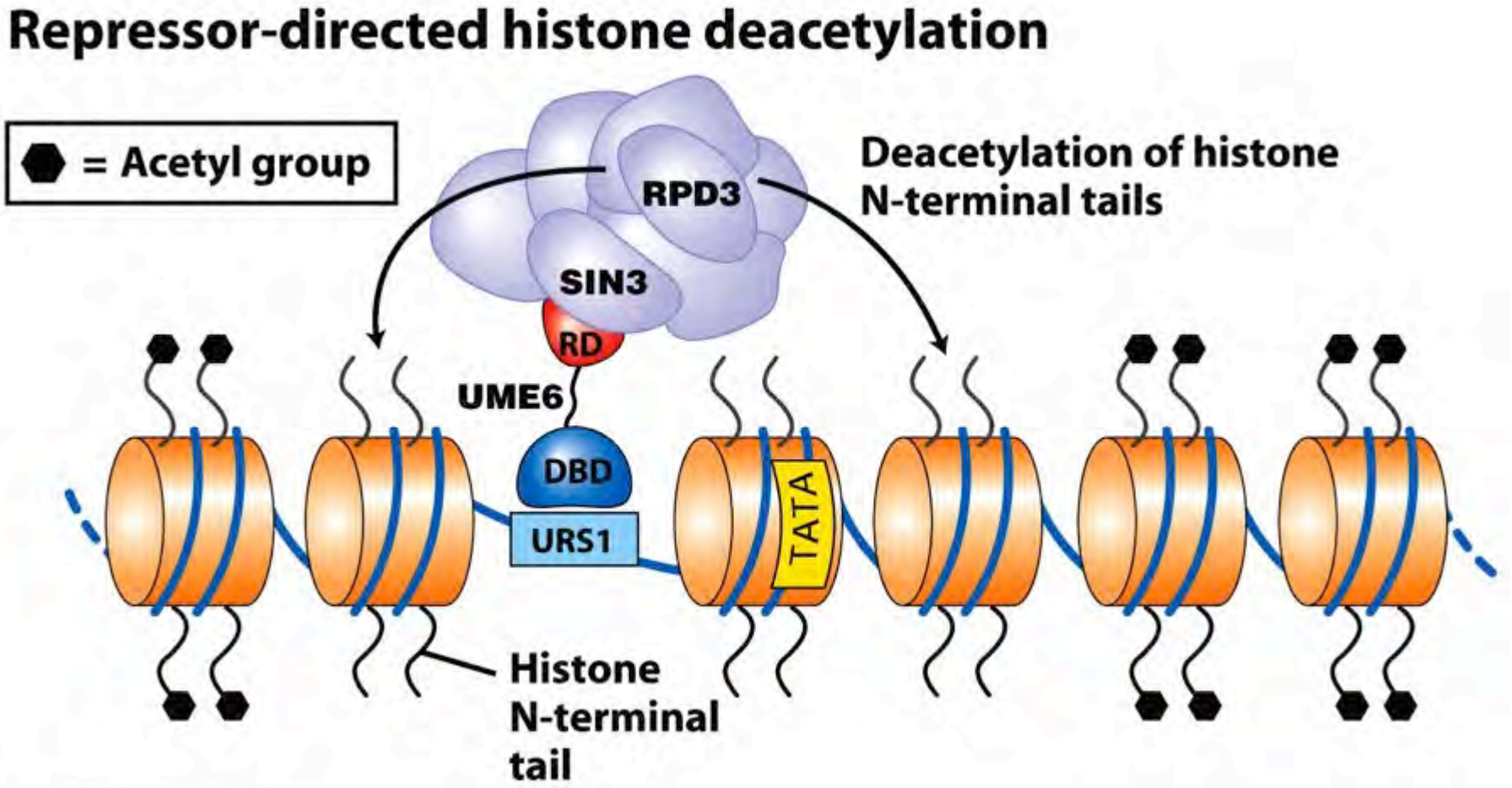
Dawson & Kouzarides *Cell* 2012

HDACs

- Reverse lysine acetylate and restore positive charge on side chain
- 4 classes based on sequence homology
- Class I: HDAC1-3 and 8
- Class II: HDAC4-7 and 9-10
- Class III: sirtuins 1-7 (are NAD dependent)
- Class IV: HDAC11

Usually part of large complexes (NuRD, Sin3A, Co-REST) which afford substrate specificity.

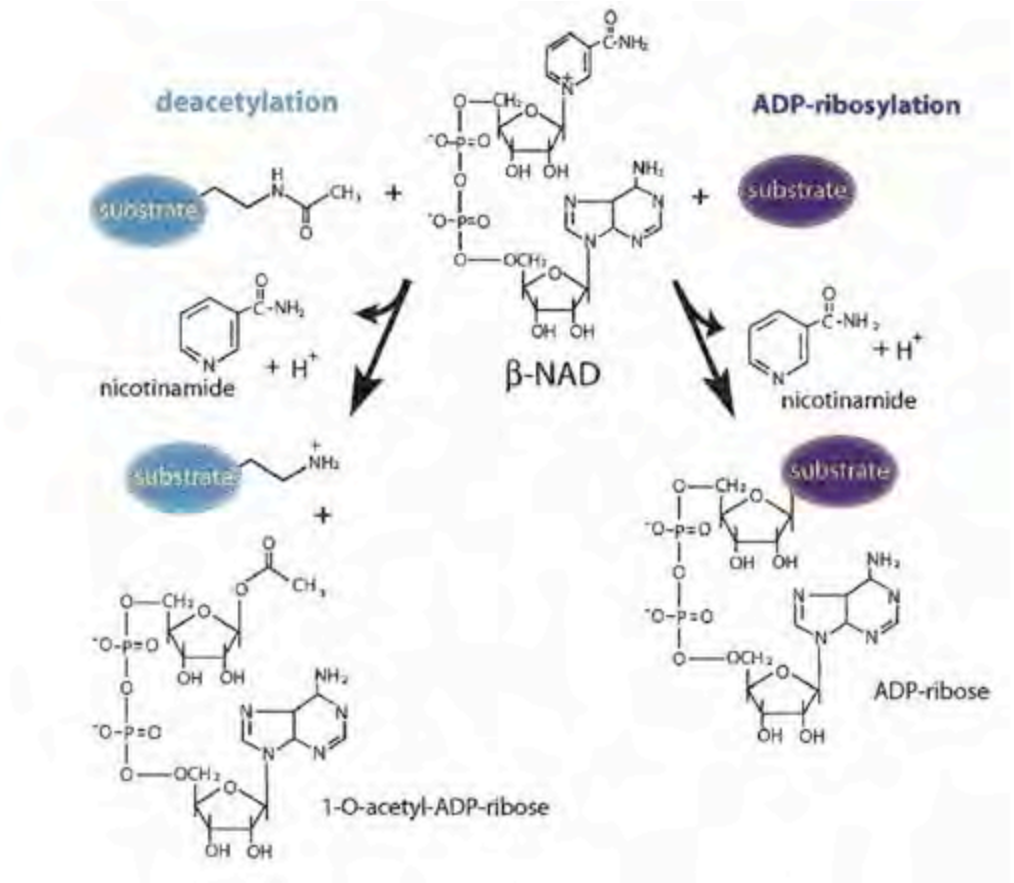
Repressors recruit histone deacetylases (HDACs)



NAD-dependent Sirtuin Deacetylation

The sirtuin-mediated deacetylation reaction couples lysine deacetylation to NAD hydrolysis.

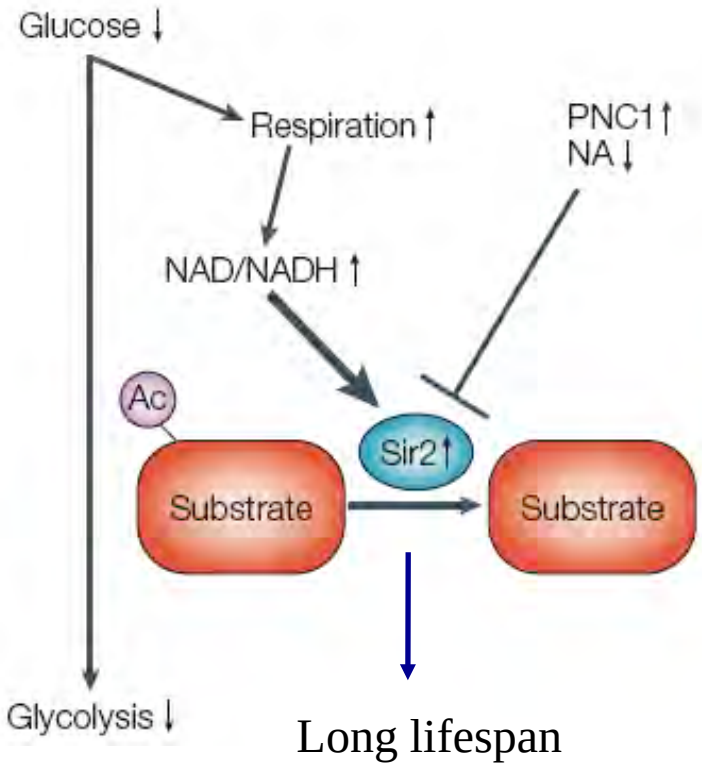
The dependence of sirtuins on NAD links their enzymatic activity directly to the energy status of the cell



Haigis and Guarente *Genes&Dev.* 2006

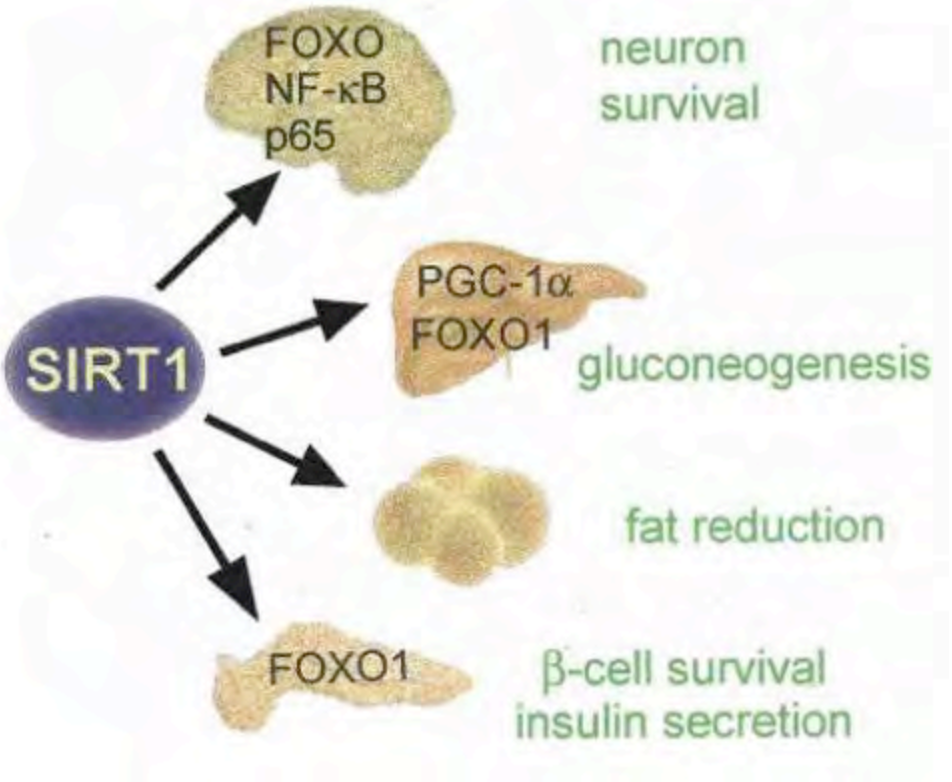
SIRT1 Has Broad Substrates And Regulates Diverse Mammalian Physiological Functions

In Yeast



Long lifespan

In Human



Bordone & Guarente *Nature Review MCB* 2005

HDAC Inhibitors (HDACi's)

-Different types: Trichostatin A (TSA), Suberoylanilide Hydroxamic Acid (SAHA) etc.

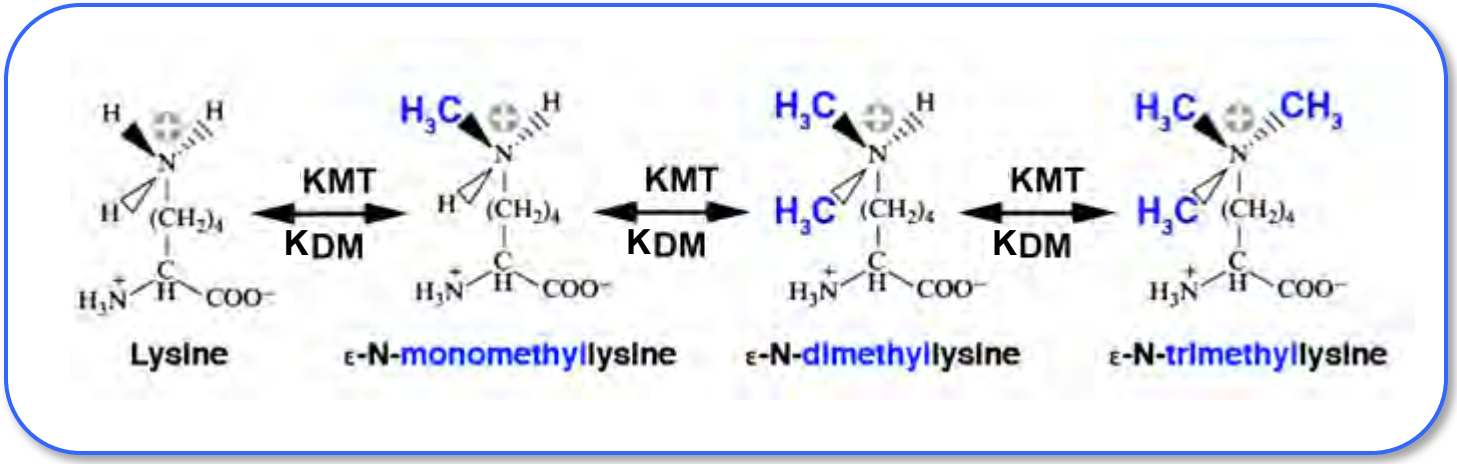
-Generally antiproliferative but effects still debated

- Demonstrable antitumor activity in both in vitro and in vivo studies in a wide range of malignancies

- HDACi treatment results in increased acetylation of histones and also transcription factors such as p53, GATA-1 and ER-alpha

-HDACi's are on Phase I–III clinical trials for treatment of cancers and other diseases.

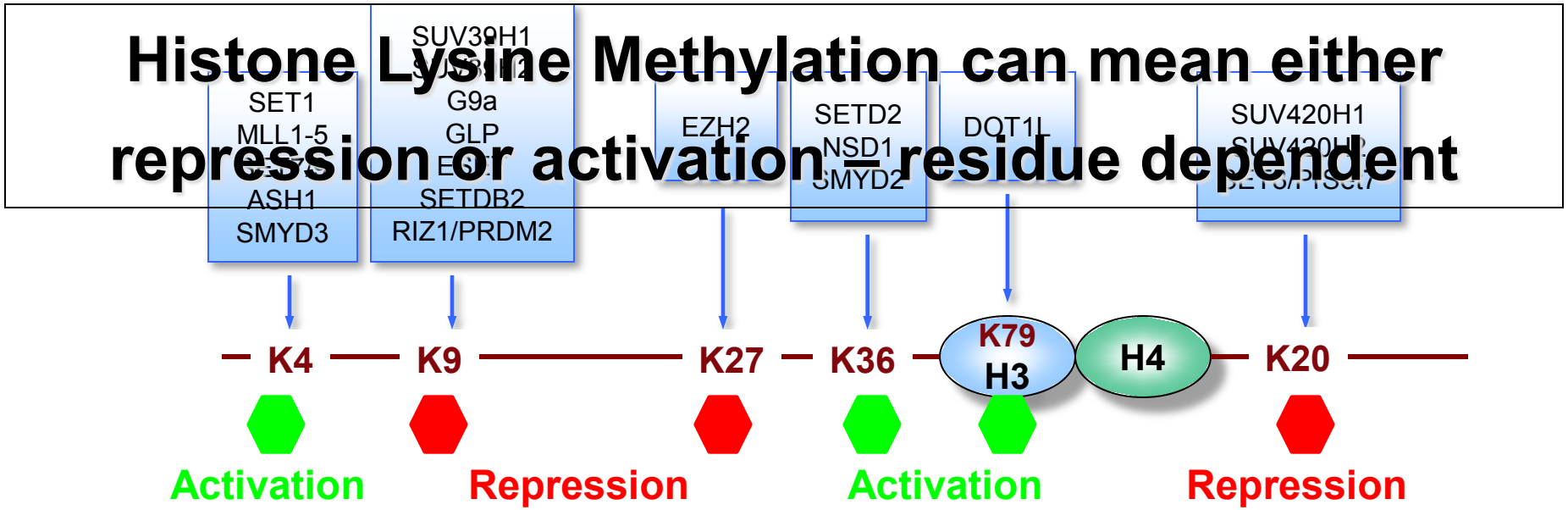
Lysine and Arginine Methylation



M. Bedford *JCS* 2007

Histone Lysine Methyltransferases (HMTs or KMTs) are specific

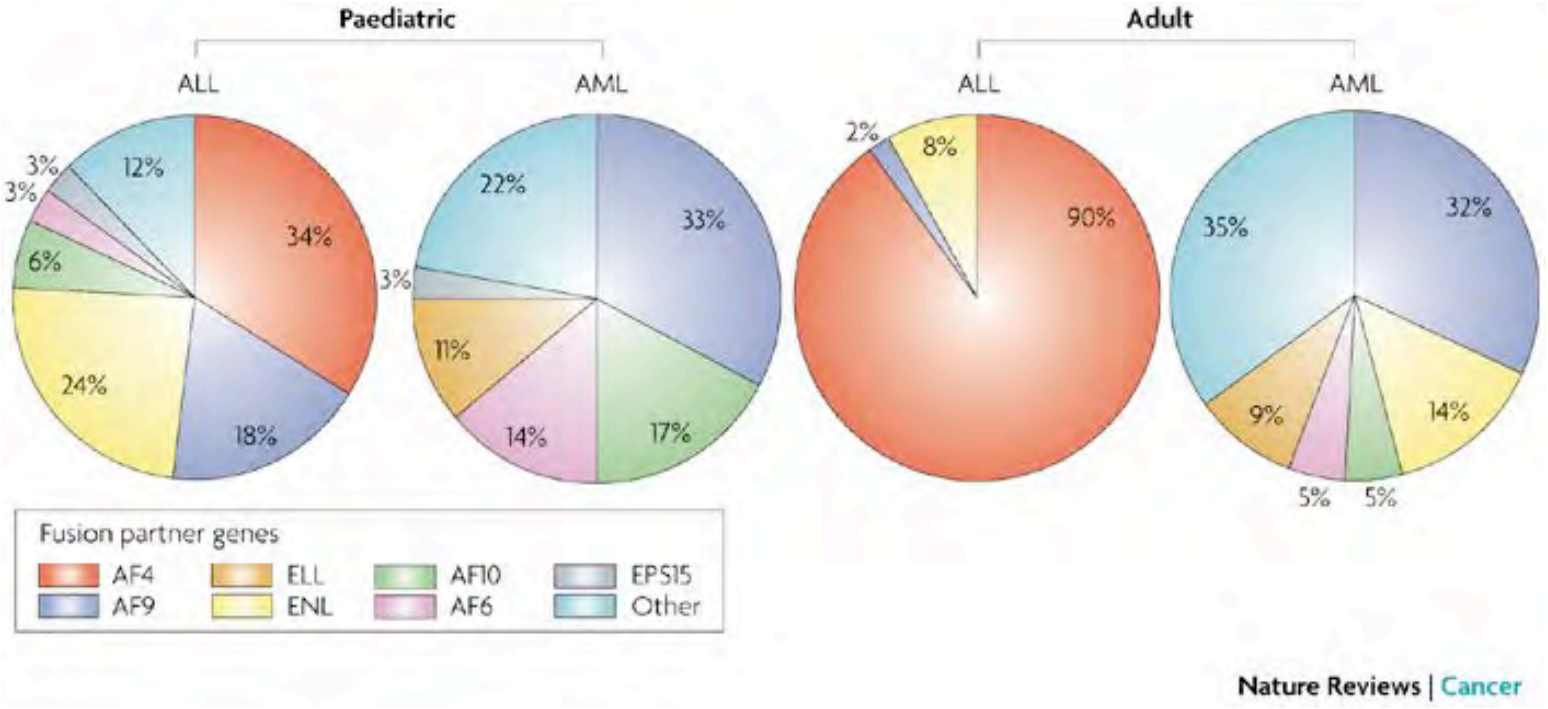
Histone Lysine Methylation can mean either repression or activation – residue dependent



MLL translocations define a subset of acute leukemias

MLL: mixed lineage leukemia gene

- Encodes a DNA binding protein that methylates H3K4
- MLL fusion proteins lose methyltransferase ability



Distribution of major MLL fusion partner genes in de novo childhood and adult leukaemias. Mixed lineage leukaemia (MLL) rearrangements are found in approximately 5% of acute lymphoblastic leukaemias (ALL), approximately 5–10% of acute myeloid leukaemias (AML) and virtually all cases of mixed lineage (or biphenotypic) leukaemias (MLL)7, 8, 119. Major MLL fusion partner genes are AF4, which is predominantly found in ALL; AF9, which is predominantly found in AML; and ENL, which is found in both ALL and AML.

Krivtsov and Armstrong
Nov. 2007; Vol. 7; p823

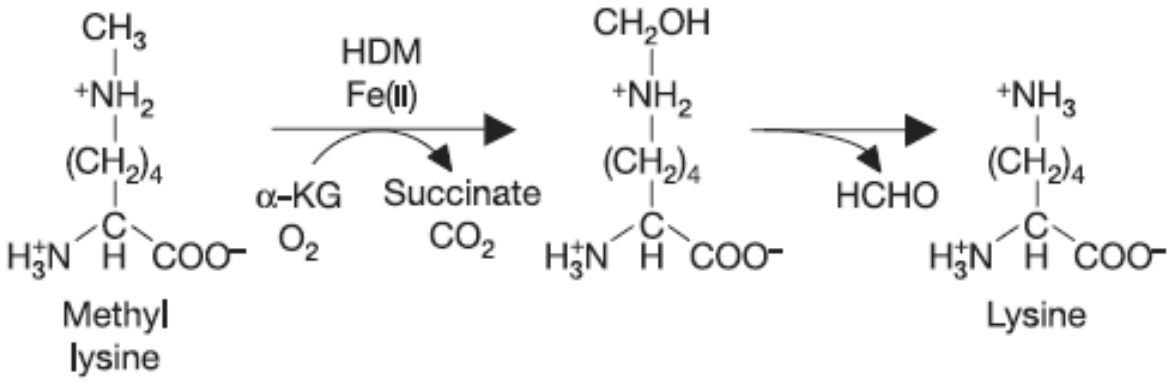
Jumonji Protein: A Family of KDM

doi:10.1038/nature04433 nature

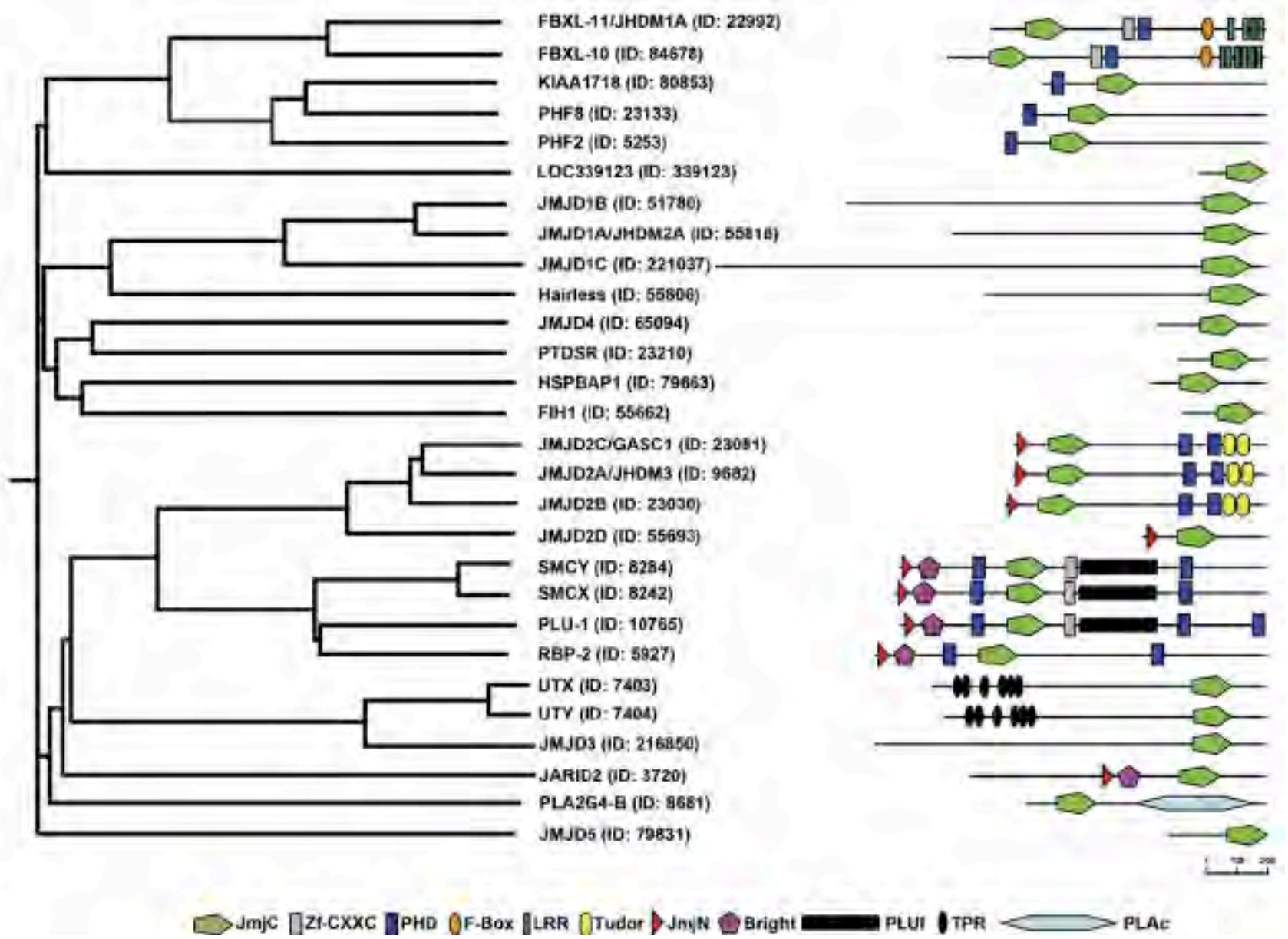
ARTICLES

Histone demethylation by a family of JmjC domain-containing proteins

Yu-ichi Tsukada[1,2], Jia Fang[1,2], Hediye Erdjument-Bromage[3], Maria E. Warren[2], Christoph H. Borchers[2], Paul Tempst[3] & Yi Zhang[1,2]

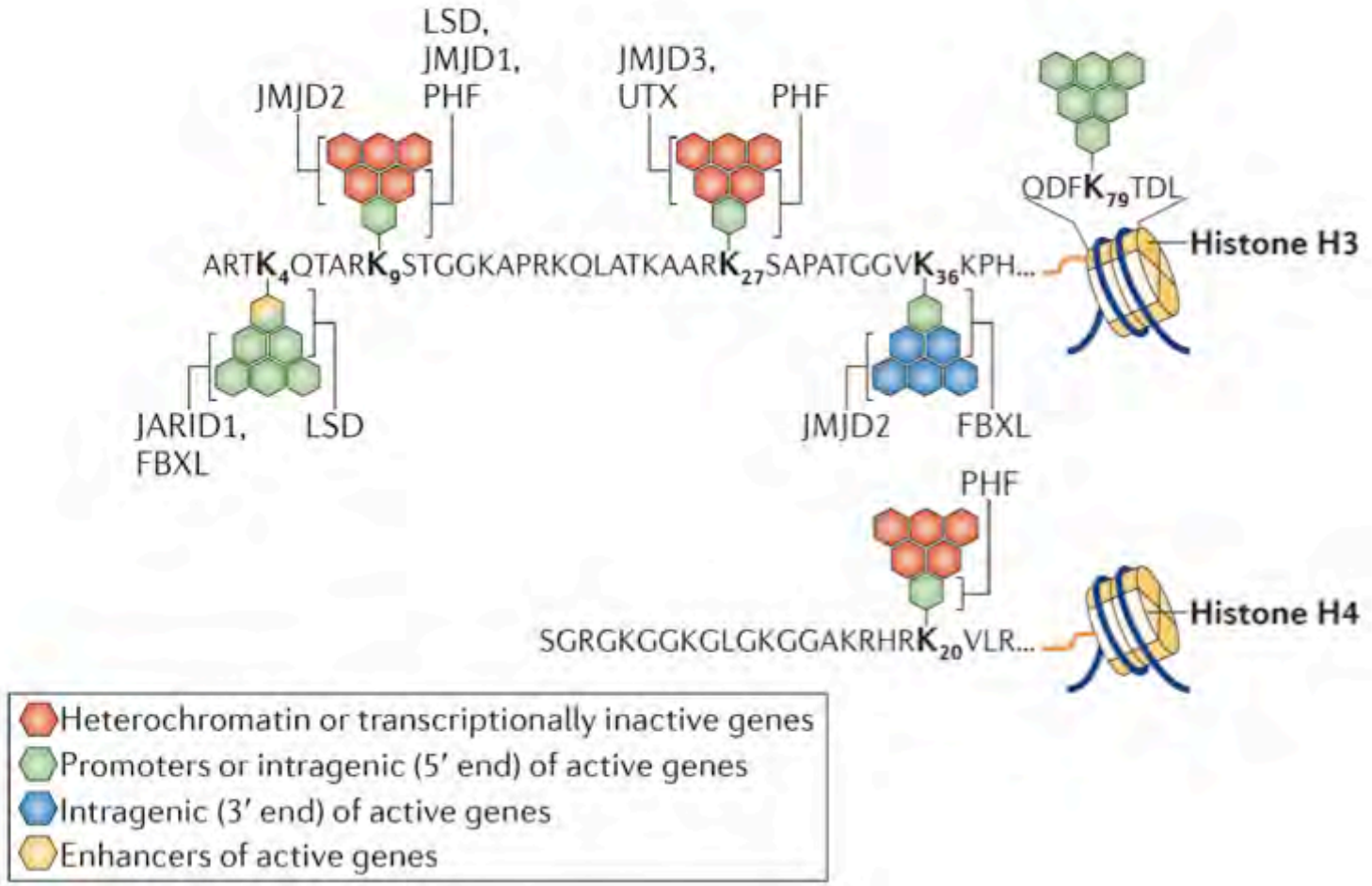


Jumonji Protein: A Family of KDM



Shi *Mol Cell* 2007

Activities of KDM Family Proteins



Shi *Mol Cell* 2007

Mutations of KMT's (writers) and KDM's (erasers) in Cancer

Methyltransferases

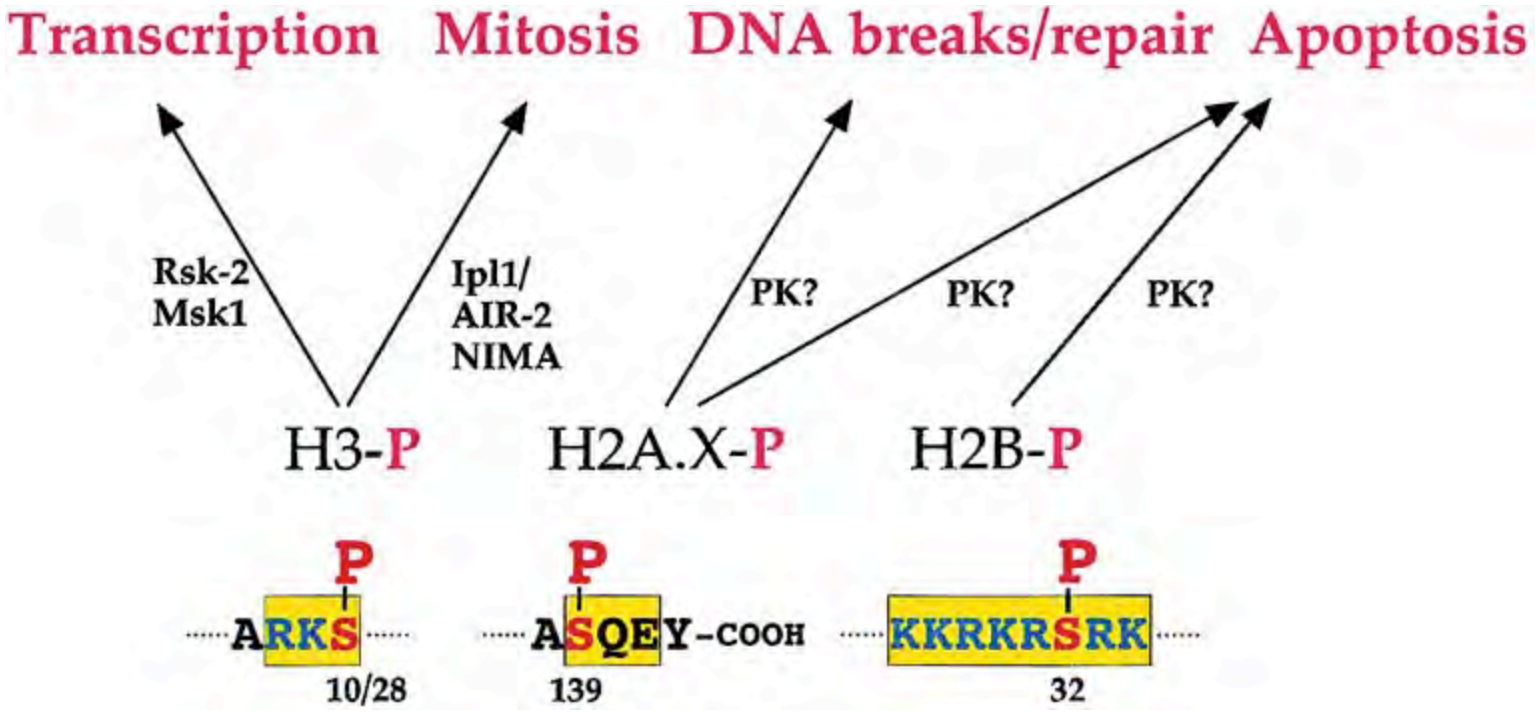
Enzyme	Mutation	Tumor
KMT2A (MLL1**+**)	T, PTD	AML, ALL, TCC
KMT2B (MLL2*)	N, F, M	Medulloblastoma, Renal, DLBCL, FL
KMT2C (MLL3*)	N	Medulloblastoma, TCC, Breast
KMT3A (SETD2)	N, F, S, M	Renal, Breast
KMT3B (NSD1+^)	T	AML
NSD2+^	T	Multiple myeloma
NSD3^	T	AML
KMT6 (EZH2)	M	DLBCL, MPD, MDS

Demethylases

Enzyme	Mutation	Tumor
KDM5A (JARID1A)+	T	AML
KDM5C (JARID1C)+	N, F, S	Renal
KDM6A (UTX)	D, N, F, S	AML, TCC, Renal, Oesophageal, Multiple myeloma

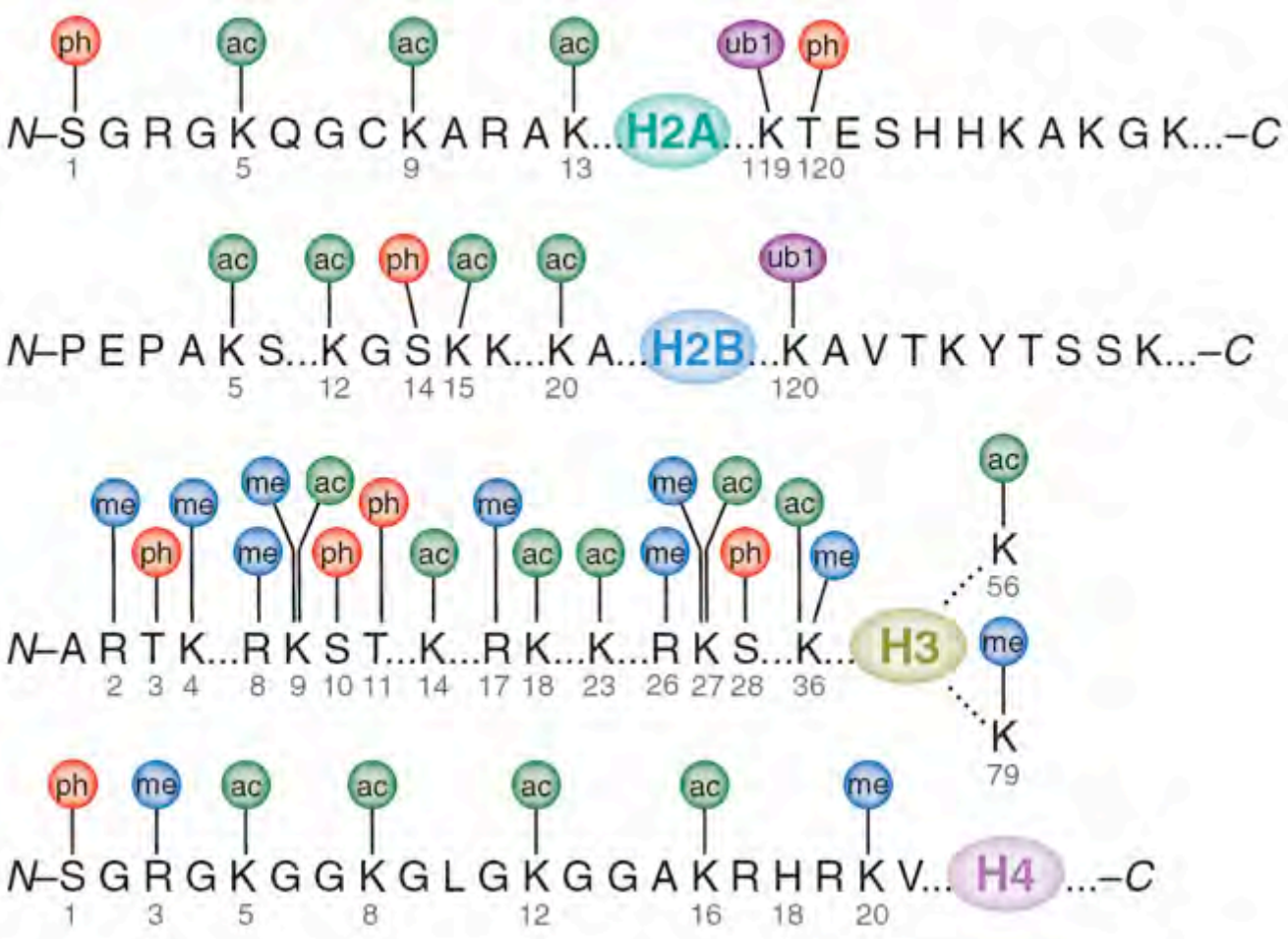
Dawson & Kouzarides *Cell* 2012

Histone Phosphorylation Is Involved in Many Processes:



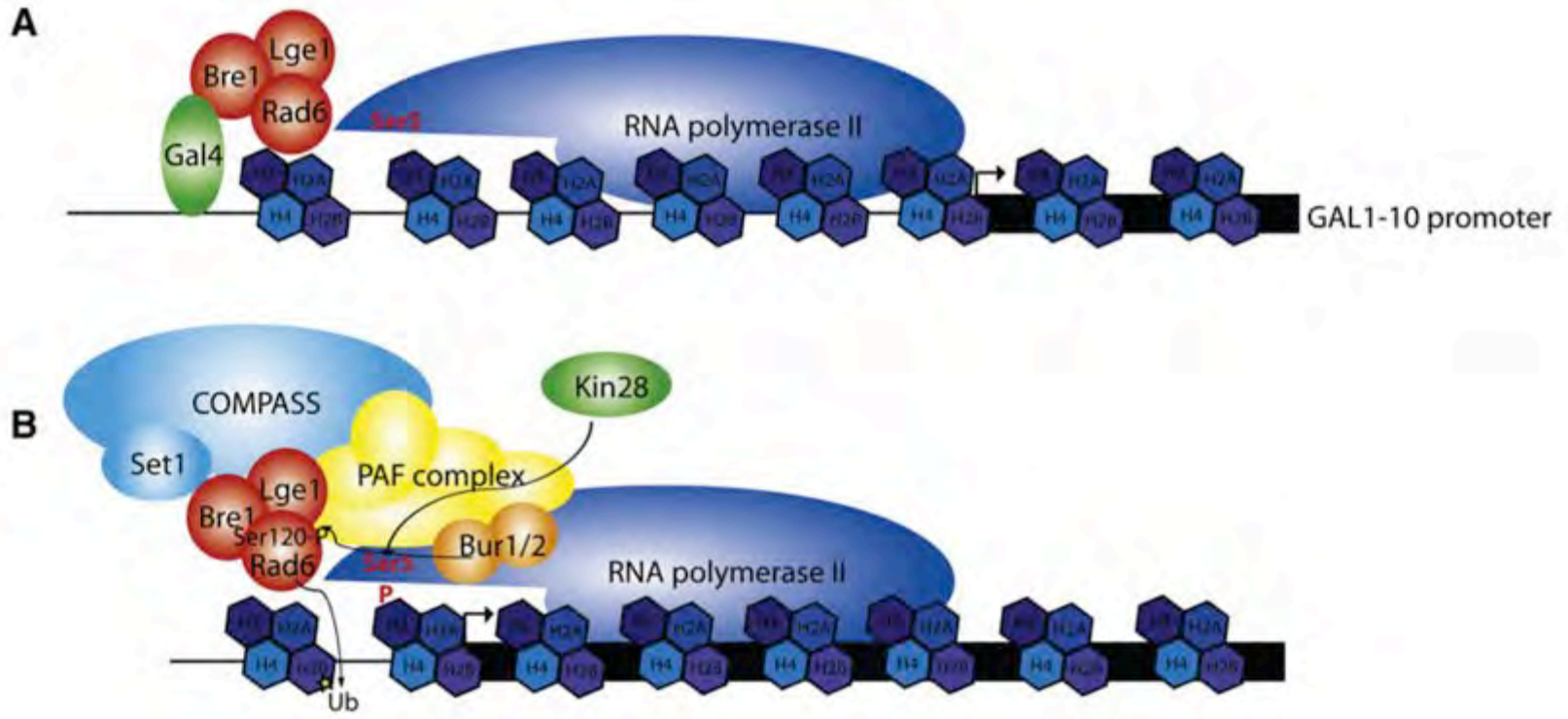
Cheung et al ***Cell*** 2000

Histone Ubiquitylation



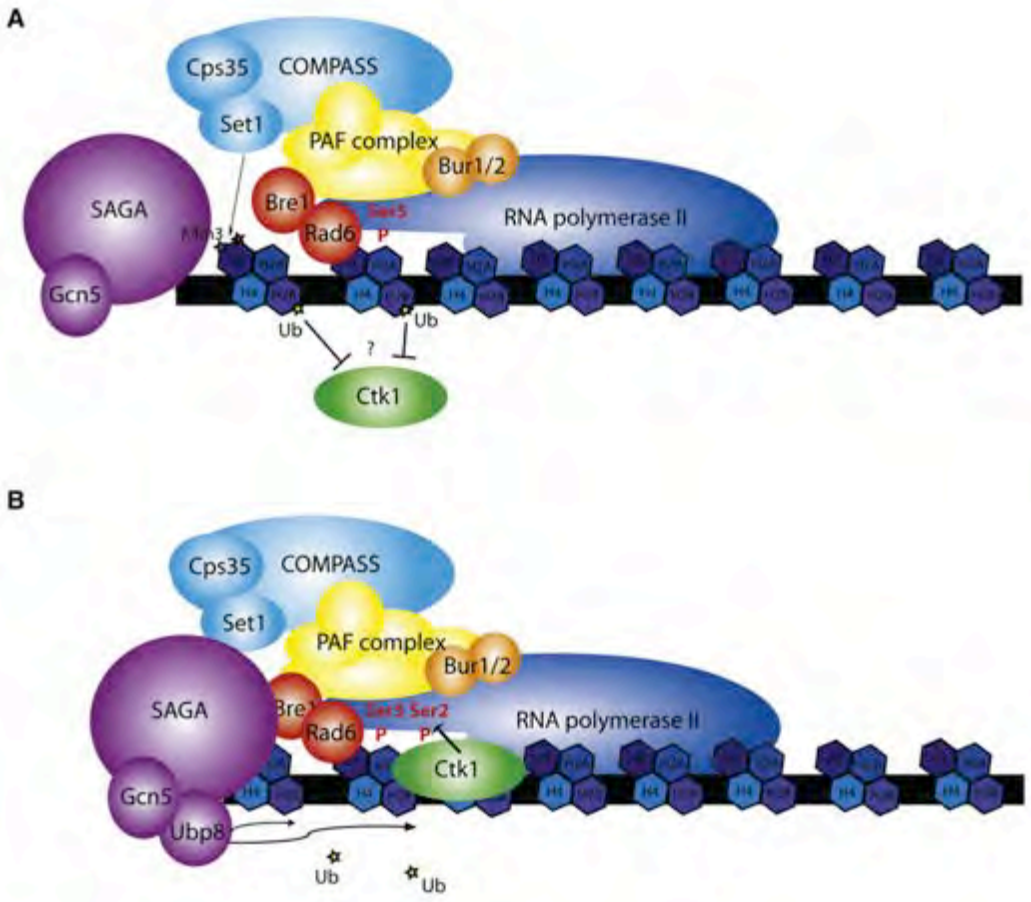
Shilatifard *Nature SMB* 2007

H2B Ubiquitination Is Required for Early Steps in Transcription Elongation



Weake & Workman Mol *Cell* 2008

UbH2B Deubiquitination Is Required for Later Stages of Transcription Elongation



Weake & Workman Mol *Cell* 2008

New Histone Modifications Discovered

Cell

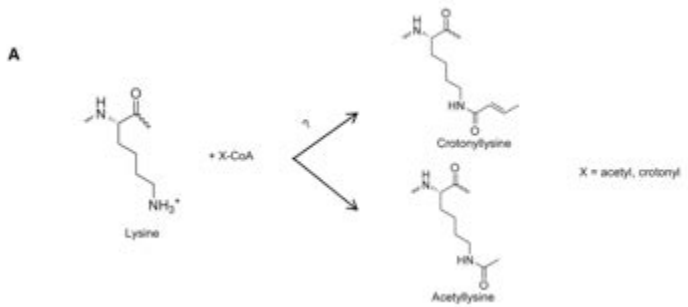
Resource

Identification of 67 Histone Marks and Histone Lysine Crotonylation as a New Type of Histone Modification

Minjia Tan,[1,6] Hao Luo,[1,6] Sangkyu Lee,[1,6] Fulai Jin,[2] Jeong Soo Yang,[1] Emilie Montellier,[3] Thierry Buchou,[3] Zhongyi Cheng,[1] Sophie Rousseaux,[3] Nisha Rajagopal,[2] Zhike Lu,[1] Zhen Ye,[2] Qin Zhu,[4] Joanna Wysocka,[5] Yang Ye,[4] Saadi Khochbin,[3] Bing Ren,[2] and Yingming Zhao[1,*]

[1]Ben May Department of Cancer Research, The University of Chicago, Chicago, IL 60637, USA

Lysine crotonylation



Tan et al Cell (2011) 146, 1016–1028

New Histone Modifications Discovered

LETTER

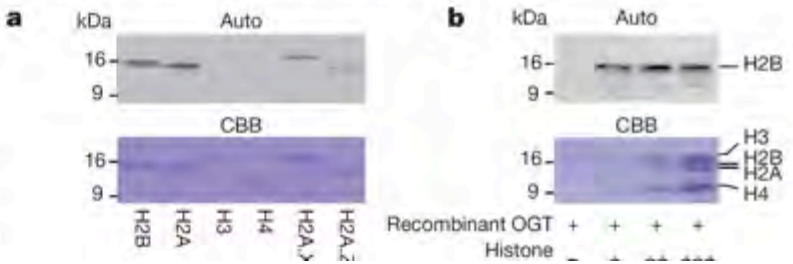
doi:10.1038/nature10656

GlcNAcylation of histone H2B facilitates its monoubiquitination

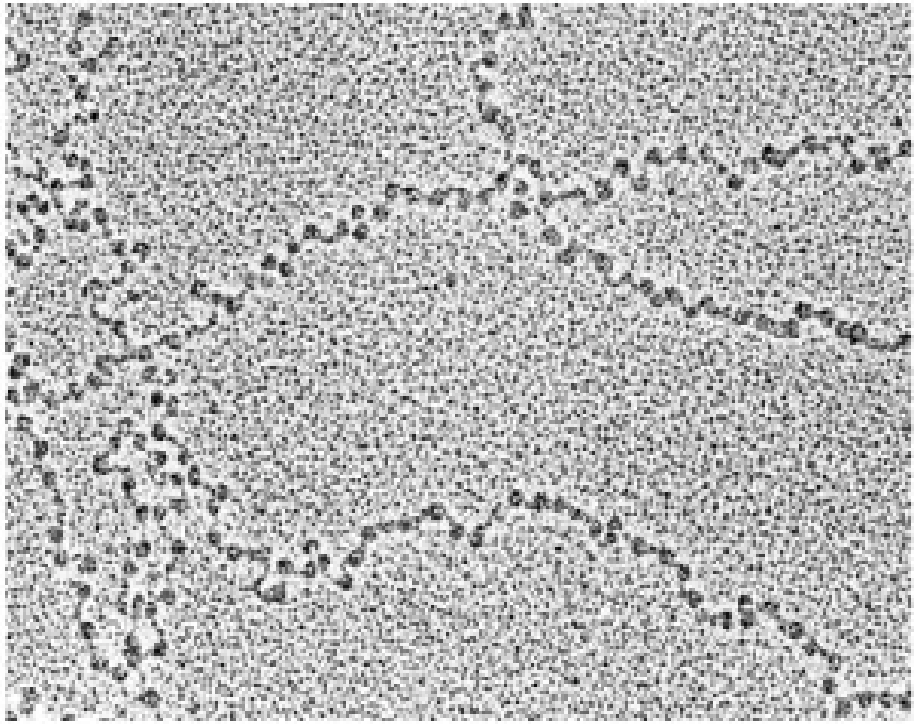
Ryoji Fujiki[1], Waka Hashiba[1], Hiroki Sekine[1], Atsushi Yokoyama[1], Toshihiro Chikanishi[1], Saya Ito[1], Yuuki Imai[1], Jaehoon Kim[2], Housheng Hansen He[3], Katsuhide Igarashi[4], Jun Kanno[4], Fumiaki Ohtake[1], Hirochika Kitagawa[1], Robert G. Roeder[2], Myles Brown[3] & Shigeaki Kato[1,5]

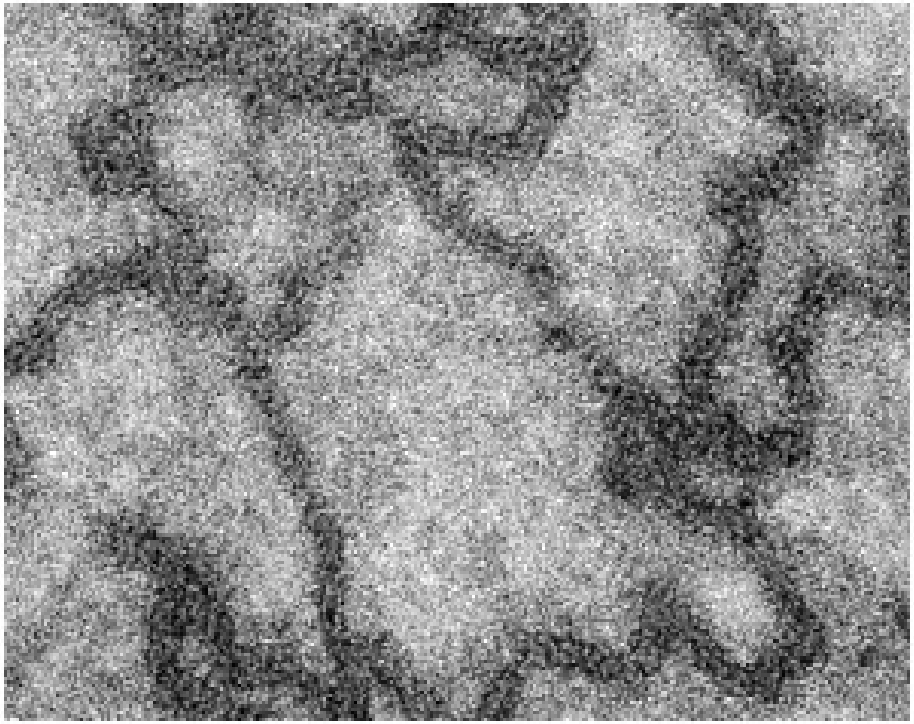
Chromatin reorganization is governed by multiple post-translational modifications of chromosomal proteins and DNA[1,2]. These histone modifications are reversible, dynamic events that can regulate DNA-driven cellular processes[3,4]. However, the molecular mechanisms that coordinate histone modification patterns remain largely unknown. In metazoans, reversible protein modification by *O*-linked *N*-acetylglucosamine (GlcNAc) is catalysed by two enzymes, *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA)[5,6]. However, the significance of GlcNAcylation in chromatin reorganization remains elusive. Here we report that histone H2B is GlcNAcylated at residue S112 by OGT *in vitro* and in living cells. Histone GlcNAcylation fluctuated in response to extracellular glucose

detect the reported sites in H2B S36 and H4 S47. However, H2A T101 was detected as a GlcNAc site when H2A protein alone was used (data not shown). This discrepancy in identified GlcNAc sites might be due to differences in experimental approaches.



Finding PTMs with real chromatin: EM is not possible

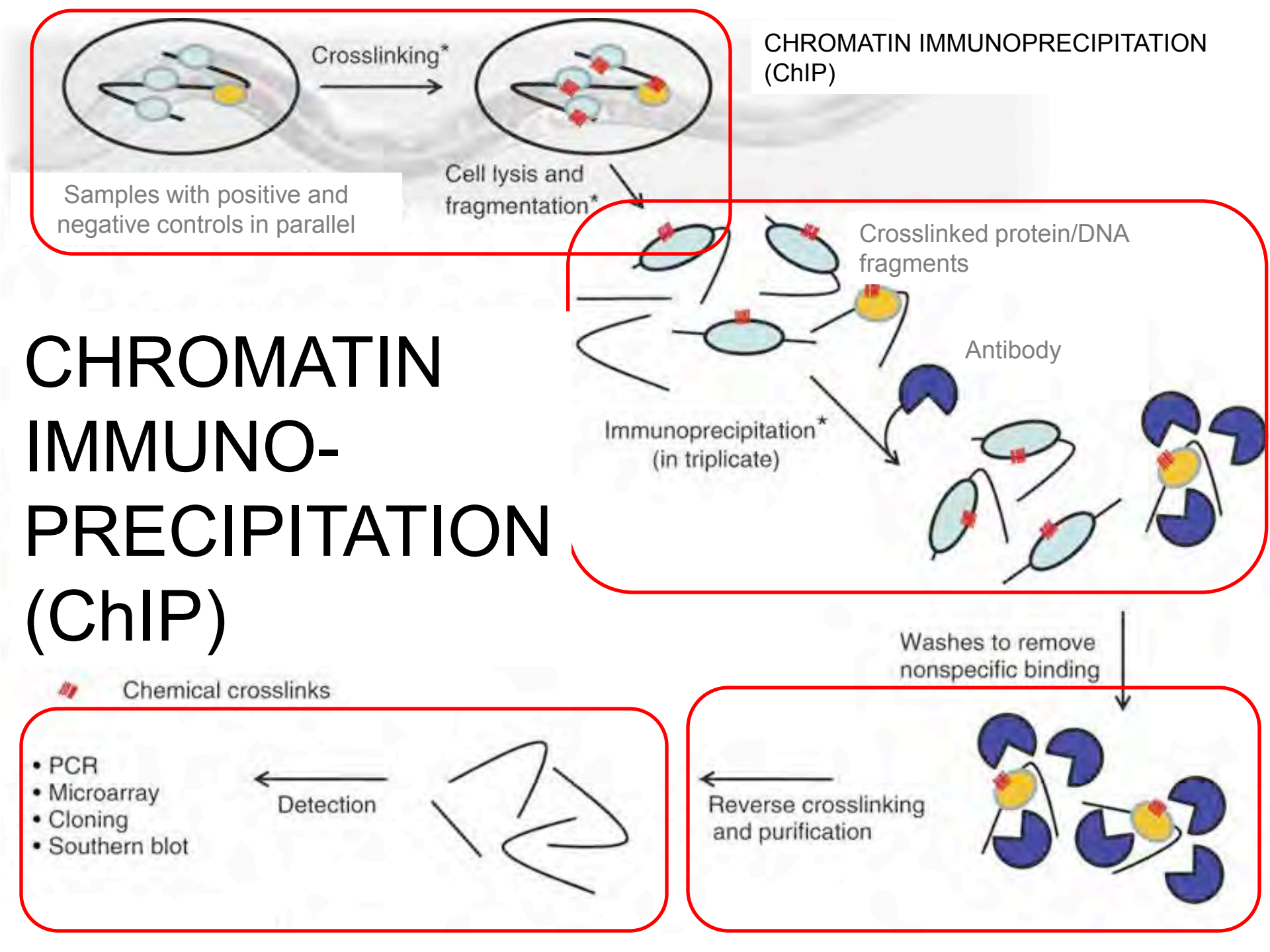




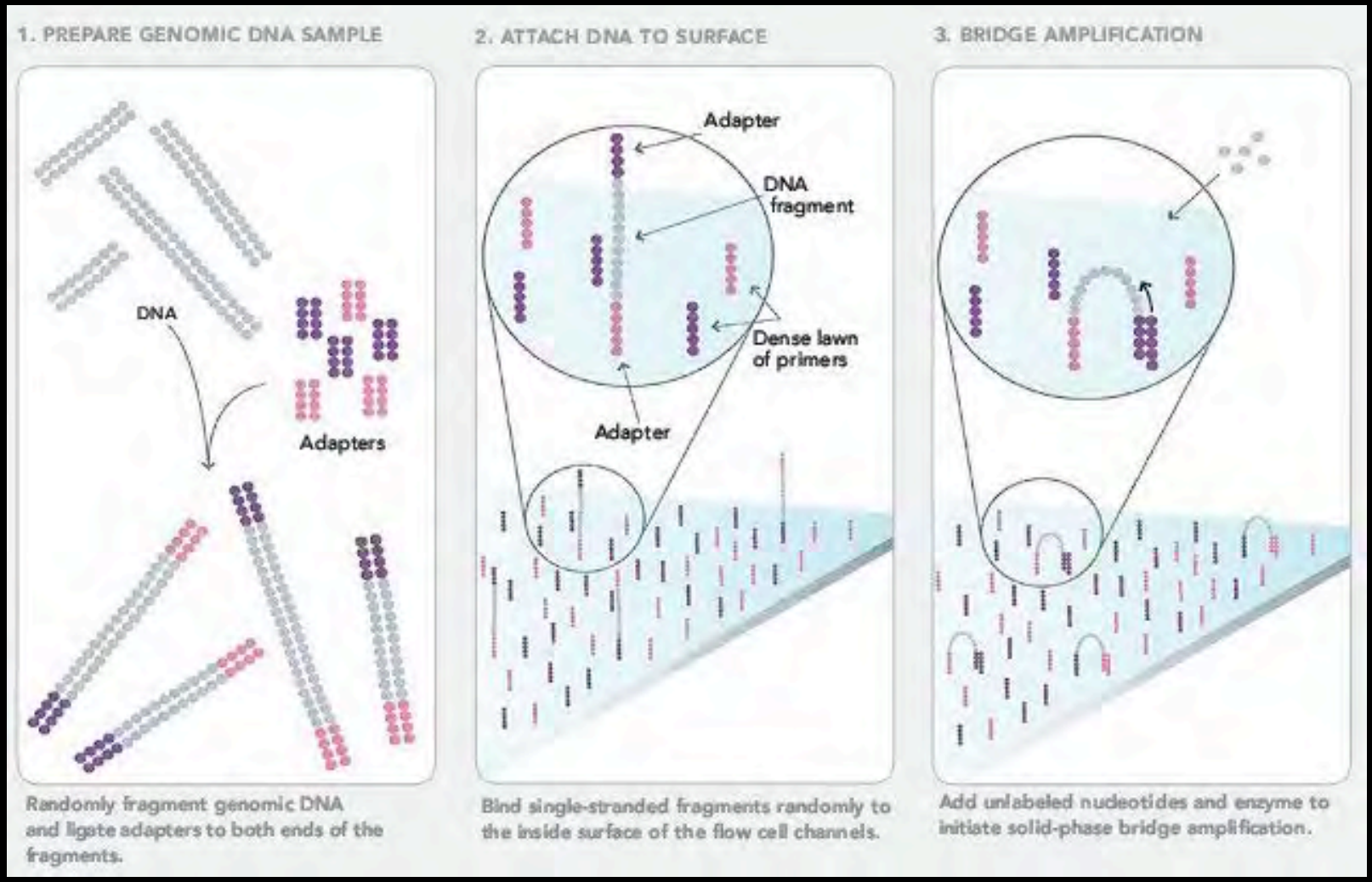
10nm fiber → 30nm fiber

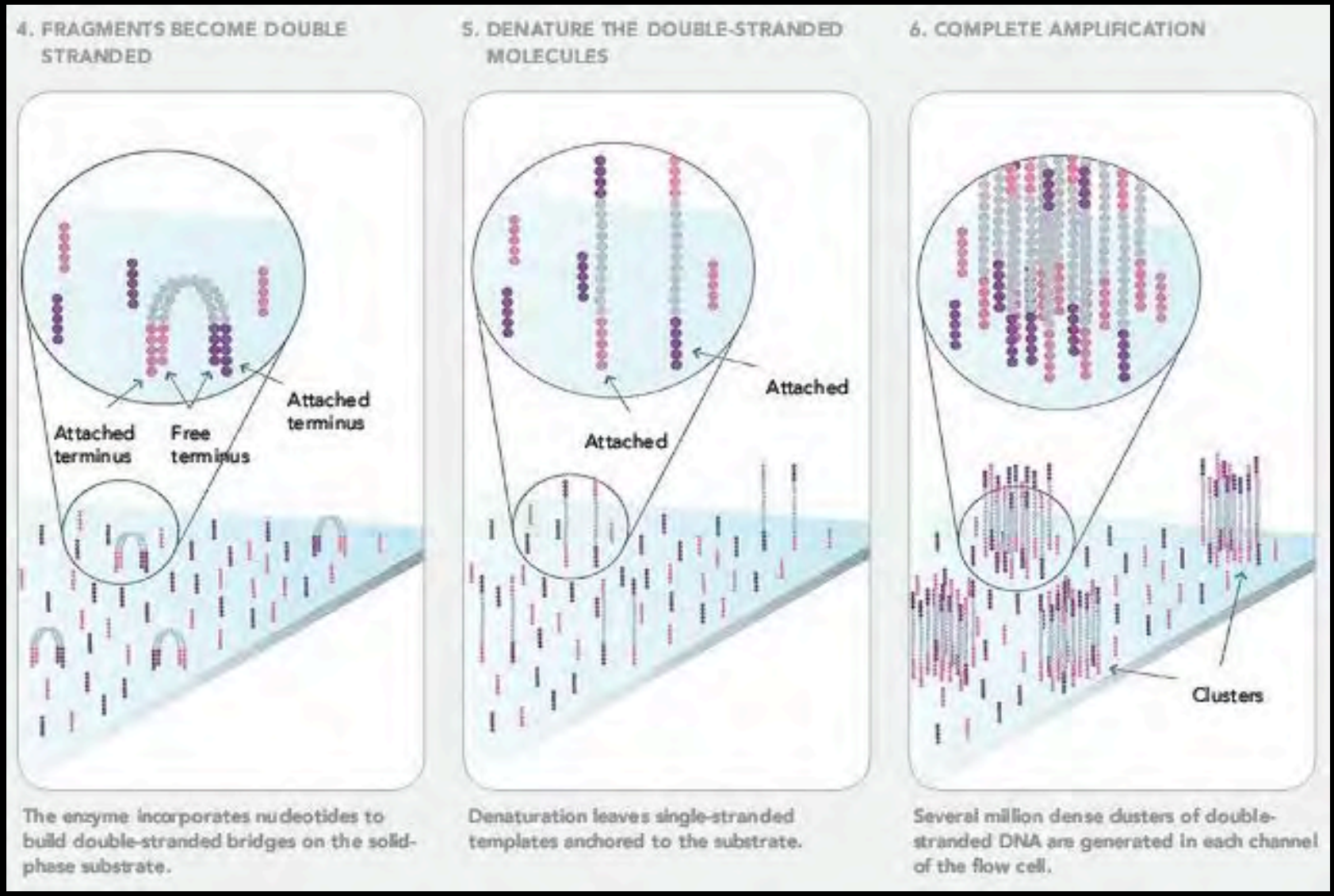
DNA compaction ratio=36

CHROMATIN IMMUNO-PRECIPITATION (ChIP)

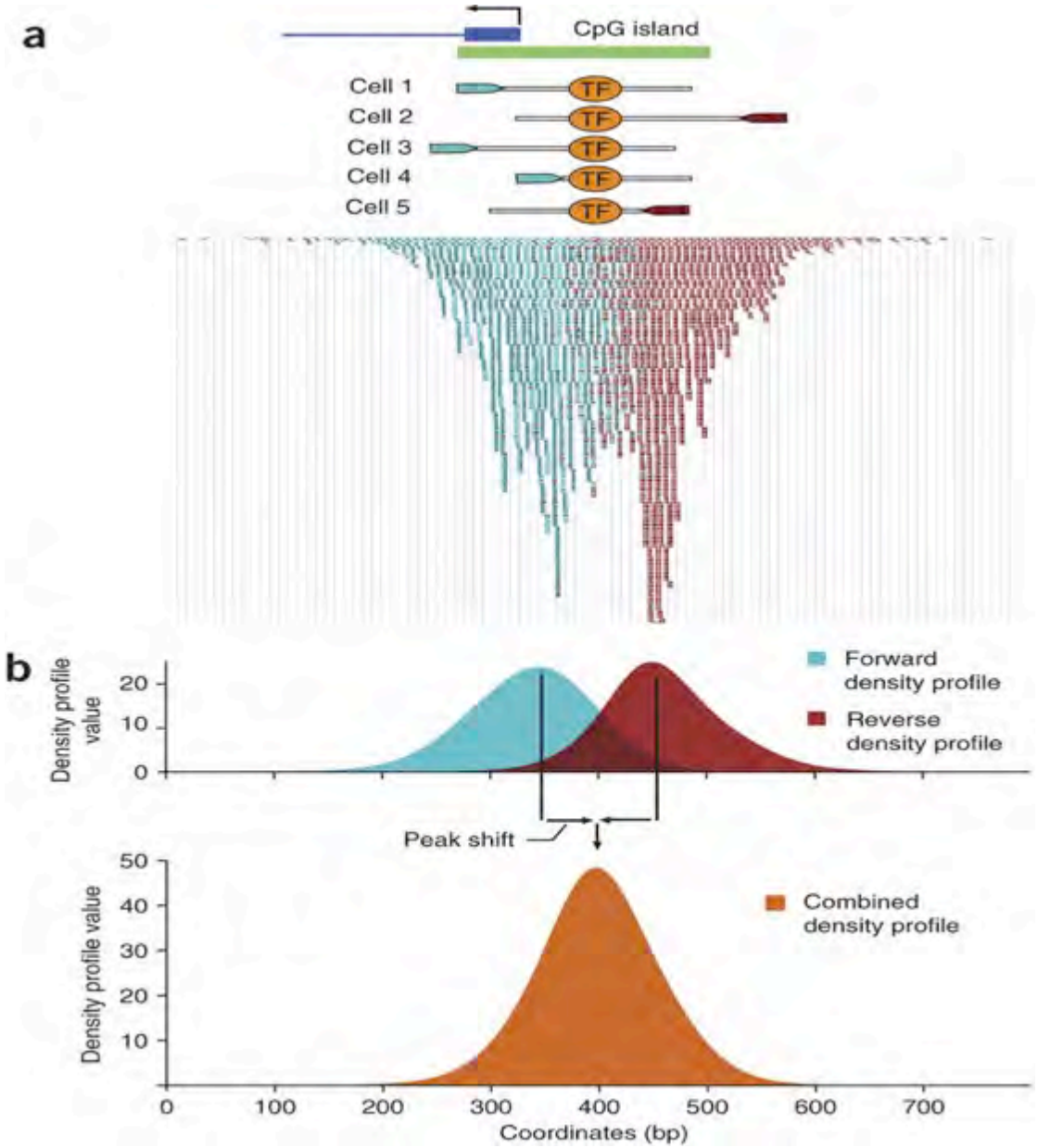


FROM ILLUMINA'S WEBSITE ON DEEP SEQUENCING

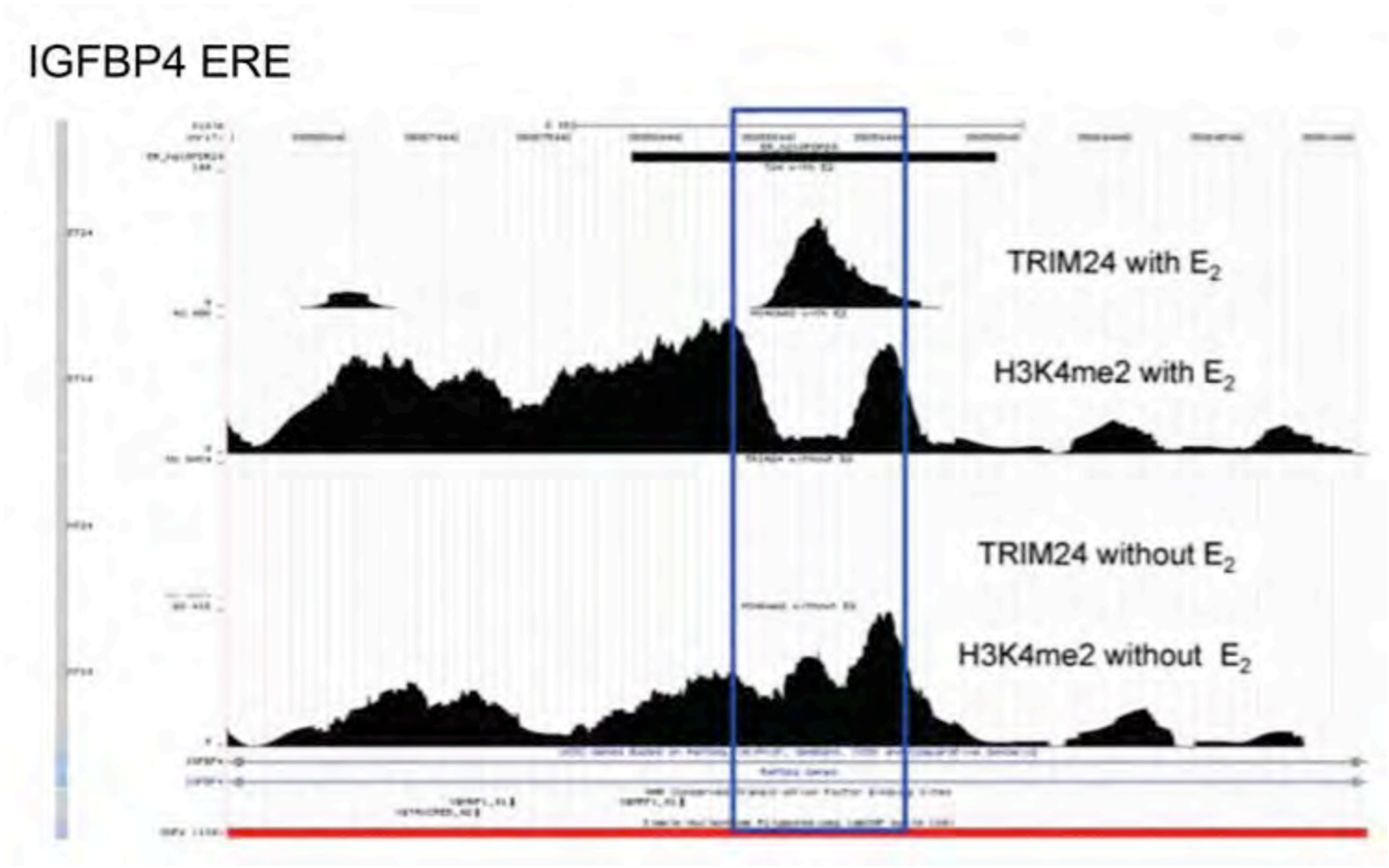



4. FRAGMENTS BECOME DOUBLE STRANDED
5. DENATURE THE DOUBLE-STRANDED MOLECULES
6. COMPLETE AMPLIFICATION
Attached terminus
Free terminus
Attached terminus
Attached
Attached
Clusters
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.
Denaturation leaves single-stranded templates anchored to the substrate.
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

ChIP-seq data coupled with bioinformatic analyses

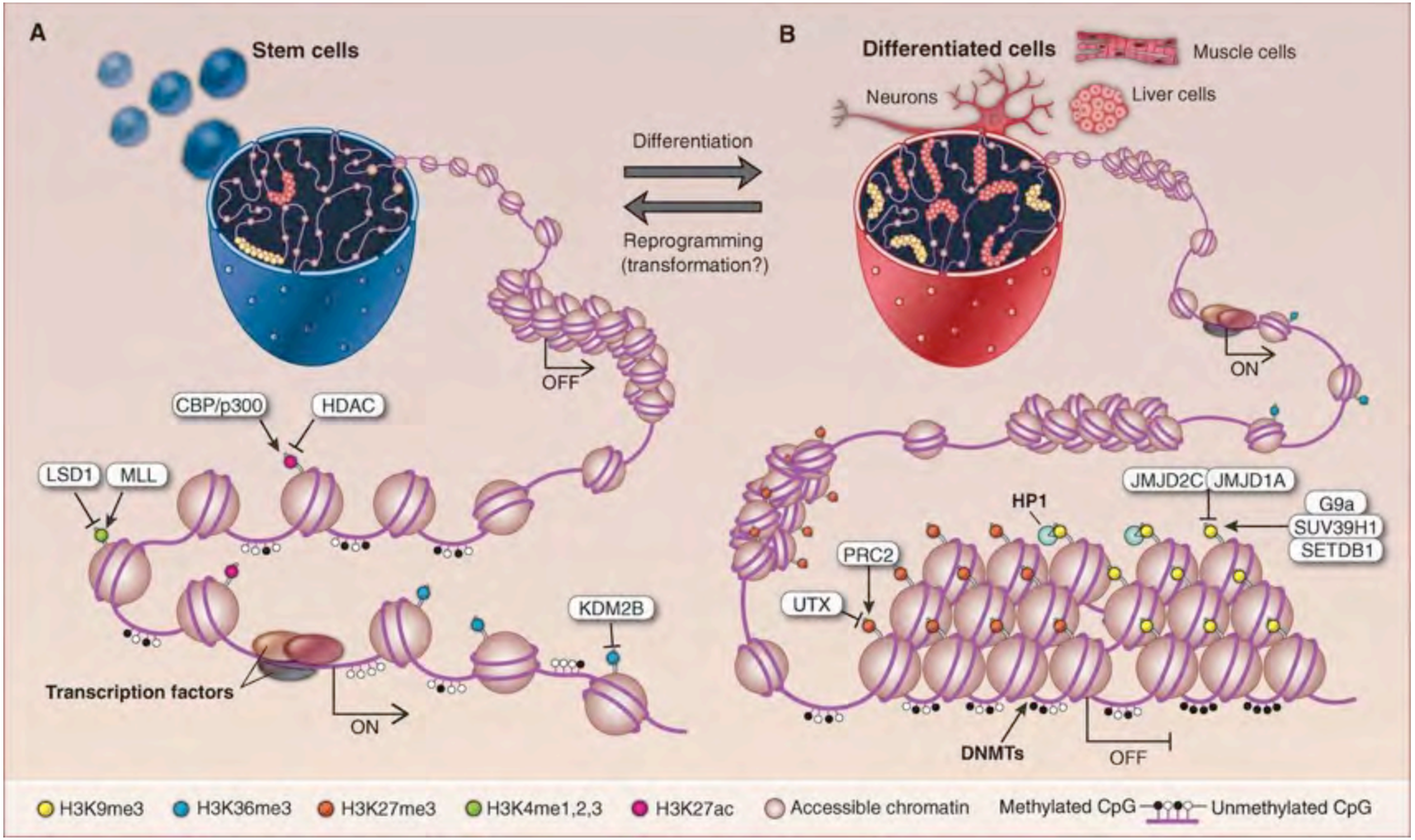


A Snapshot of ChIP-sequencing Data



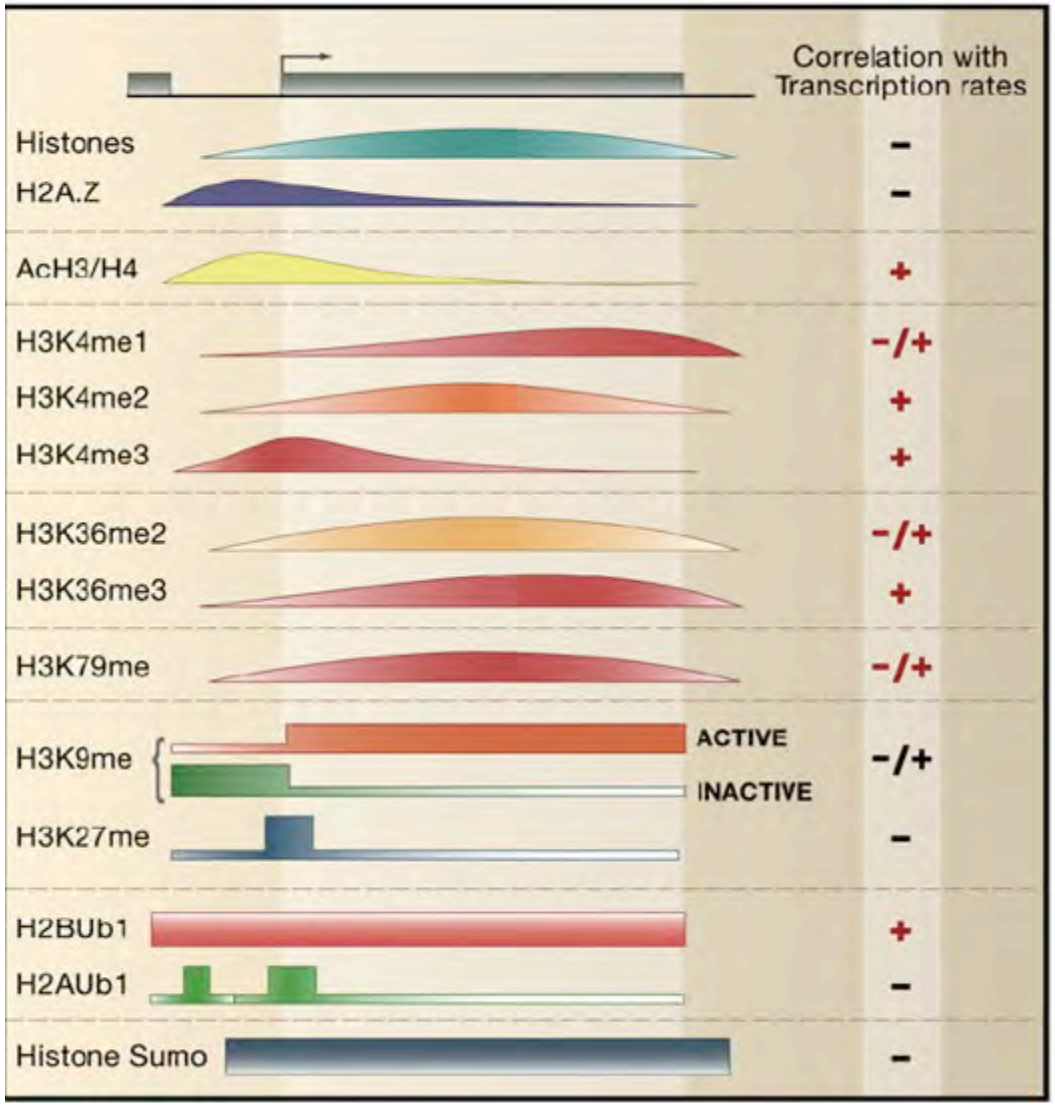
Data from *Nature 2010*
Barton lab

Epigenetic alterations distinguish between stem cells and differentiated cells



Science. 2013 Mar 29;339(6127):1567-70..
Epigenetic reprogramming in cancer.
Suvà ML, Riggi N, Bernstein BE.

Histone Modifications During Transcription
-A Global view across a gene locus



Take home:

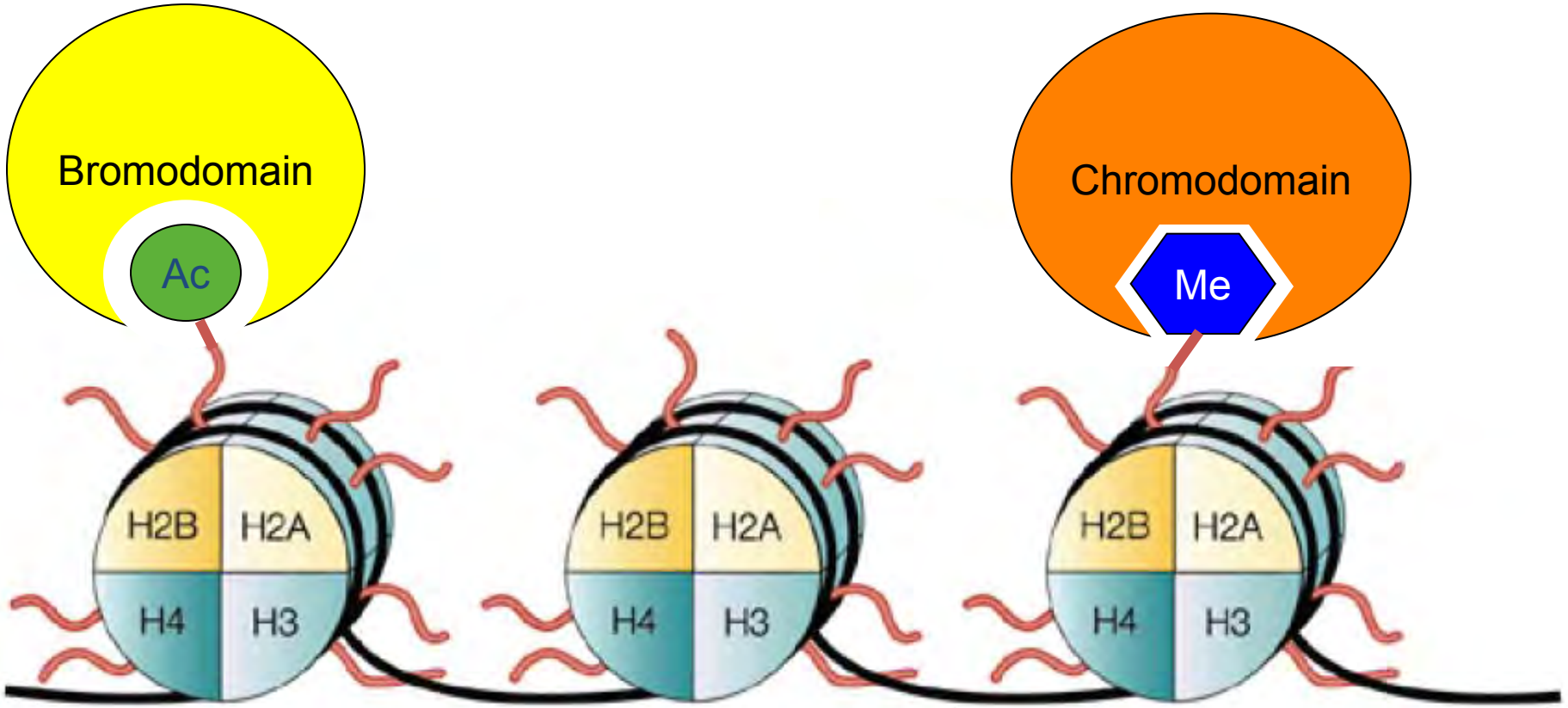
Different distributions for different modifications

Locations of modifications give clues to functions

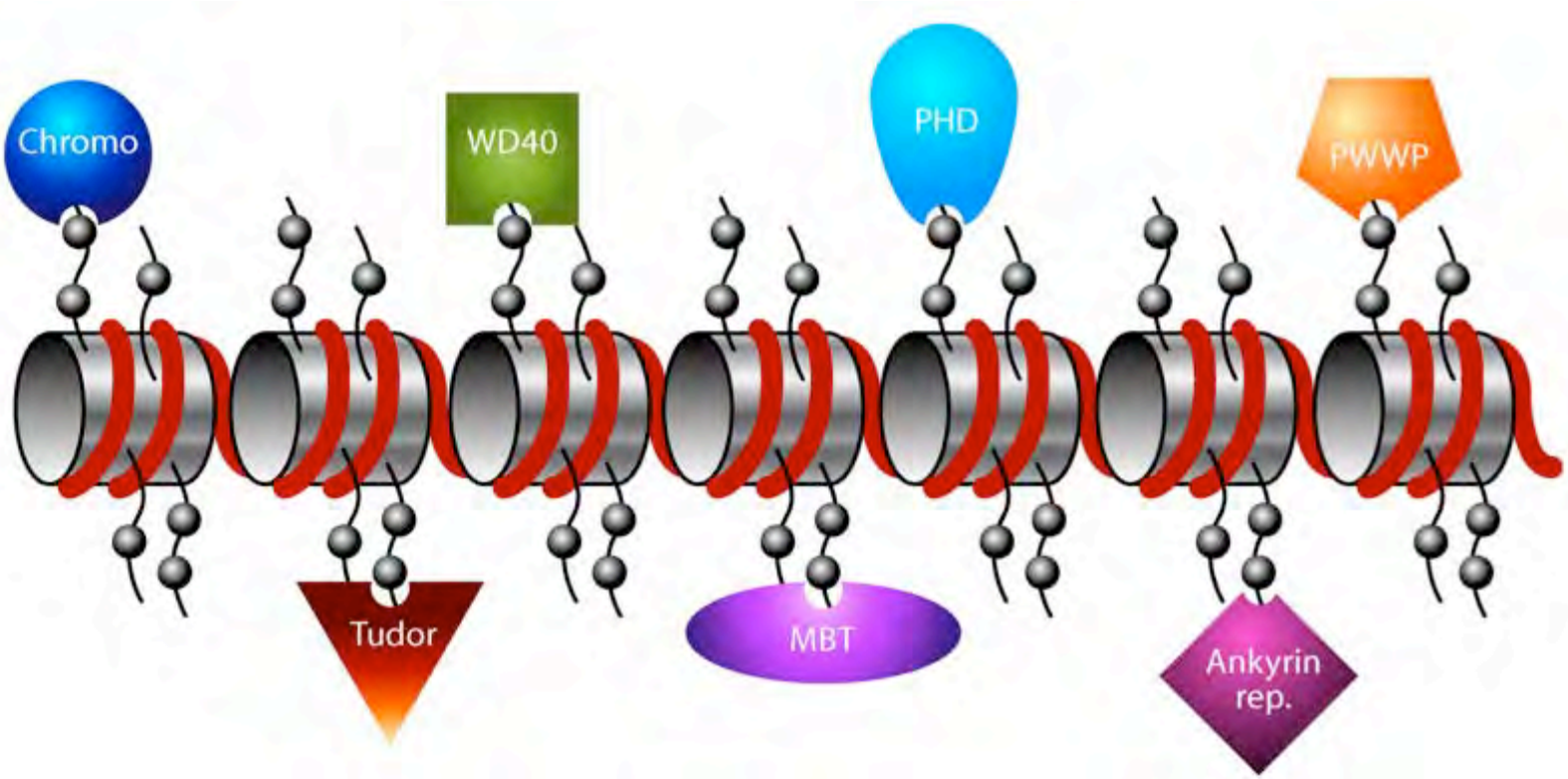
Similar distributions seen in yeast and mammals

Li, Carey and Workman *Cell* 2007

Histone modifications are generally landing sites for specific recognition domains - Readers

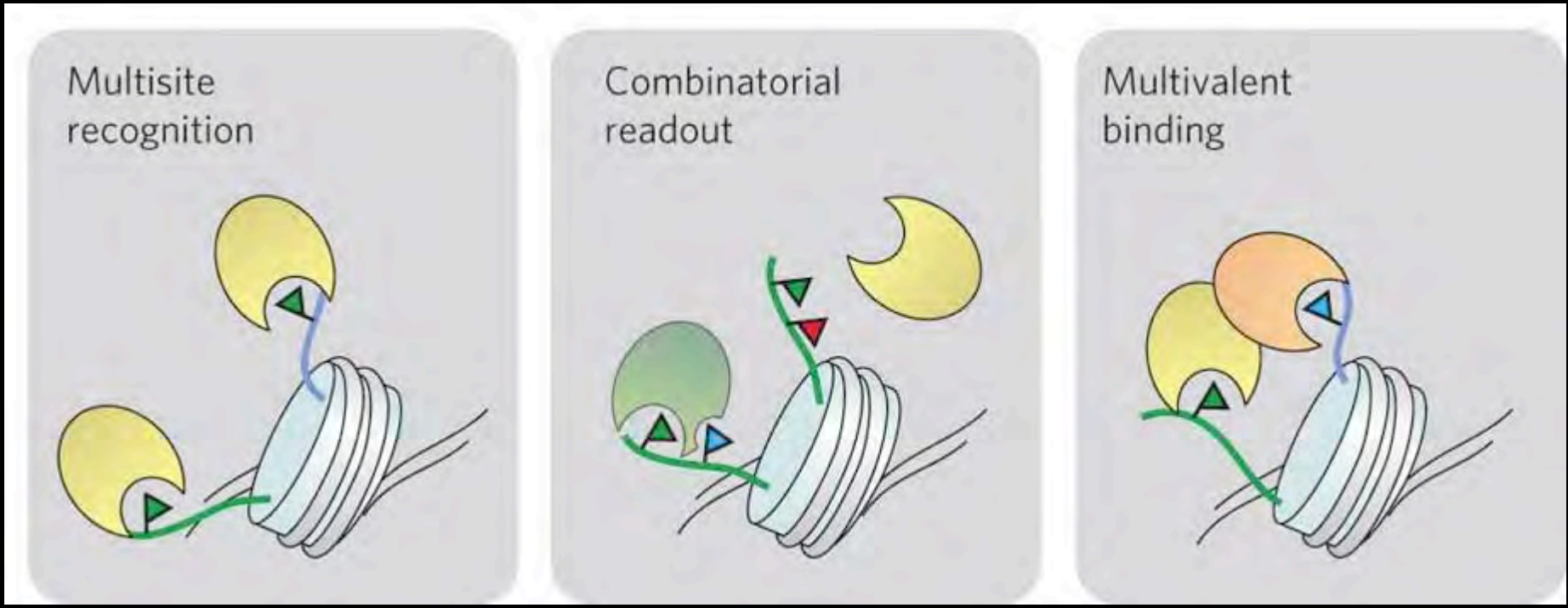


Protein Domains Recognize Histone Methylation



Mark Bedford

Combinatorial histone modification recognition



One domain recognizes combination of modifications

Different domains in same protein recognizing combination of modifications

Different Readers of Chromatin Modifications

Table 1. Chromatin Modifications, Readers, and Their Function

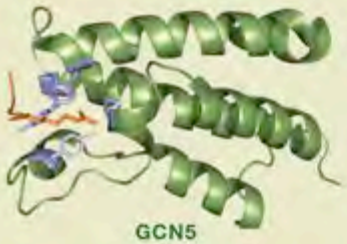
Chromatin Modification	Nomenclature	Chromatin-Reader Motif	Attributed Function
DNA Modifications			
5-methylcytosine	5mC	MBD domain	transcription
5-hydroxymethylcytosine	5hmC	unknown	transcription
5-formylcytosine	5fC	unknown	unknown
5-carboxylcytosine	5caC	unknown	unknown
Histone Modifications			
Acetylation	K-ac	BromodomainTandem, PHD fingers	transcription, repair, replication, and condensation
Methylation (lysine)	K-me1, K-me2, K-me3	Chromodomain, Tudor domain, MBT domain, PWWP domain, PHD fingers, WD40/β propeller	transcription and repair
Methylation (arginine)	R-me1, R-me2s, R-me2a	Tudor domain	transcription
Phosphorylation (serine and threonine)	S-ph, T-ph	14-3-3, BRCT	transcription, repair, and condensation
Phosphorylation (tyrosine)	Y-ph	SH2[a]	transcription and repair
Ubiquitylation	K-ub	UIM, IUIM	transcription and repair
Sumoylation	K-su	SIM[a]	transcription and repair
ADP ribosylation	E-ar	Macro domain, PBZ domain	transcription and repair
Deimination	R → Cit	unknown	transcription and decondensation
Proline isomerisation	P-cis ⇔ P-trans	unknown	transcription

Dawson & Kouzarides *Cell* 2012

SnapShot: Histone Readers

Tatiana G. Kutateladze[1]
[1]Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045, USA

Cell



Bromodomain

H3/H4Kac
ANCCA (H3K14)
BDF1
BRD2,3,4,7
BRDT(H4K5K8; H3K18)
BRG1
CBP/p300 (H4K20; H3K36)
GCN5 (H4K16)
PB-2 (H3K14)
PCAF
RSC4 (H3K14)
TAF1
TRIM24 (H3K23)

Chromodomain

H3K9me3/2
HP1
CBX1,3,5
CDY
CHP1
MPP8
Tip60

H3K27me3/2
Pc

H3K27/K9me3/2
CBX2,4,6,7,8

Chromo Barrel

H3K36me3/2
EAF3
MRG15
MSL3
(H4K20me1-DNA)

Double Chromodomain

H3K4me
CHD1(h)

PHD

H3K4me3/2
BPTF
ING1,2,3,4,5
JARID1A
KDM7A(ce)
KIAA1718
Lid
MLL1
PHF2,8
PHO23
Pygo
RAG2
TAF3
YNG1,2

H3un
AIRE
BHC80
CHD4
DNMT3L
JADE1
TRIM24

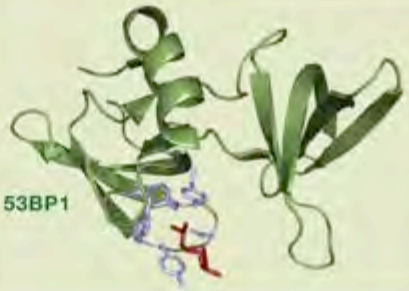
H3K9me
CHD4
ICBP90
SMCX

ZF-CW

H3K4me3
ZCWPW1

Tandem PHD

H3K14ac
DPF3

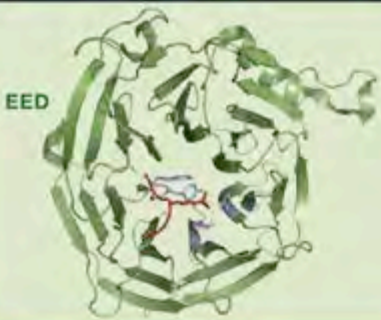


Tudor/ Tandem Tudor

H4K20me2
53BP1
CRB2

H3/H4Rme2
TDRD3

H3/H4Kme3
FXR1,2 (H4K20)
JMJD2A (H3K4)
JMJD2A (H4K20)
UHRF1 (H3K9)

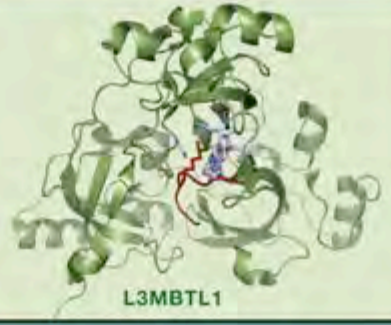


WD40/ β propeller

H3R2
WDR5

H3/H4/H1Kme3
EED

H4un (15-41)
RbAp46/48



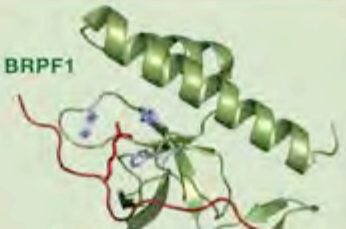
MBT

H3/H4Kme1/2
L3MBTL1,2
MBTD1
SCM
SCML2
SFMBT

H3K9me2/3
LIN61

Ankyrin

H3K9me2/1
G9a
GLP

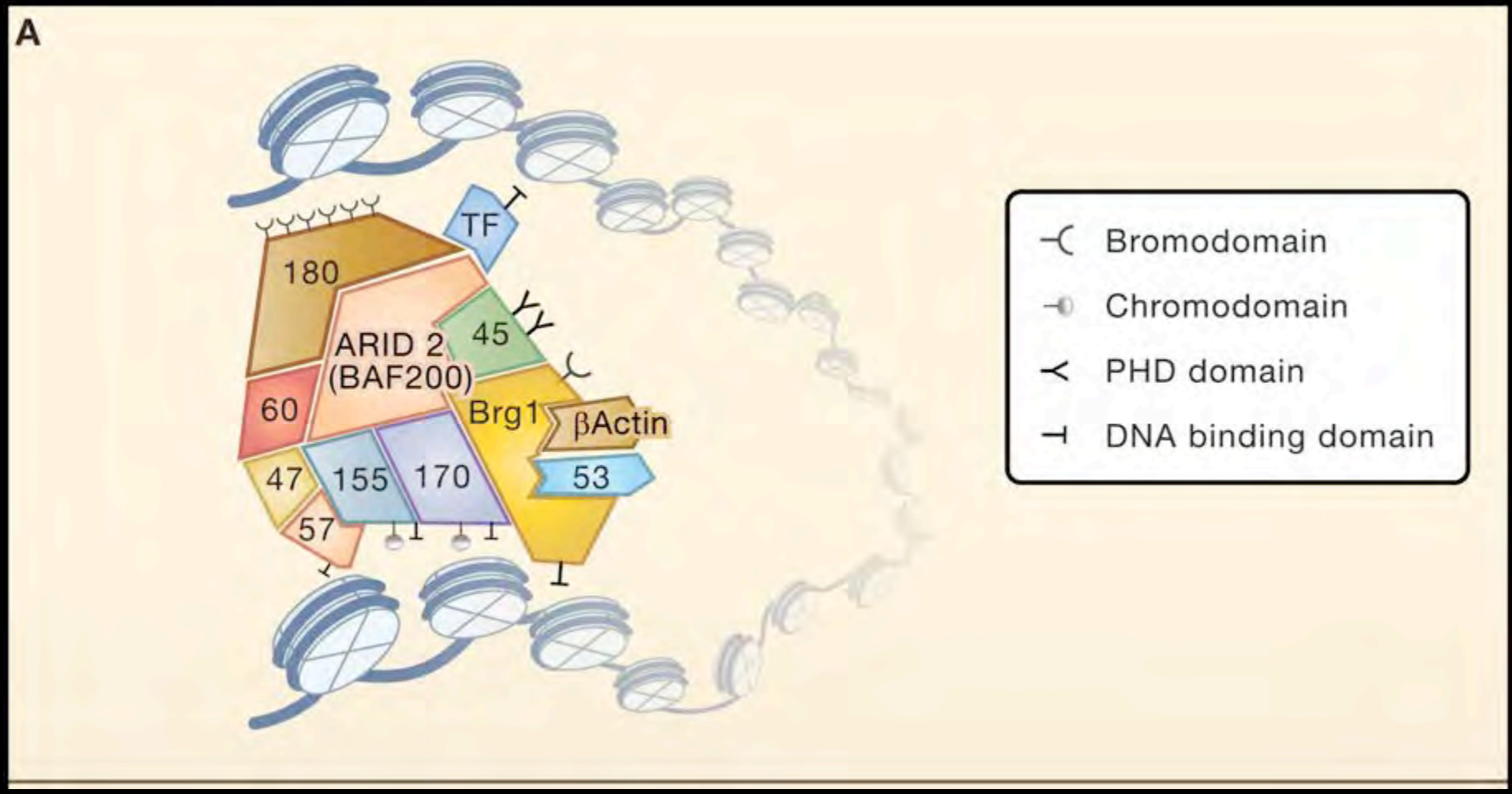


PWWP

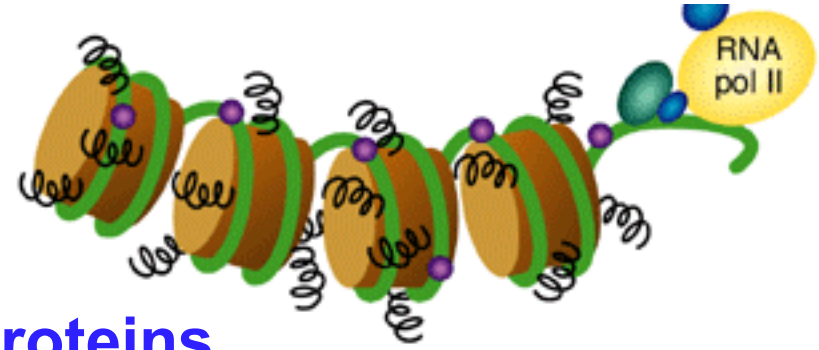
H3K36me3
BRPF1
DNMT3A

H4K20me1
PDP1

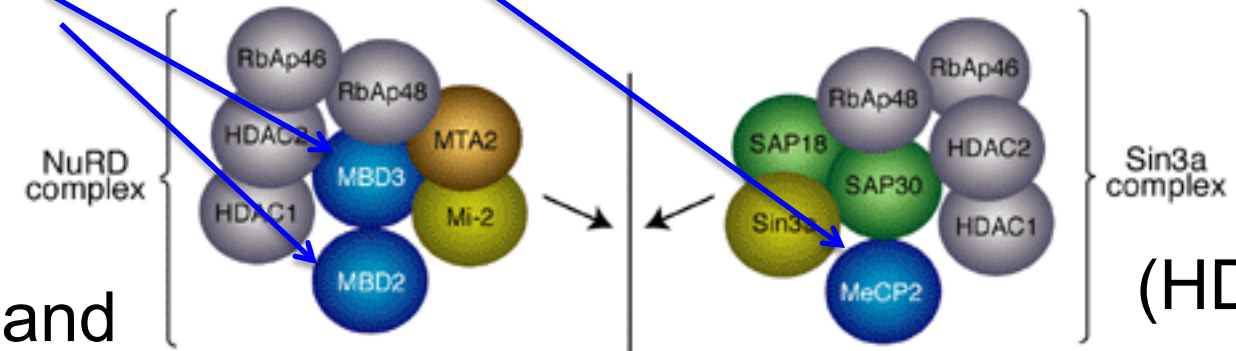
Human SWI/SNF - an ATP-dependent chromatin remodeler with “reader” functions: Subunits with DNA and histone binding domains



How does DNA methylation repress gene expression?
Co-repressors contain Methyl-C binding proteins

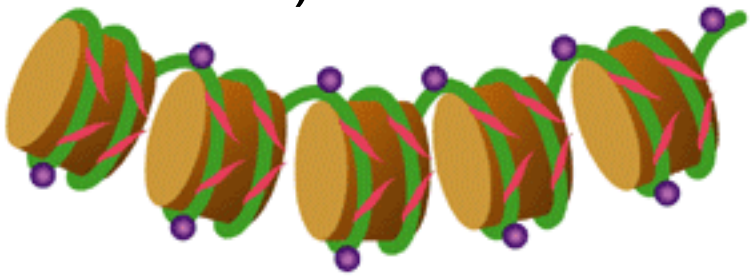


Methyl-C binding proteins



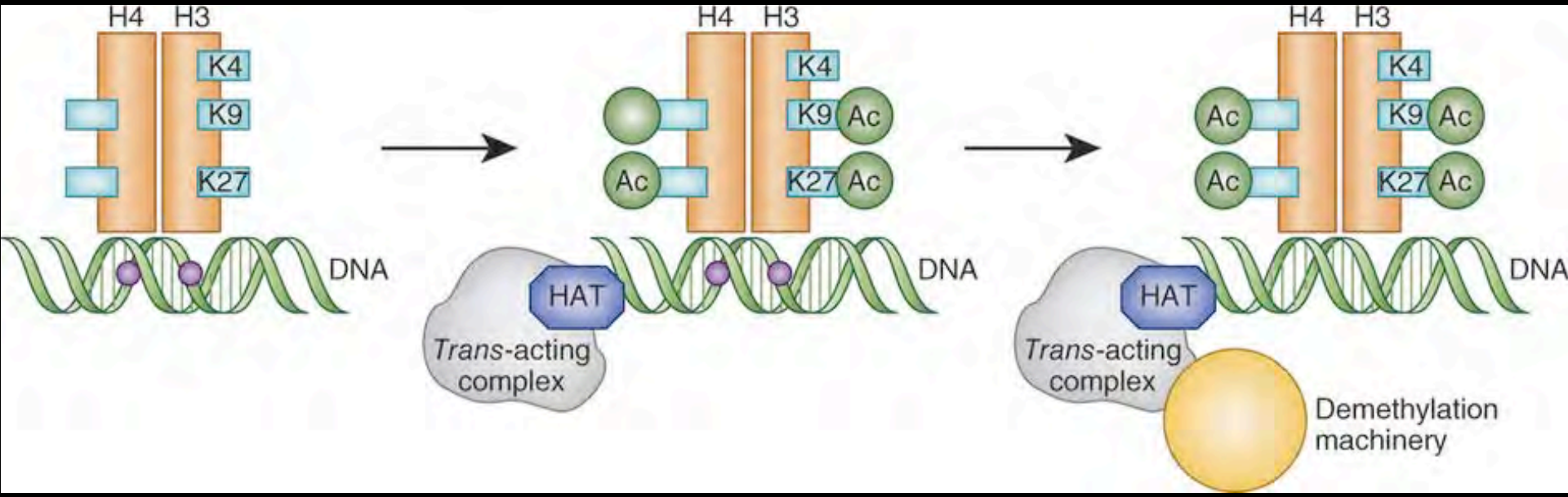
(HDACs and ATP-dependent remodeler)

(HDACs)



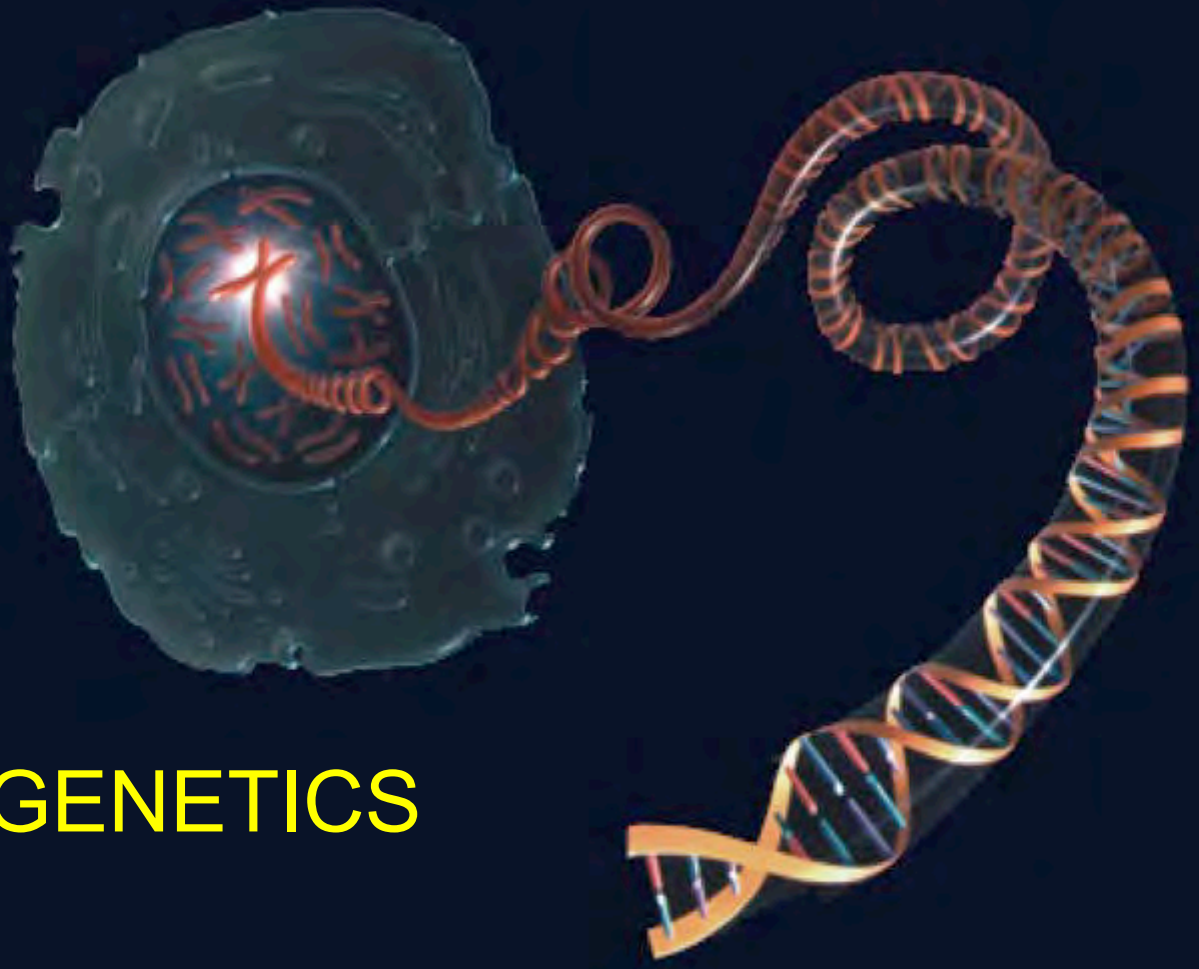
Methylated CpG pair Deacetylated histone tail Acetylated histone tail

Readers that recruit DNA demethylation machinery remain unknown



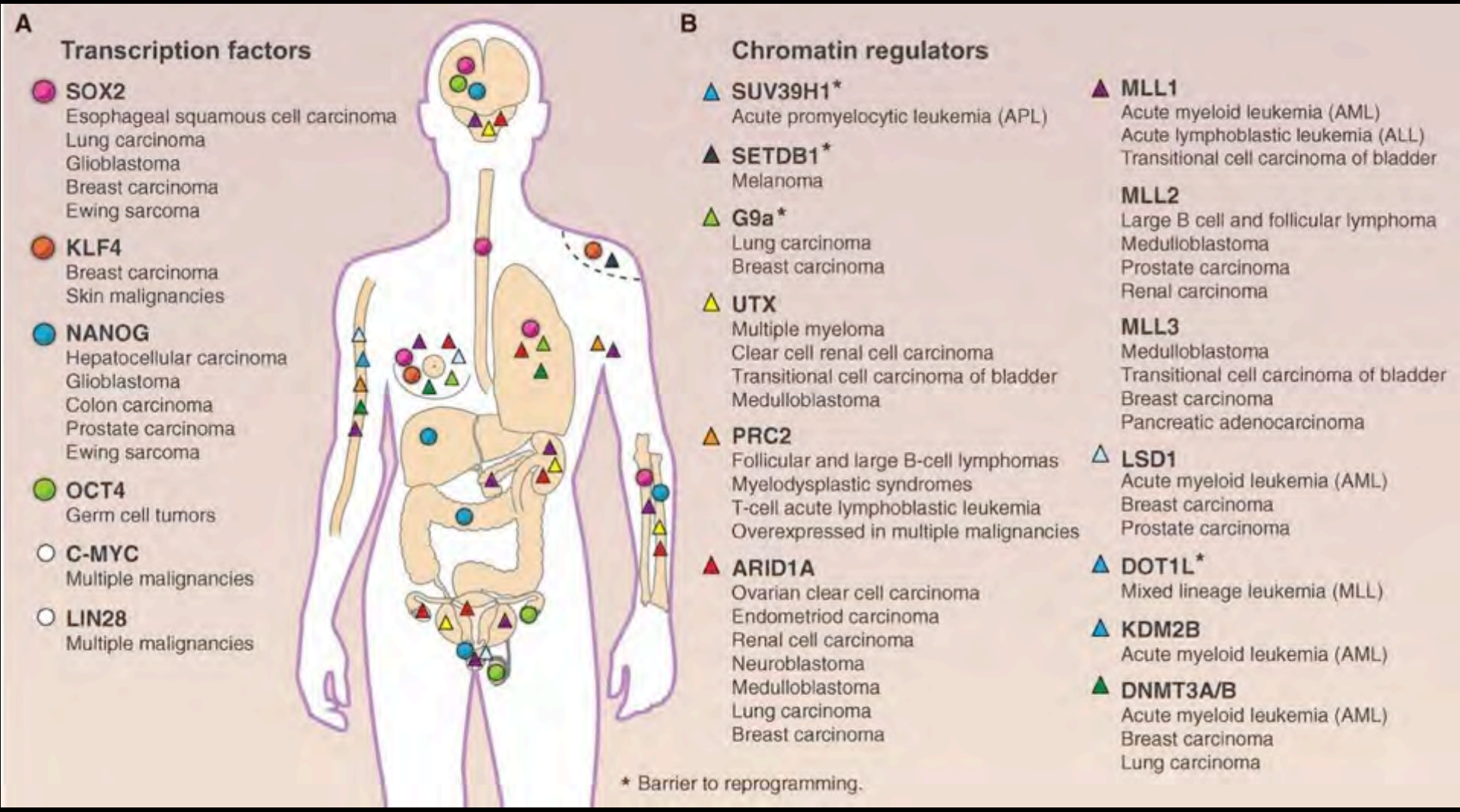
DNA methylation dynamics in health and disease, Yehudit Bergman & Howard Cedar, Nature Structural & Molecular Biology 20, 274–281(2013)

HOW CAN WE USE THIS TO FIGHT CANCER?



EPIGENETICS

EPIGENETICS X STEM CELLS = IMPACT IN CANCER



Epigenetic Reprogramming in Cancer

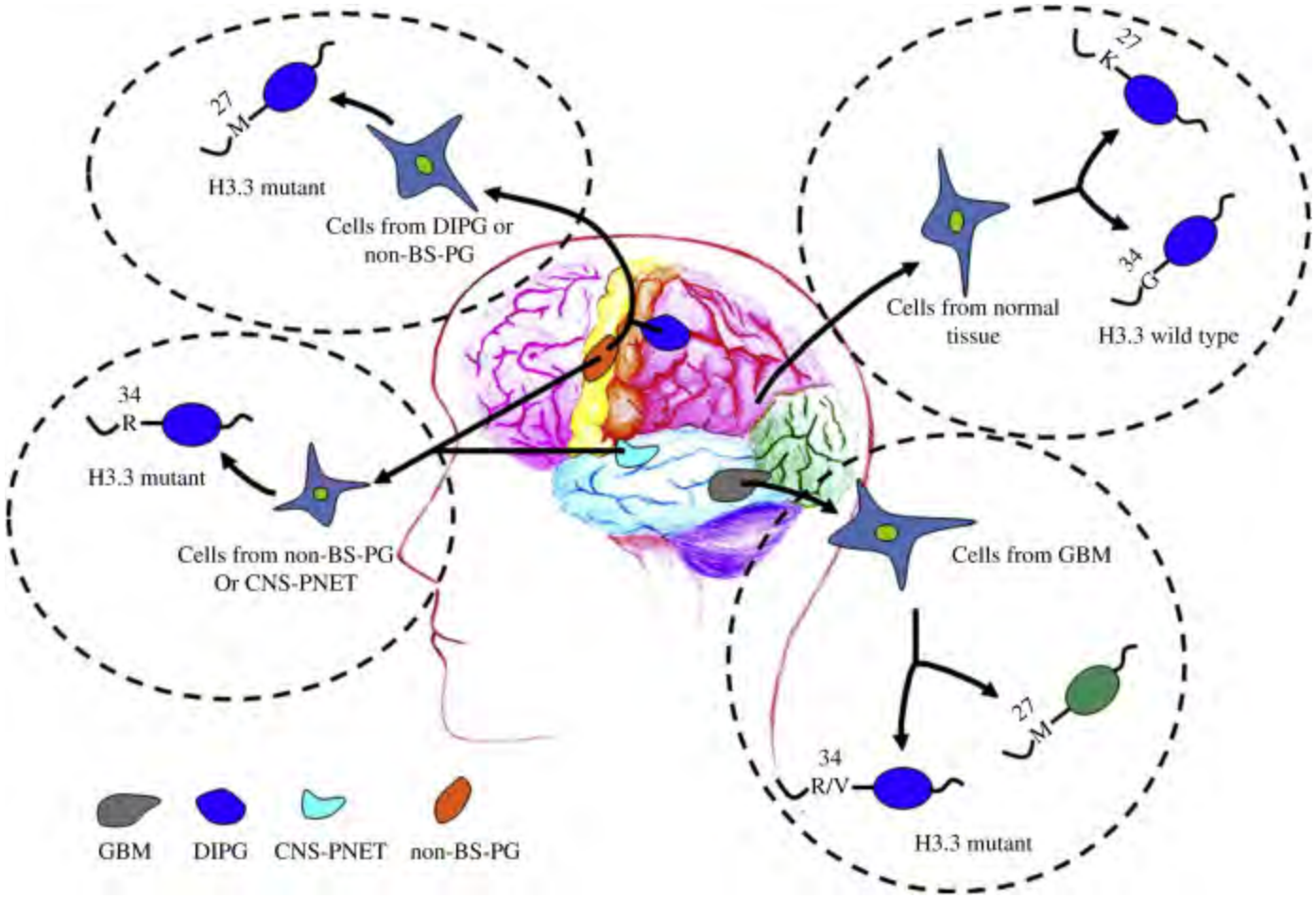
Mario L. Suvà, Nicolo Riggi, and Bradley E. Bernstein Science 2013

Cancer mutations in histone genes

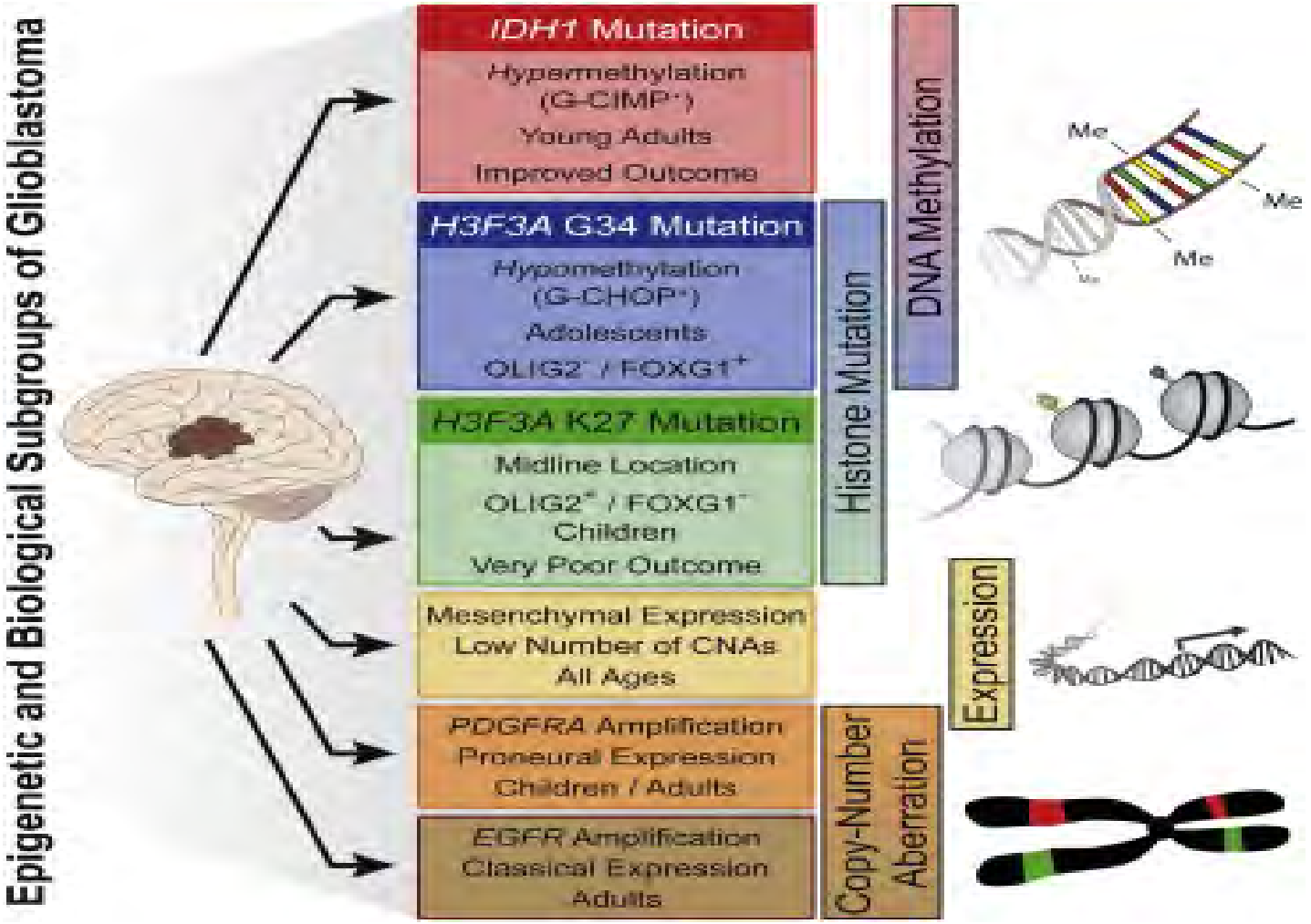
- Histone H3 mutations in pediatric glioblastomas
- Mutations cluster to yield amino acid substitutions at 2 residues in tail of histone H3 (K27M and G34R/G34V)
- K27M mutation affects methylation and acetylation
- G34V mutation caused global increase in H3K36me3
- Pedi GBM also has mutations in ATRX/DAXX chromatin

Pediatric glioblastoma are epigenetically distinct from adult glioblastoma

Histone H3.3 and H3.1 mutations in 78 % DIPG and 36% of non-brain stem gliomas: K27M and G34R: unique to high grade pedi GBM



Tumor location and age of onset are influenced by mutations

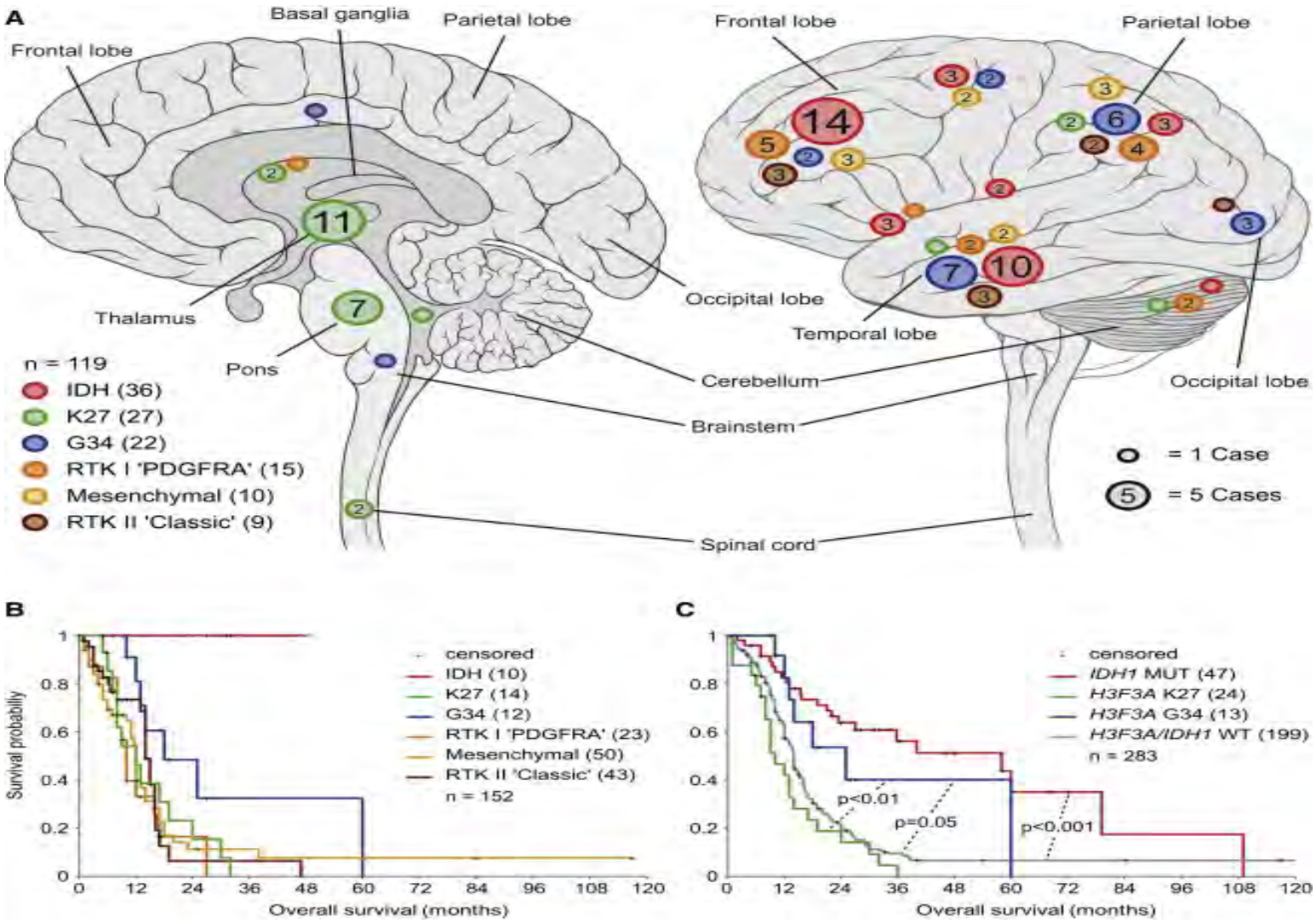


Dominik Sturm , Hendrik Witt , Volker Hovestadt , Dong-Anh Khuong-Quang , David T.W. Jones , Carolin Konermann , E...

Hotspot Mutations in *H3F3A* and *IDH1* Define Distinct Epigenetic and Biological Subgroups of Glioblastoma

Cancer Cell Volume 22, Issue 4 2012 425 - 437

Tumor location and age of onset are influenced by mutations



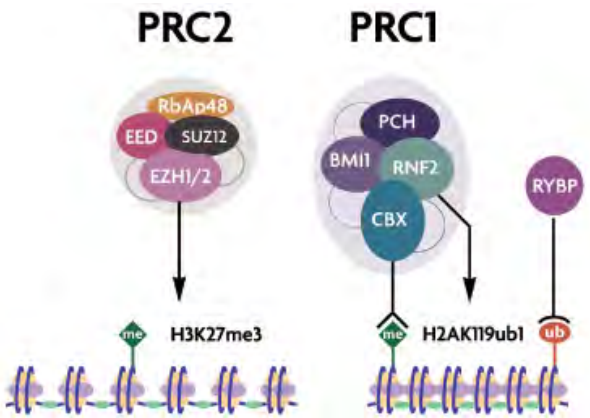
Dominik Sturm , Hendrik Witt , Volker Hovestadt , Dong-Anh Khuong-Quang , David T.W. Jones , Carolin Konermann , E...

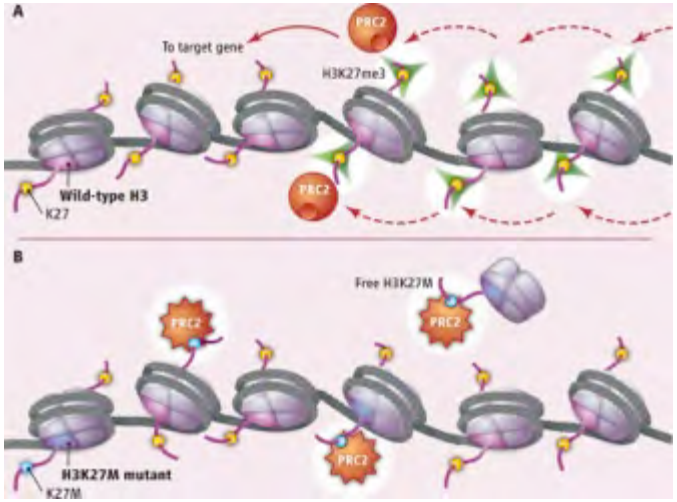
Hotspot Mutations in *H3F3A* and *IDH1* Define Distinct Epigenetic and Biological Subgroups of Glioblastoma

Cancer Cell Volume 22, Issue 4 2012 425 - 437

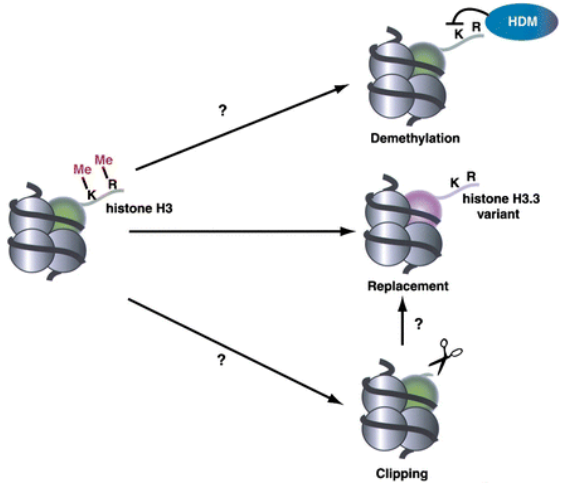
How could these mutations be targeted?

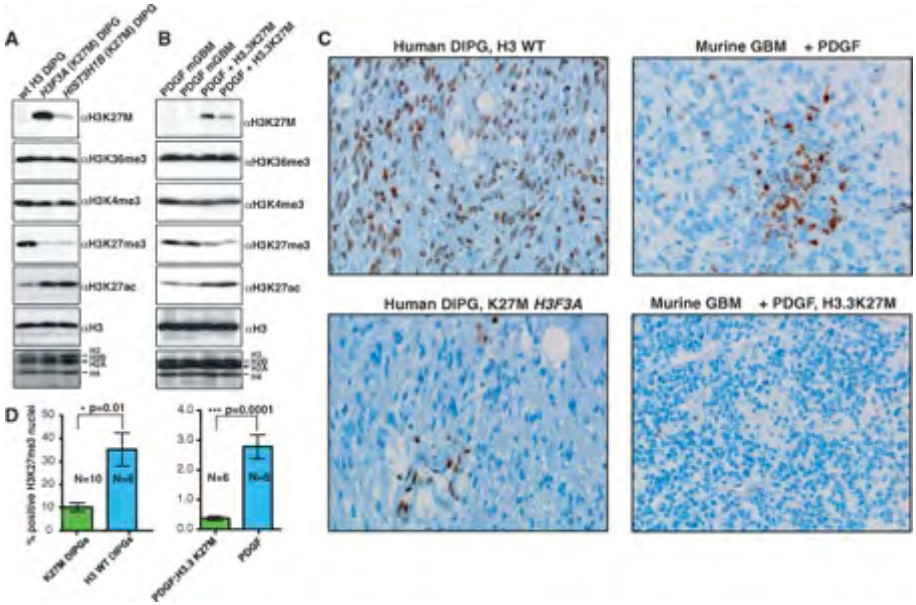
PRC: polycomb repressor complex



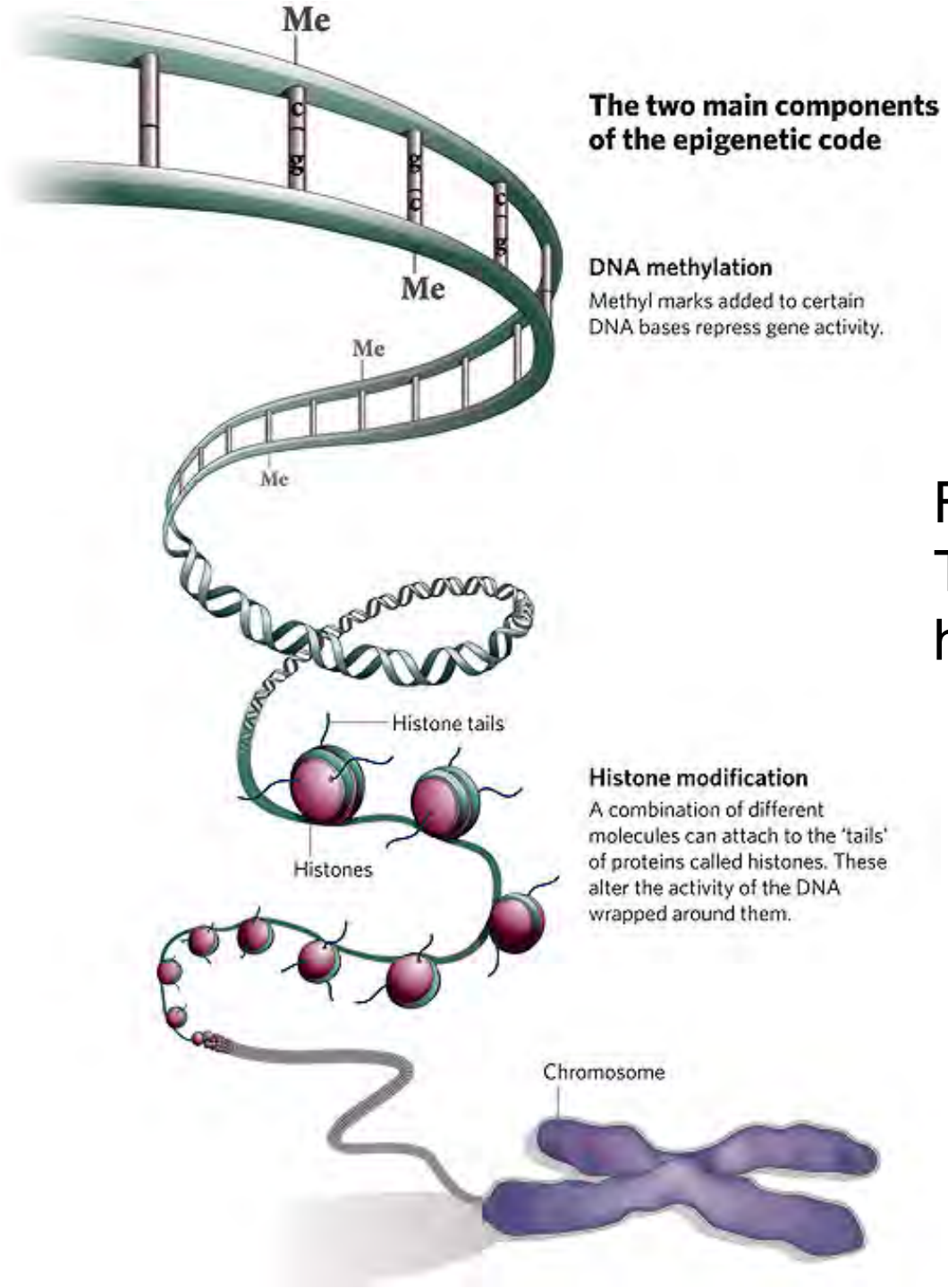


Morgan and Shilatifard
Science 17 May 2013:
Vol. 340 no. 6134 pp. 823-824





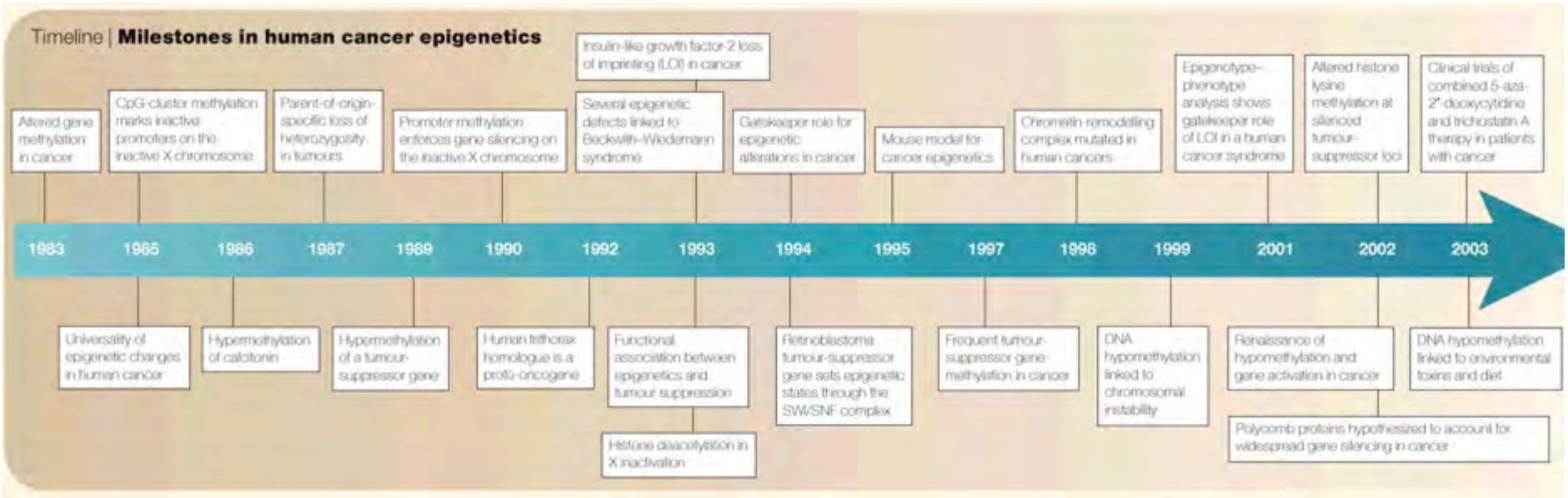
Science 17 May 2013: Vol. 340 no. 6134 pp. 857-861
Inhibition of PRC2 Activity by a Gain-of-Function H3 Mutation Found in Pediatric Glioblastoma
Lewis…Allis



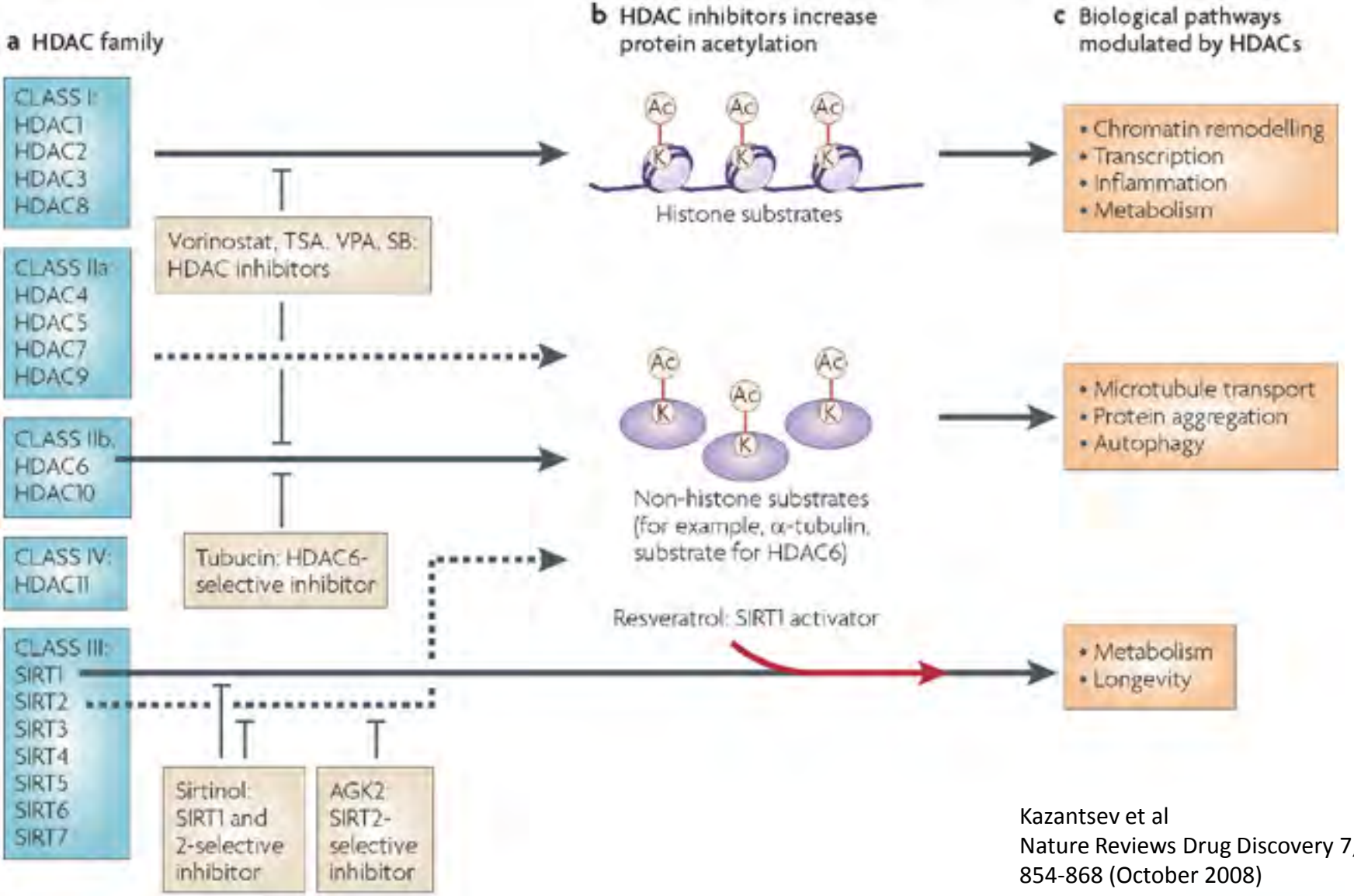
FDA Approved Therapies Target DNA methylation and histone acetylation

Translational aspects

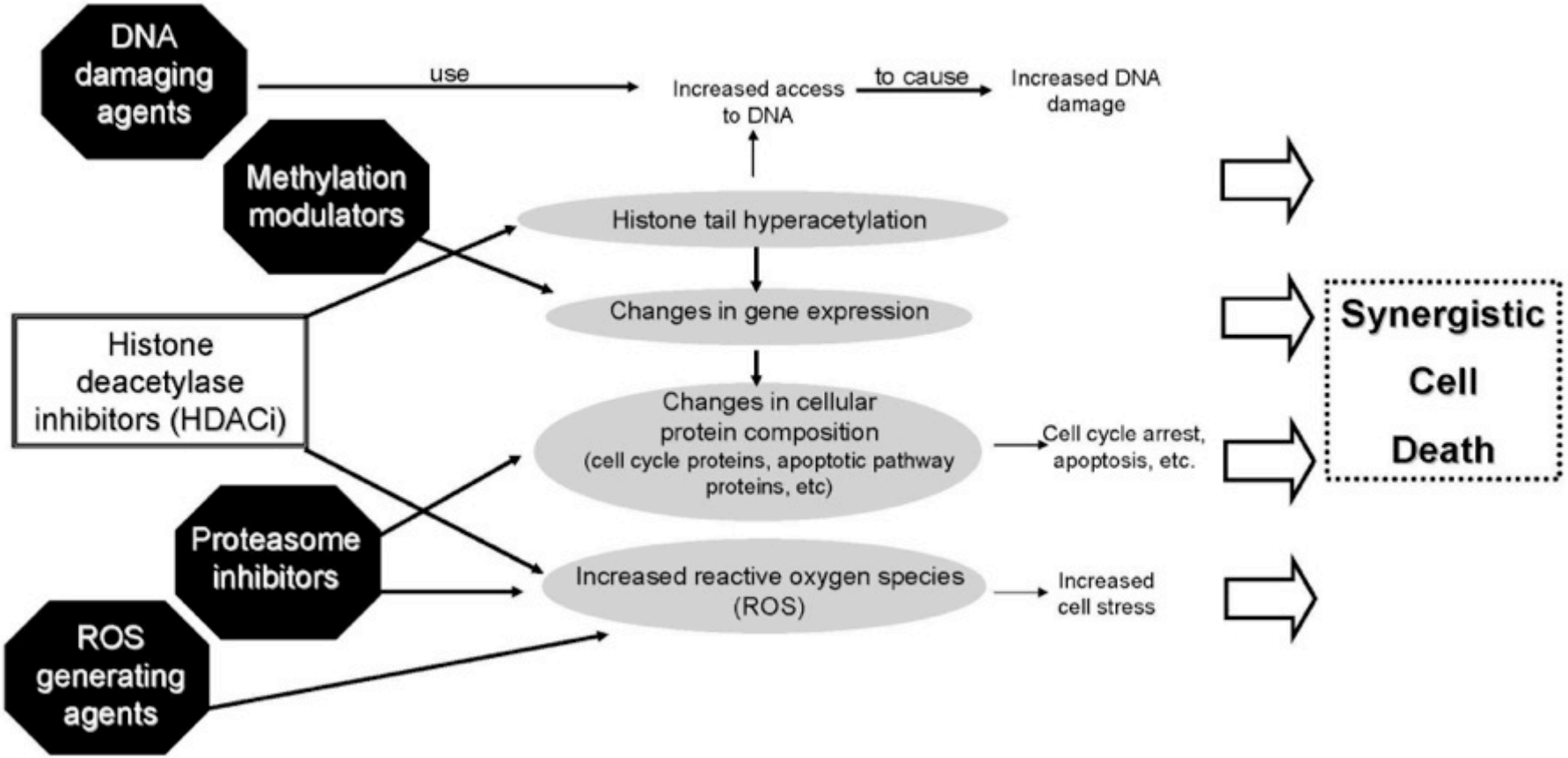
HDAC inhibitors
Hypomethylating agents



The history of cancer epigenetics
Andrew P. Feinberg & Benjamin Tycko
Nature Reviews Cancer 4, 143-153 (February 2004)



Kazantsev et al
Nature Reviews Drug Discovery 7, 854-868 (October 2008)
Therapeutic application of histone deacetylase inhibitors for central nervous system disorders

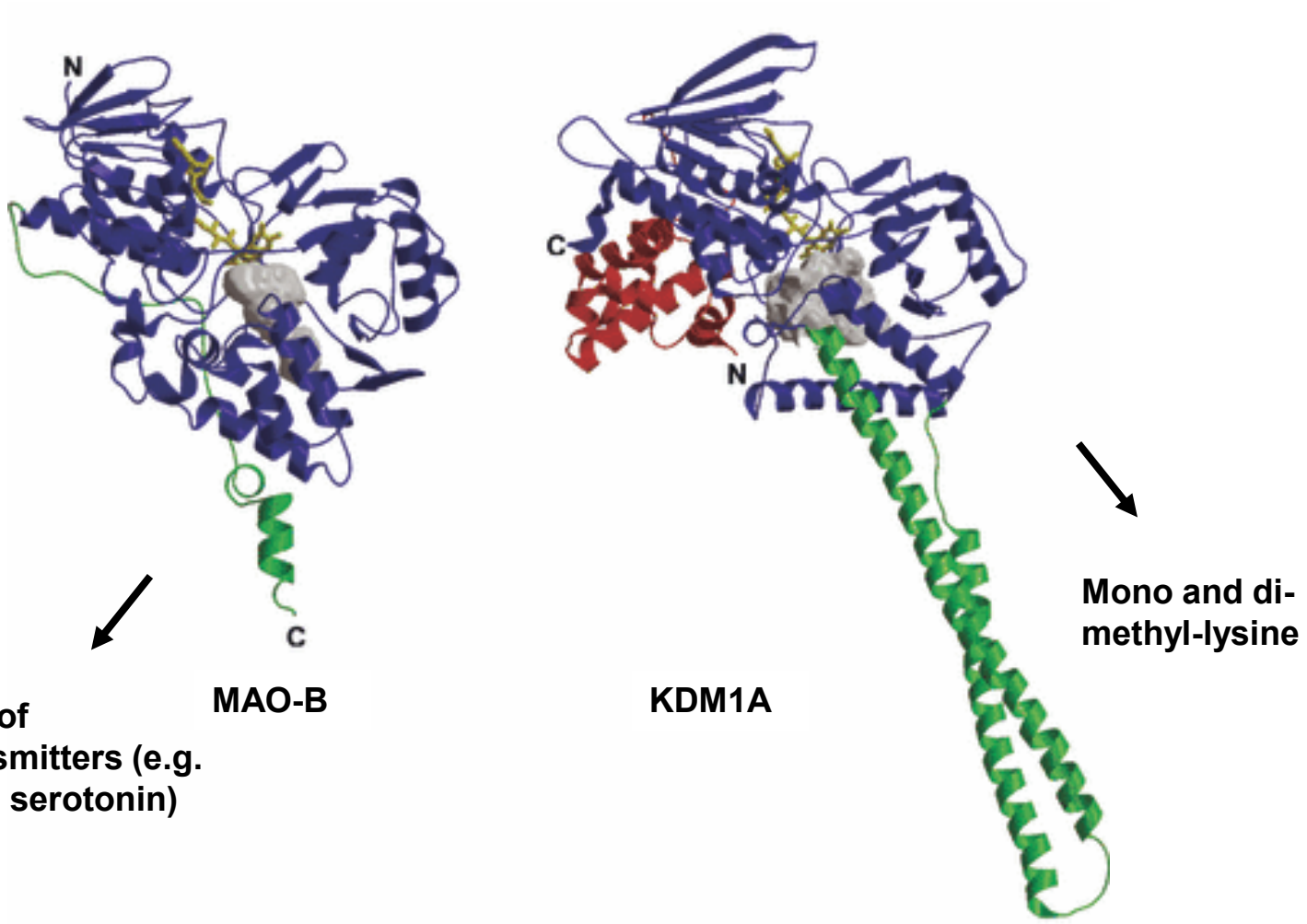

DNA damaging agents
use
Increased access to DNA
to cause
Increased DNA damage
Methylation modulators
Histone tail hyperacetylation
Changes in gene expression
Histone deacetylase inhibitors (HDACi)
Changes in cellular protein composition (cell cycle proteins, apoptotic pathway proteins, etc)
Cell cycle arrest, apoptosis, etc.
Synergistic Cell Death
Proteasome inhibitors
Increased reactive oxygen species (ROS)
Increased cell stress
ROS generating agents

HDAC inhibitors: structural features

Vorinostat & romidepsin FDA approved for use in cutaneous T cell lymphoma patients

class	compound
aliphatic acids	valproic acid
	AR-42 (OSU-HDAC42)
hydroxamic acids	vorinostat (suberoylanilide hydroxamic acid, SAHA)[a]
	belinostat (PXD101)[b]
	dacinosat (LAQ824)
	panobinostat (LBH589)
	resminostat (4SC-201)
	PCI-24781
	SB939
	CHR2845
	CHR3996
	JNJ-26481585
benzamides	entinostat (MS-275)
	mocetinostat (MGCD0103)
	4SC-202
cyclic peptides	romidepsin (depsipeptide, FK228, FR901228)[a]

Mono- and polyamine oxidase inhibitors also target KDM1A



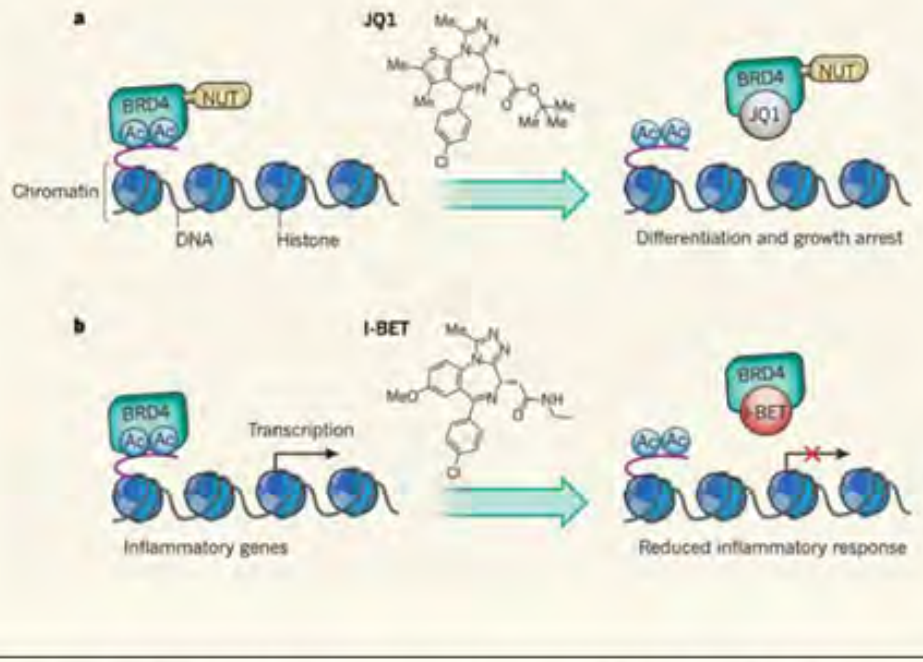
Forneris R. *et al*. FEBS J. **2009**. 276(16):4304-4312.

Bromodomain inhibitors

Bromodomains are acetyl-lysine binding pockets that target bromodomain containing proteins to histones

BRD4-NUT: NUT (Nuclear protein in testis) midline carcinoma (NMC) belongs to a class of highly lethal and poorly differentiated epithelial cancers arising mainly in human midline organs

Figure

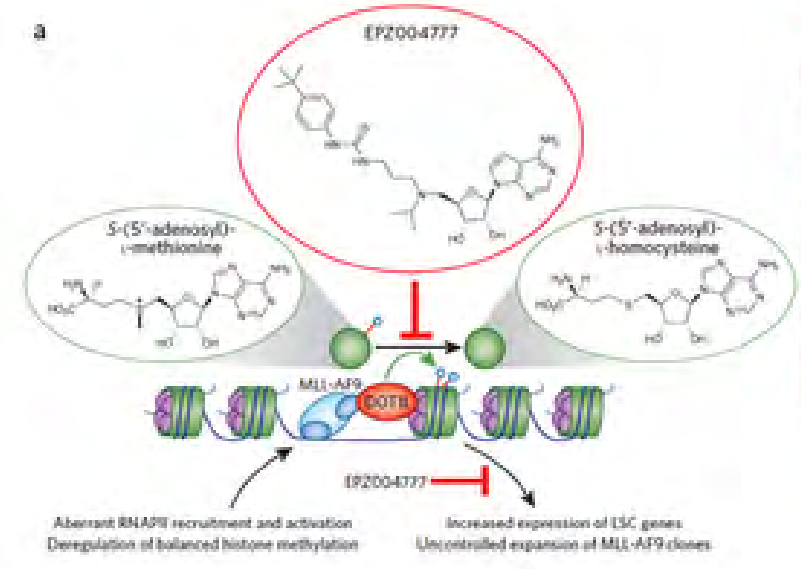


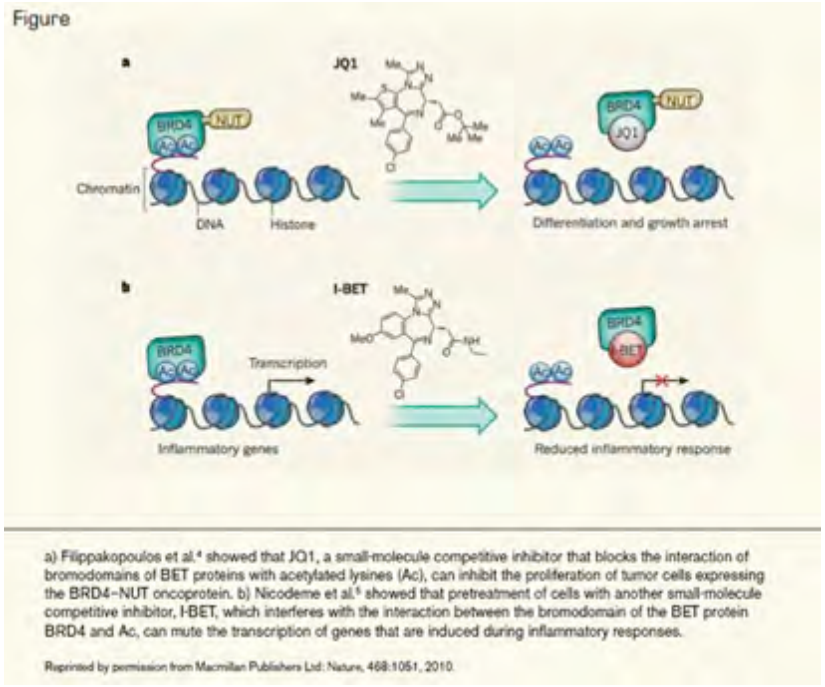
a) Filippakopoulos et al.[4] showed that JQ1, a small-molecule competitive inhibitor that blocks the interaction of bromodomains of BET proteins with acetylated lysines (Ac), can inhibit the proliferation of tumor cells expressing the BRD4–NUT oncoprotein. b) Nicodeme et al.[5] showed that pretreatment of cells with another small-molecule competitive inhibitor, I-BET, which interferes with the interaction between the bromodomain of the BET protein BRD4 and Ac, can mute the transcription of genes that are induced during inflammatory responses.

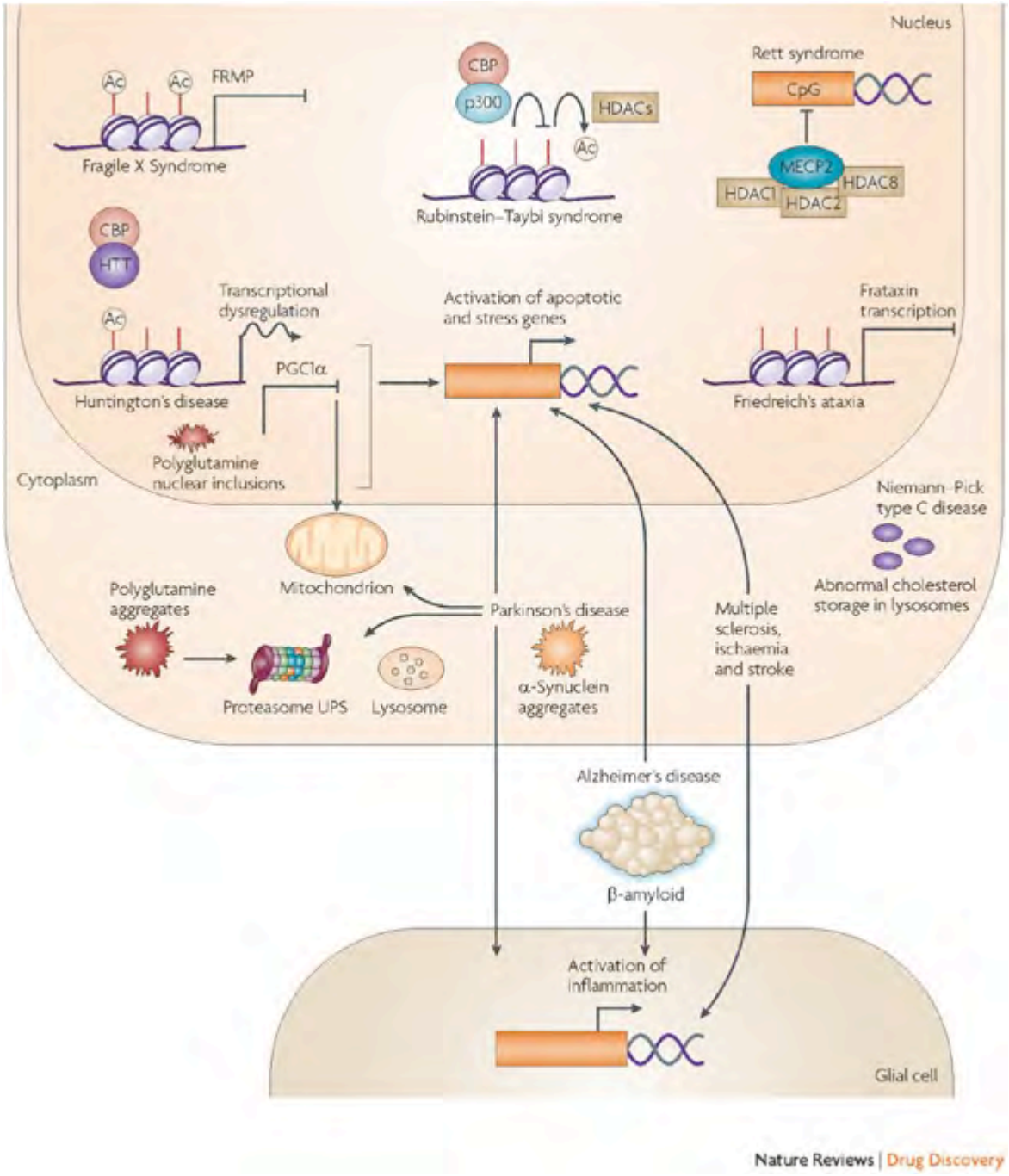
Strategies for targeting MLL-rearranged leukemias

- DOT1L inhibitors

- Bromodomain inhibitors







Roles for epigenetic enzymes in CNS disorders

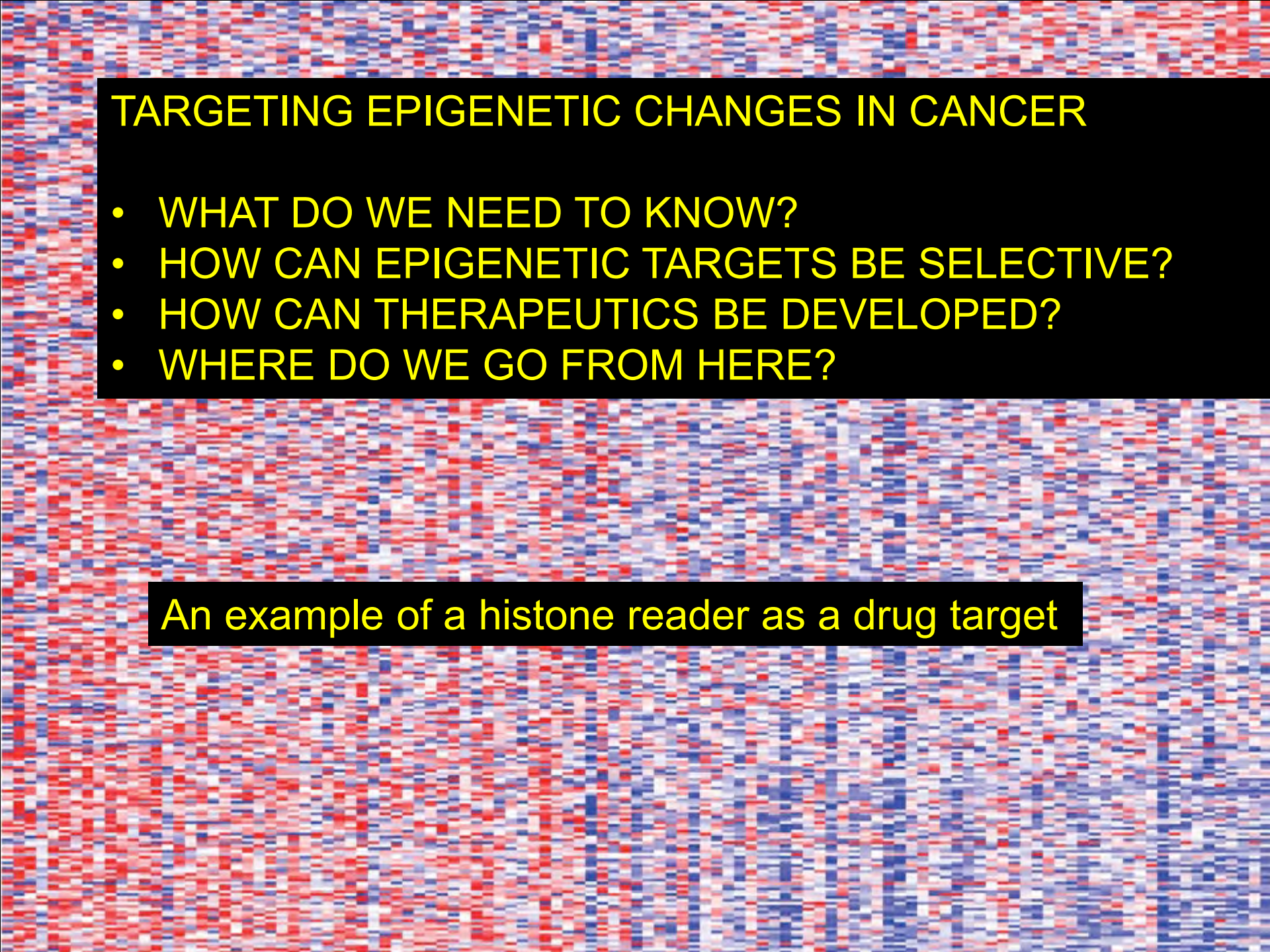
HDAC inhibitors are being tested in neurodegenerative diseases

Kazantsev et al
Nature Reviews Drug Discovery 7, 854-868 (October 2008) Therapeutic application of histone deacetylase inhibitors for central nervous system disorders

Epigenetic Alterations in Alzheimer's Disease

Epigenetic Mark	Change	Reference
HDAC2	Increase	Graff et al. (2012)
HDAC6	Increase	Ding et al. (2008)
SIRT1	Decrease	Julien et al. (2009)
DNMT1	Decrease	Mastroeni et al. (2010)
MicroRNA-101	Decrease	Hebert et al. (2008)
MicroRNA-107	Decrease early in AD	Wang et al. (2008b)
BACE1-AS	Upregulated	Faghihi et al. (2008)
Methylation on APP gene	Hypomethylation	West et al. (1995)
Methylation on APP, PS1, tau	No change	Barrachina & Ferrer (2009)

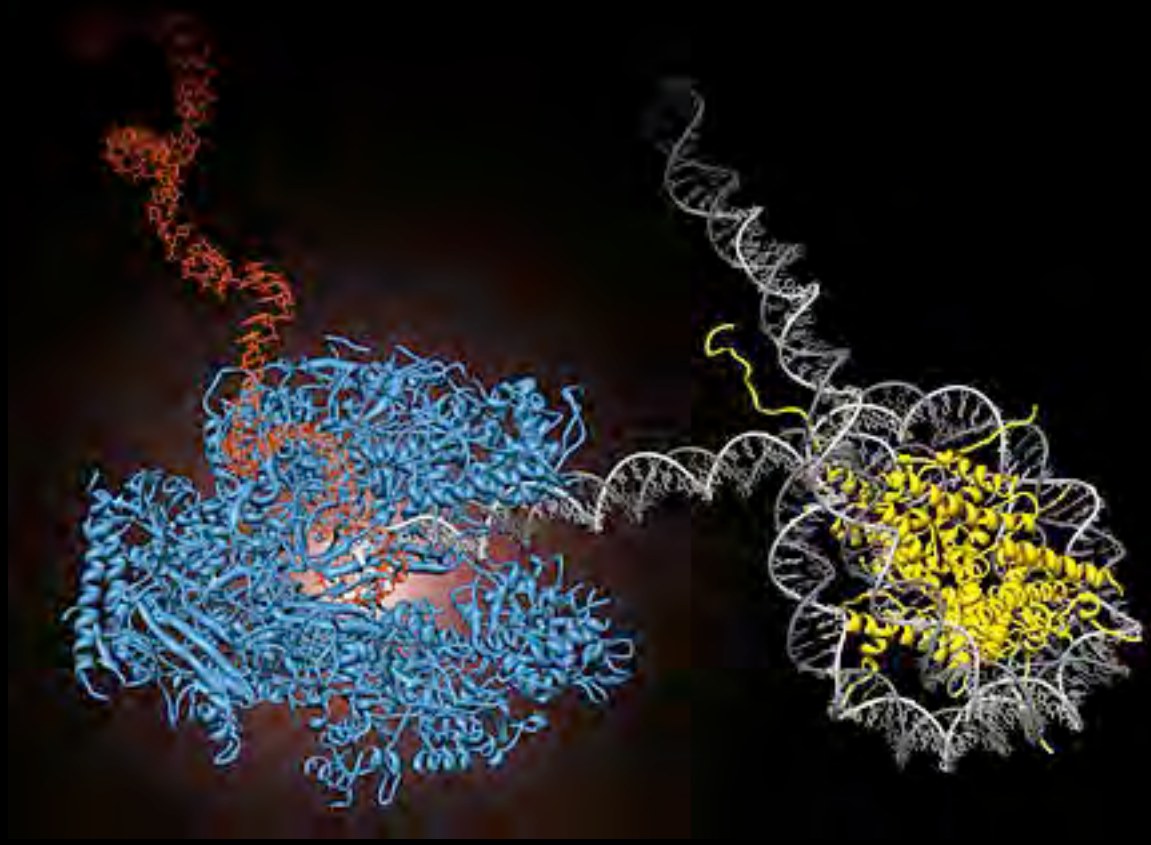
Pharmacology & Therapeutics
Volume 139, Issue 1, July 2013, Pages 41–50
Epigenetics: A novel therapeutic approach for the treatment of Alzheimer's disease
Lina Adwan[a],


TARGETING EPIGENETIC CHANGES IN CANCER
• WHAT DO WE NEED TO KNOW?
• HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
• HOW CAN THERAPEUTICS BE DEVELOPED?
• WHERE DO WE GO FROM HERE?
An example of a histone reader as a drug target

TARGETING EPIGENETIC CHANGES IN CANCER

- WHAT DO WE NEED TO KNOW?
- HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
- HOW CAN THERAPEUTICS BE DEVELOPED?
- WHERE DO WE GO FROM HERE?

WHY WE NEED EPIGENETIC REGULATORS FOR NORMAL GENE EXPRESSION



A RNA POLYMERASE II – NUCLEOSOME FACE-OFF

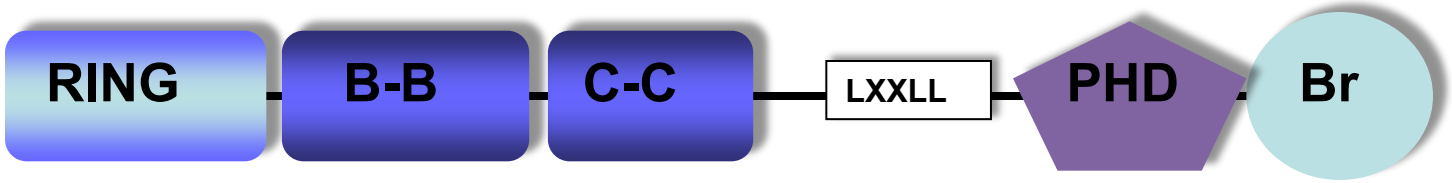
EPIGENETIC MODIFIERS – MAJOR HITS IN GLOBAL UNBIASED ANALYSES: EXPRESSION, MUTATIONS, etc.

Google images

TARGETING EPIGENETIC CHANGES IN BREAST CANCER

- WHAT DO WE NEED TO KNOW?
  - Expression correlates
  - Functional impact
- HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
- HOW CAN THERAPEUTICS BE DEVELOPED?
- WHERE DO WE GO FROM HERE?

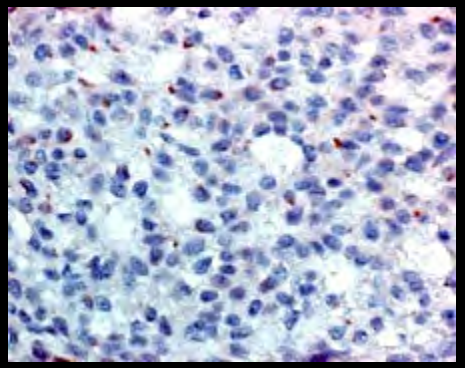
TRIM24: Transcription intermediary 1 alpha
Tripartite motif family member 24



- Over expressed in several different cancers
- Papillary thyroid carcinomas: RET-TRIM24 fusion
- Myeloid leukemias: B-RAF-TRIM24 fusion

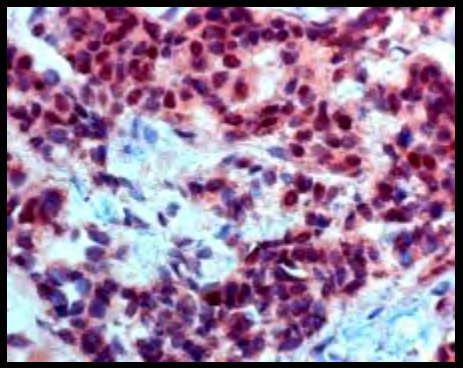
Patient survival: Breast cancer and TRIM24 levels

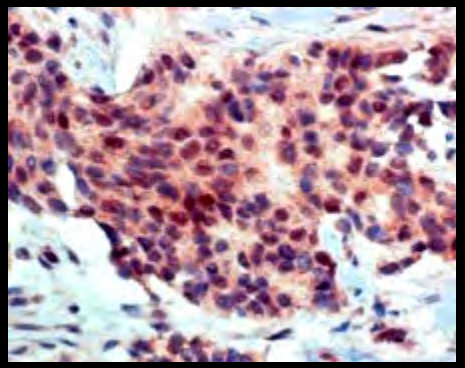
Trim 24 antibody - IHC



N –
Undetectable
Or low expression

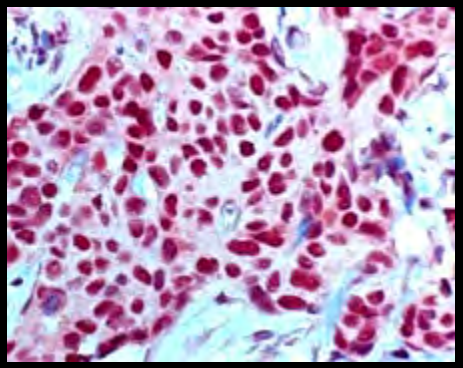
N ++
Hi expression
Cytoplasmic &
Nuclear





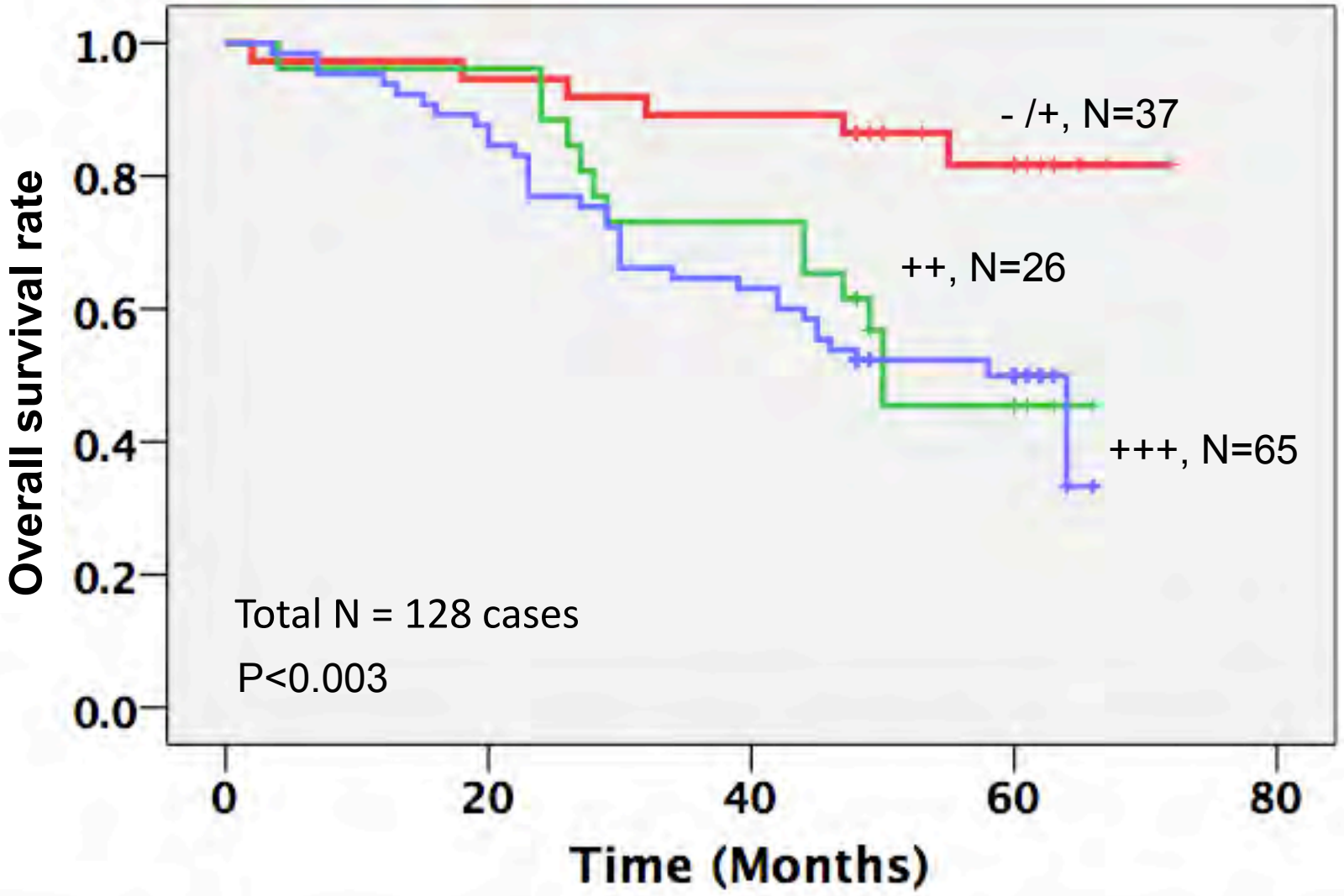
N +
Low expression
In few foci
Cytoplasmic &
Nuclear

N +++
Hi expression
Nuclear



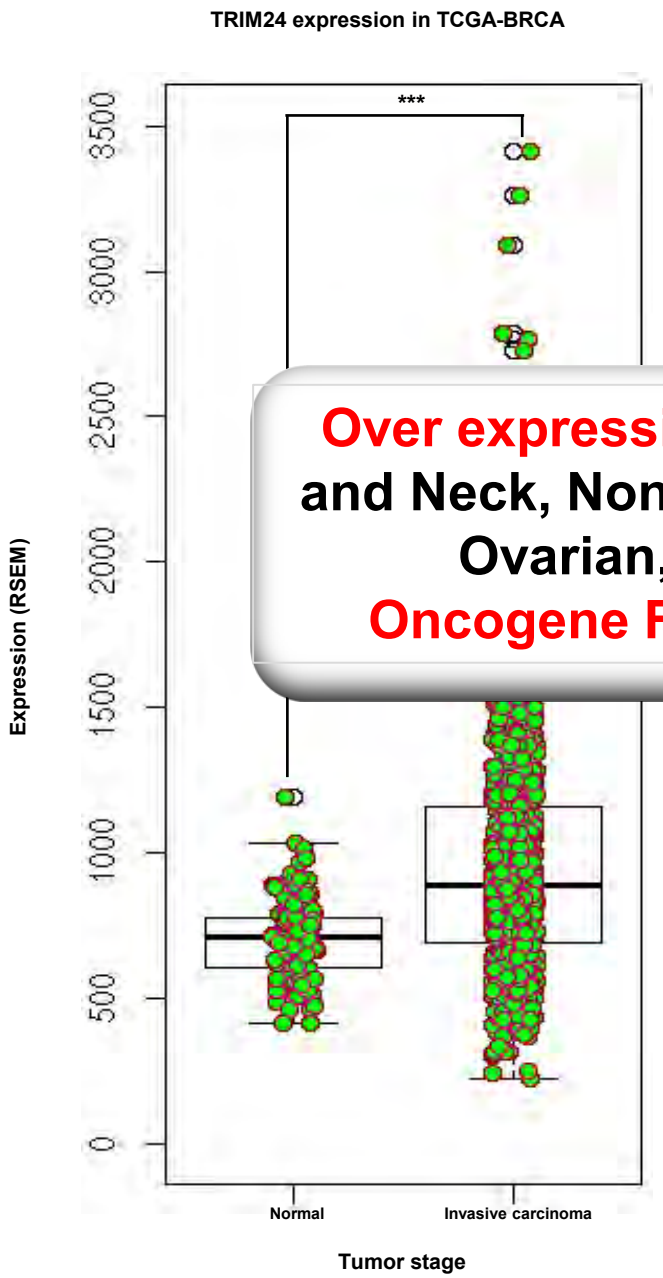
A collaboration with Drs. Weiya Xia and Mien-Chie Hung
UT MD Anderson Cancer Center

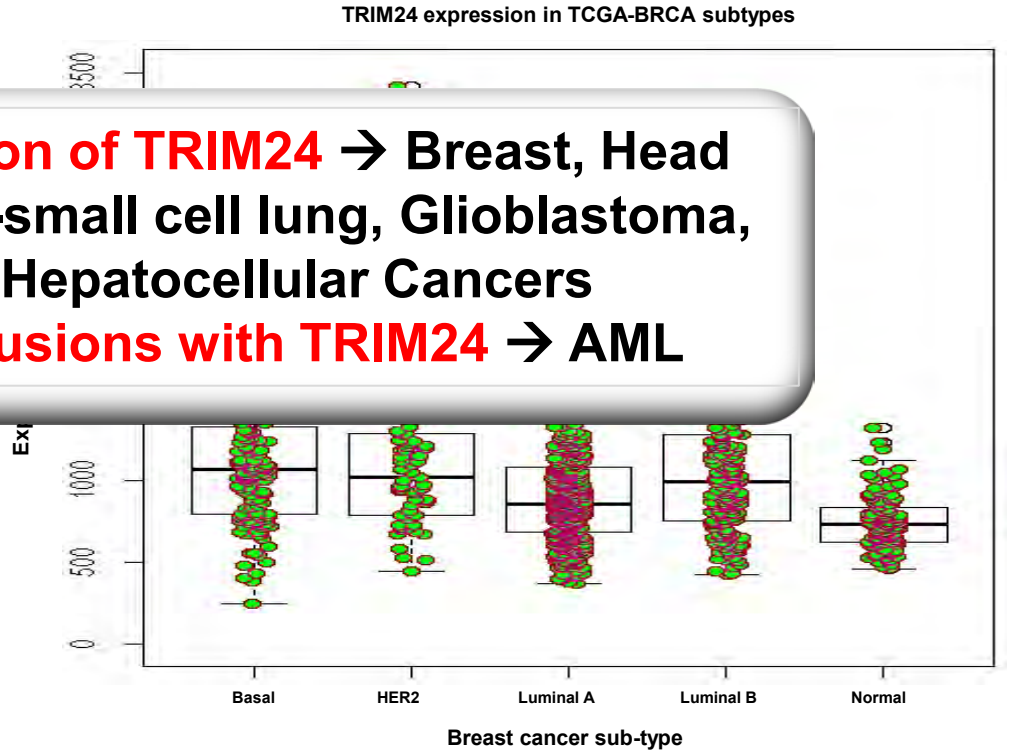
Patient survival: Breast cancer and TRIM24 levels



A collaboration with Drs. Weiya Xia and Mien-Chie Hung

TRIM24 EXPRESSION: TCGA data

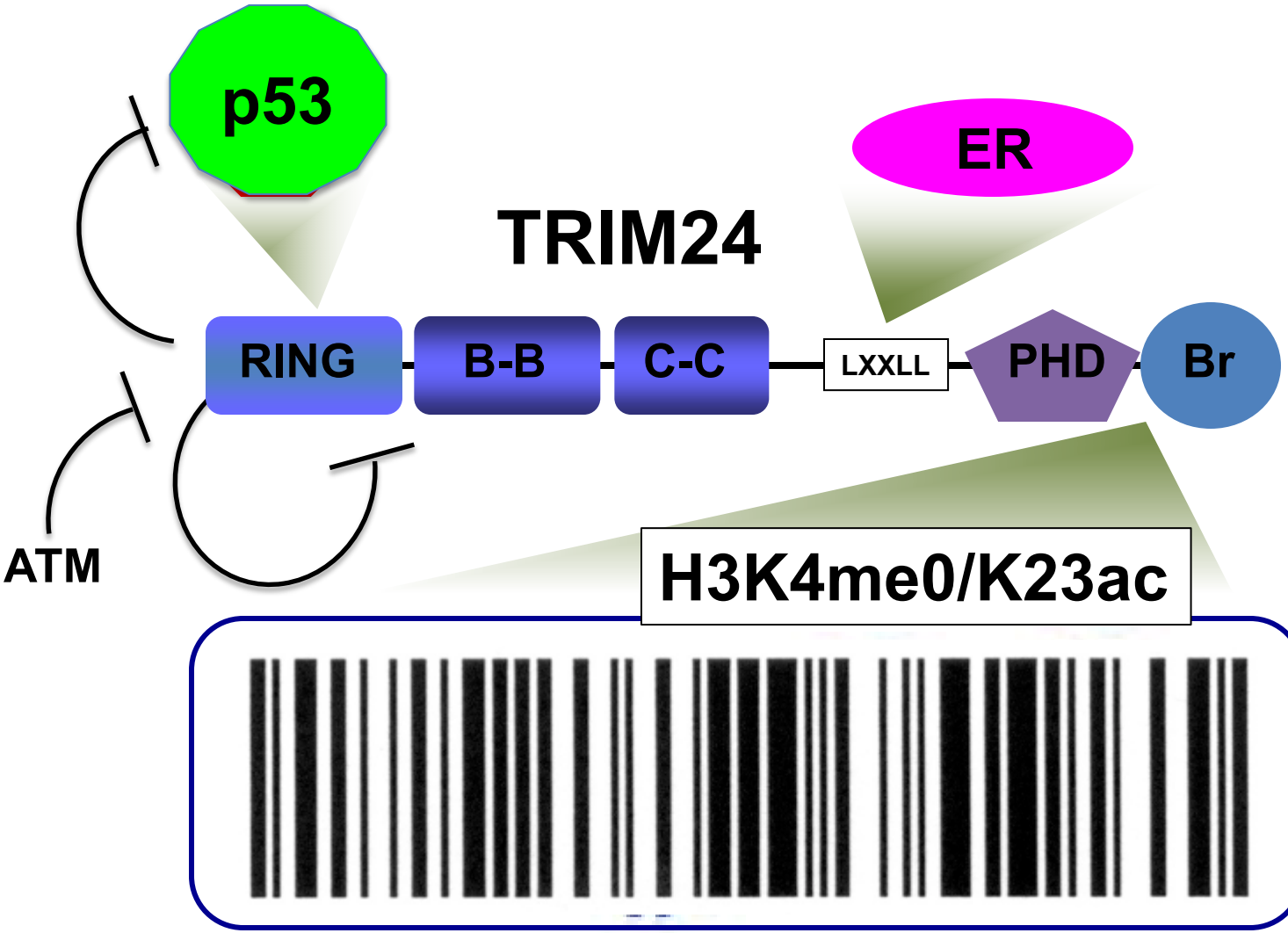




Over expression of TRIM24 → Breast, Head and Neck, Non-small cell lung, Glioblastoma, Ovarian, Hepatocellular Cancers

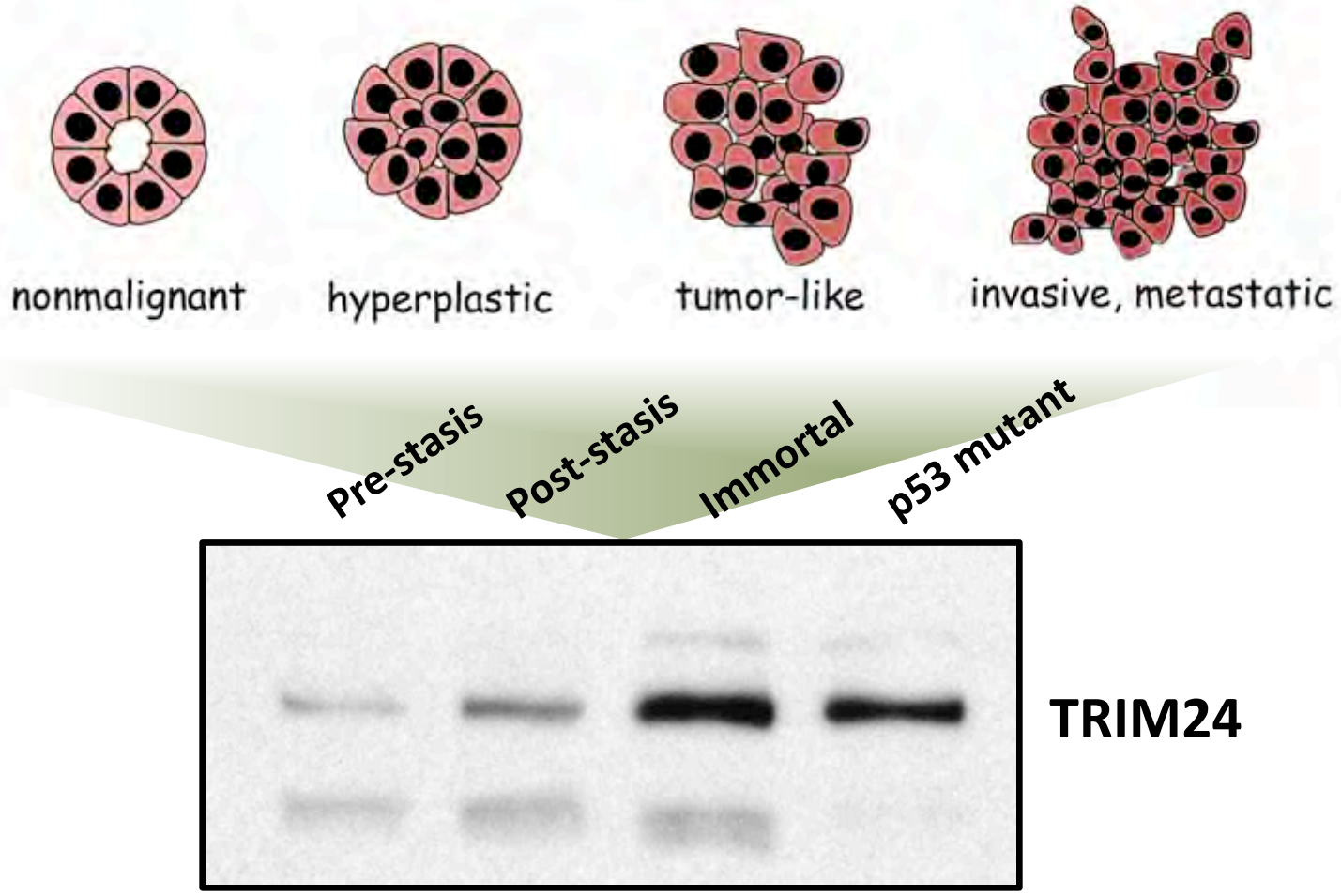
Oncogene Fusions with TRIM24 → AML

Multi-domain and Multi-functional: TRIM24



PNAS 2009, Nature 2010, MCB 2014

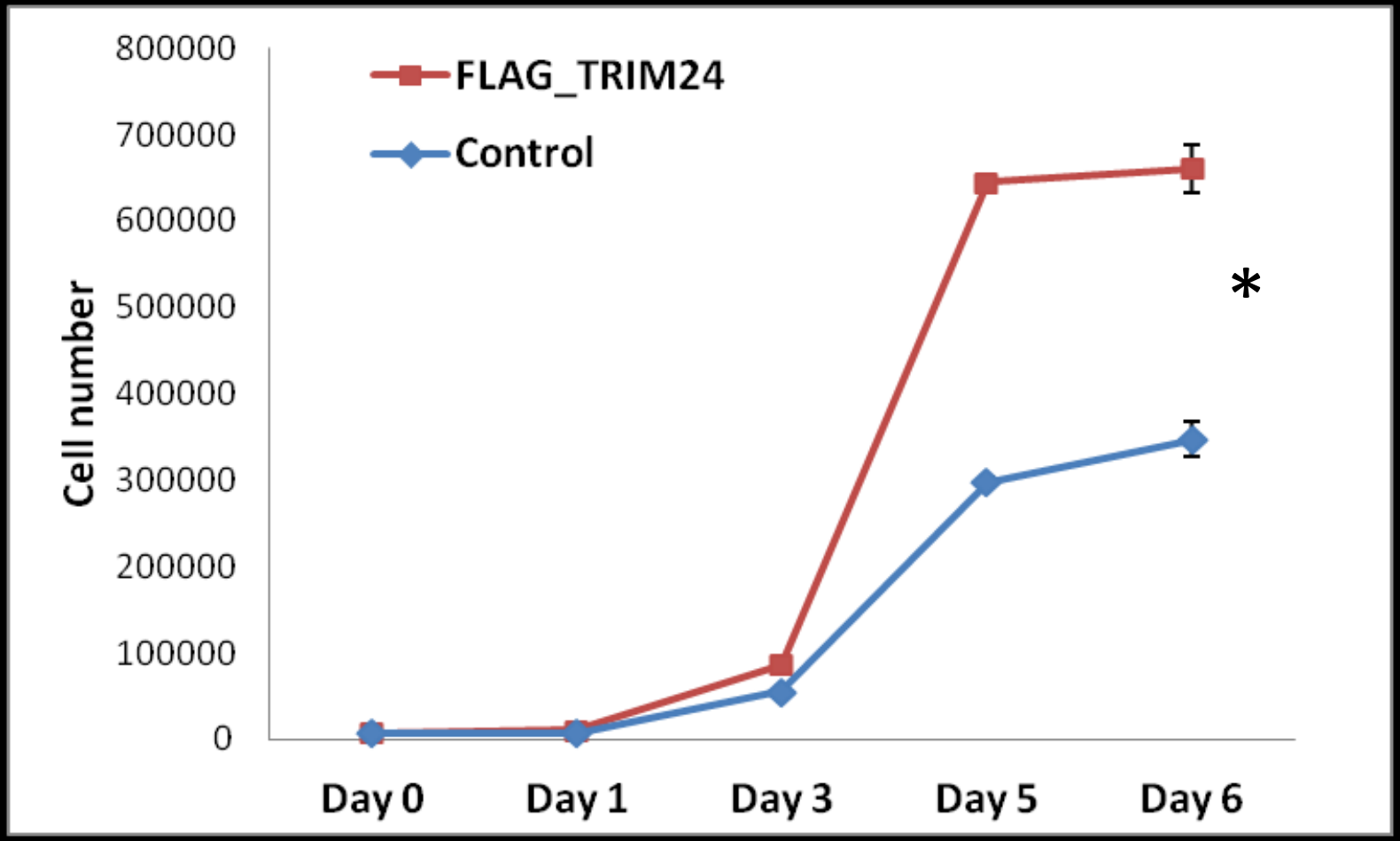
TUMOR PROGRESSION AND TRIM24 EXPRESSION: HUMAN MAMMARY EPITHELIAL CELLS



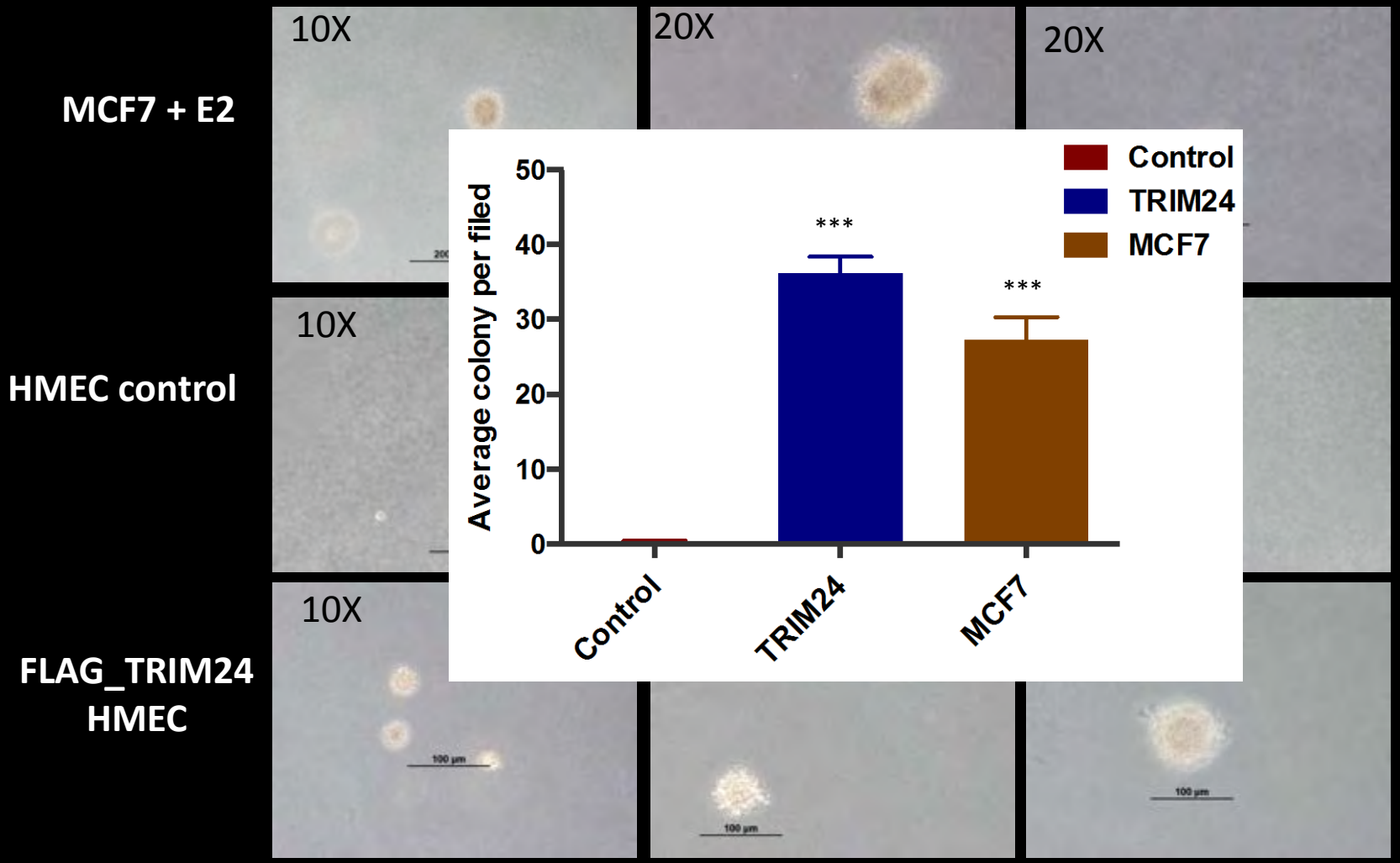
Kaushik Thakkar
Thushangi Patharaja

Martha Stampfer, Berkeley Laboratories

TRIM24 stably over-expressing HMECs show increased growth rate

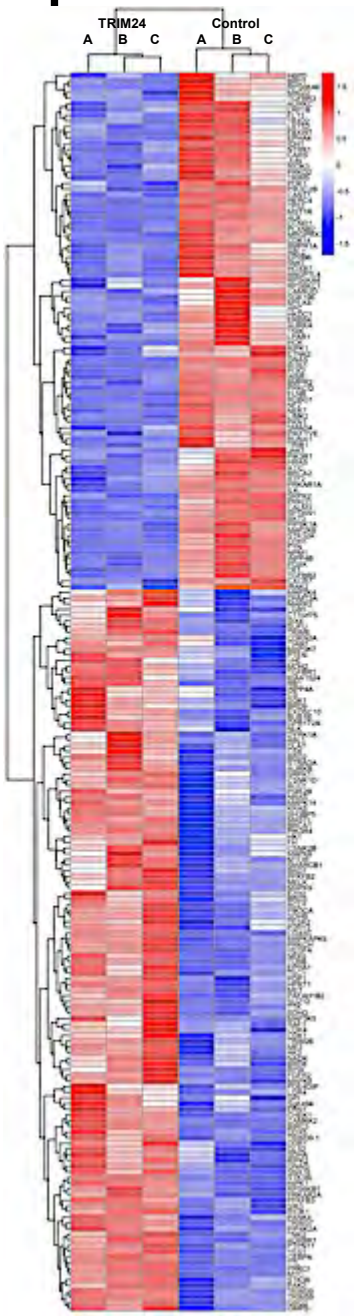


Transformation assay : TRIM24 overexpressing HMECs show anchorage independent growth



7 days

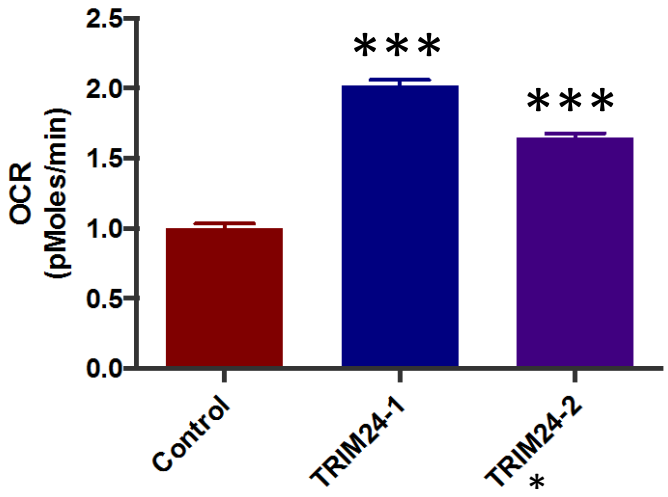
Multiple cancer-associated pathways are deregulated in TRIM24-HMECs



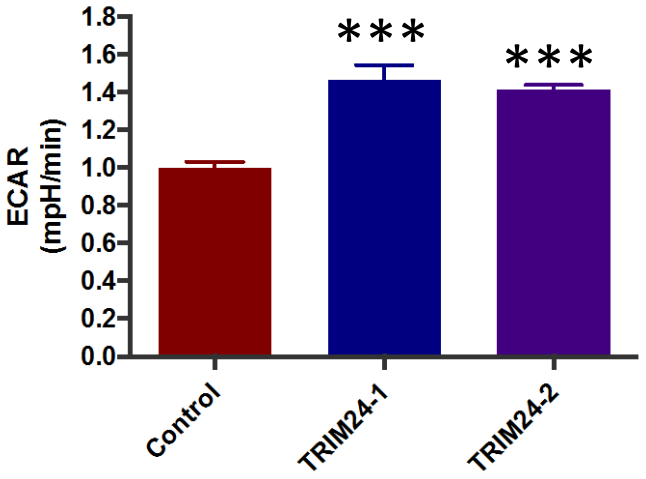
Pathways and Functional categories	p value
Aerobic respiration	1.70E-11
Citrate cycle (TCA cycle)	6.78E-11
ErbB signaling pathway	1.42E-09
Regulation of cell cycle	2.55E-07
Regulation of apoptosis	2.97E-06
MAPK signaling pathway	3.29E-06
Insulin signaling pathway	3.88E-06
Adherens junction	1.01E-04
Glucose metabolic process	1.90E-04

TRIM24-HMECs have higher basal metabolic rate

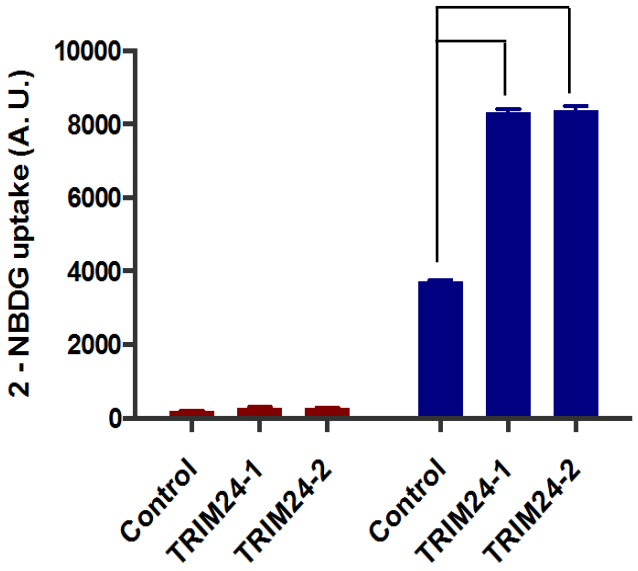
Oxygen consumption Rate - OCR (measure of TCA cycle)



Extracellular Acidification Rate - ECAR (measure of Glycolysis)



***p value < 0.001



No treatment

2 - NBDG (2h)

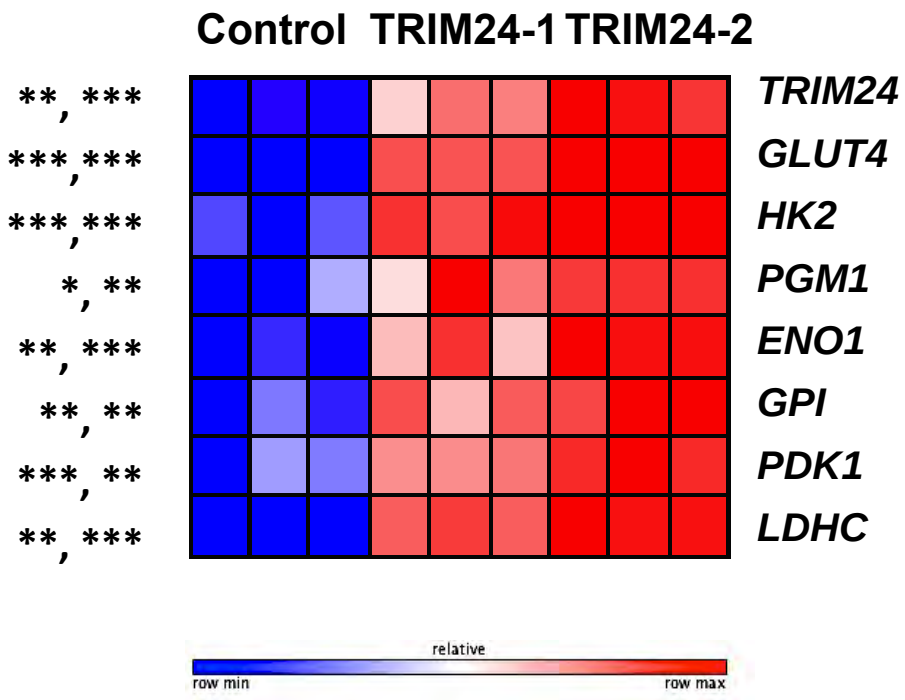
ahorseBioscience

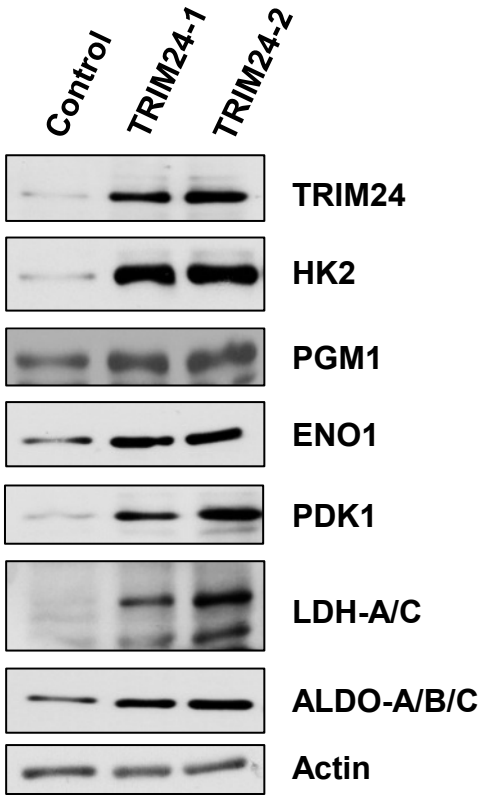
2- NBDG uptake assay (non-metabolizable Fluorescent Glucose)

Kaushik Thakkar

*p value < 0.001

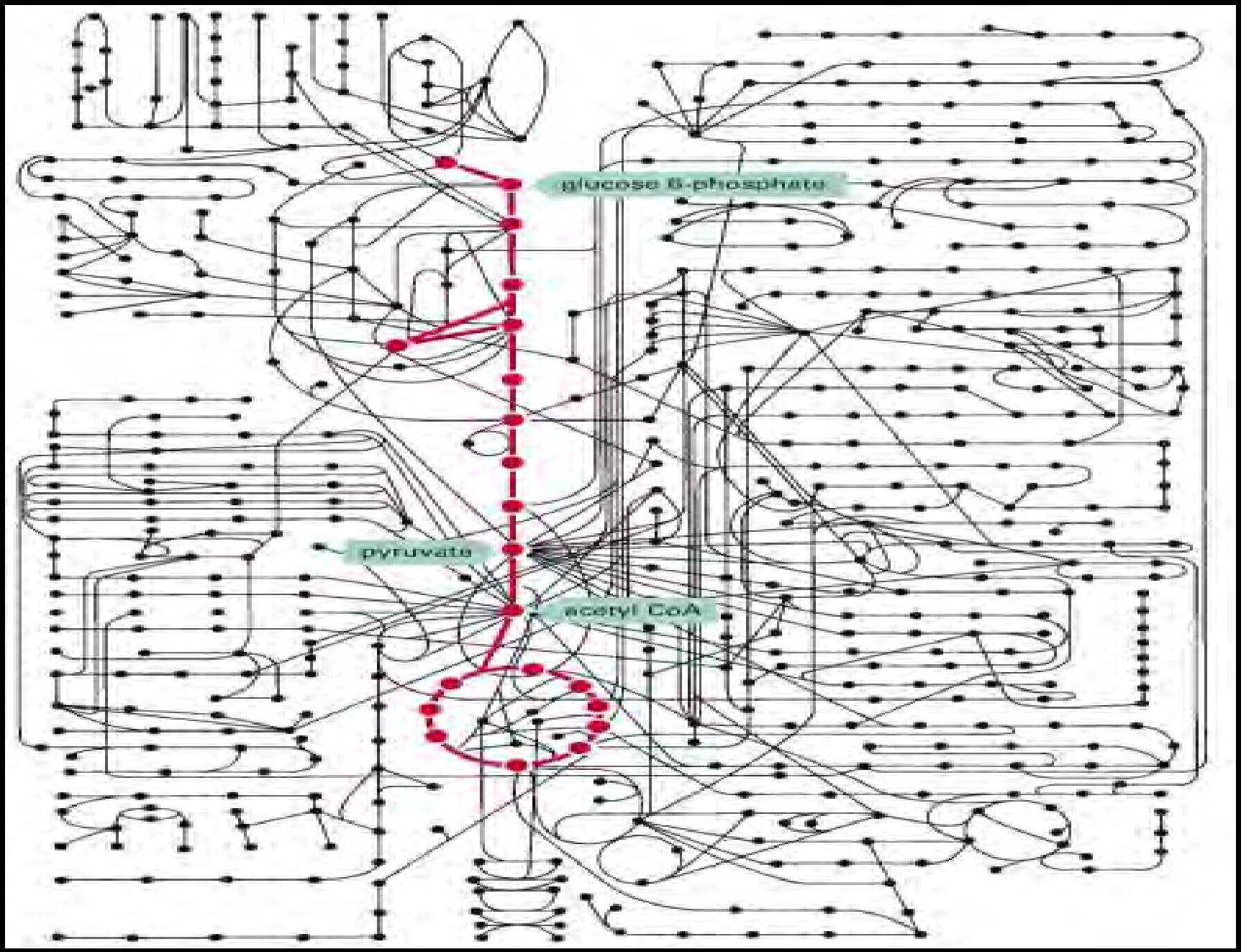
TRIM24 over-expression leads to an upregulation of glycolytic pathway





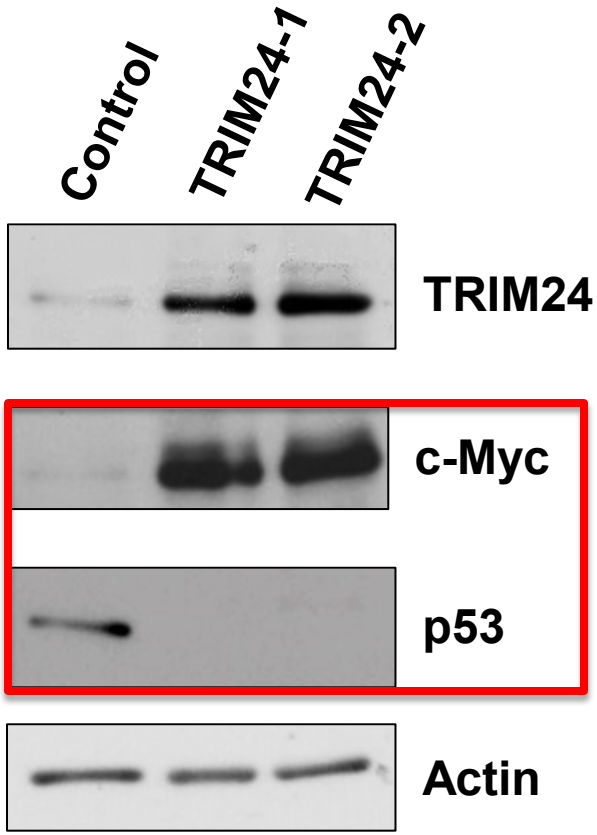
***p value < 0.001
**p value < 0.01
*p value < 0.05

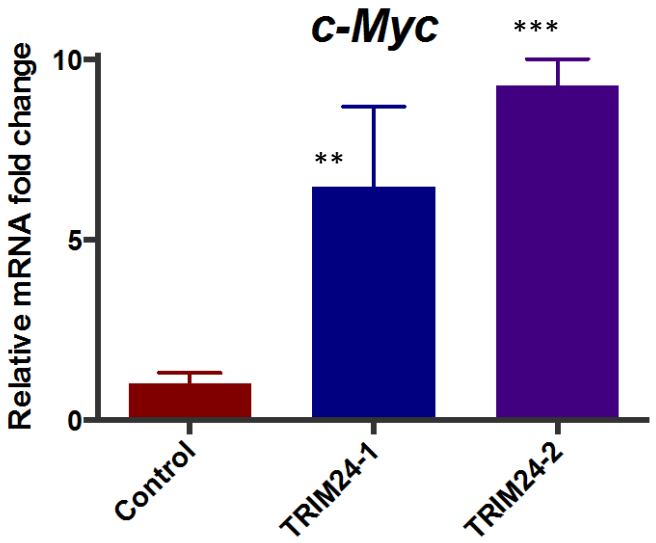
METABOLISM



darwins-god.blogspot.com/2011/07/glycolysis-and-citric-acid-cycle.html

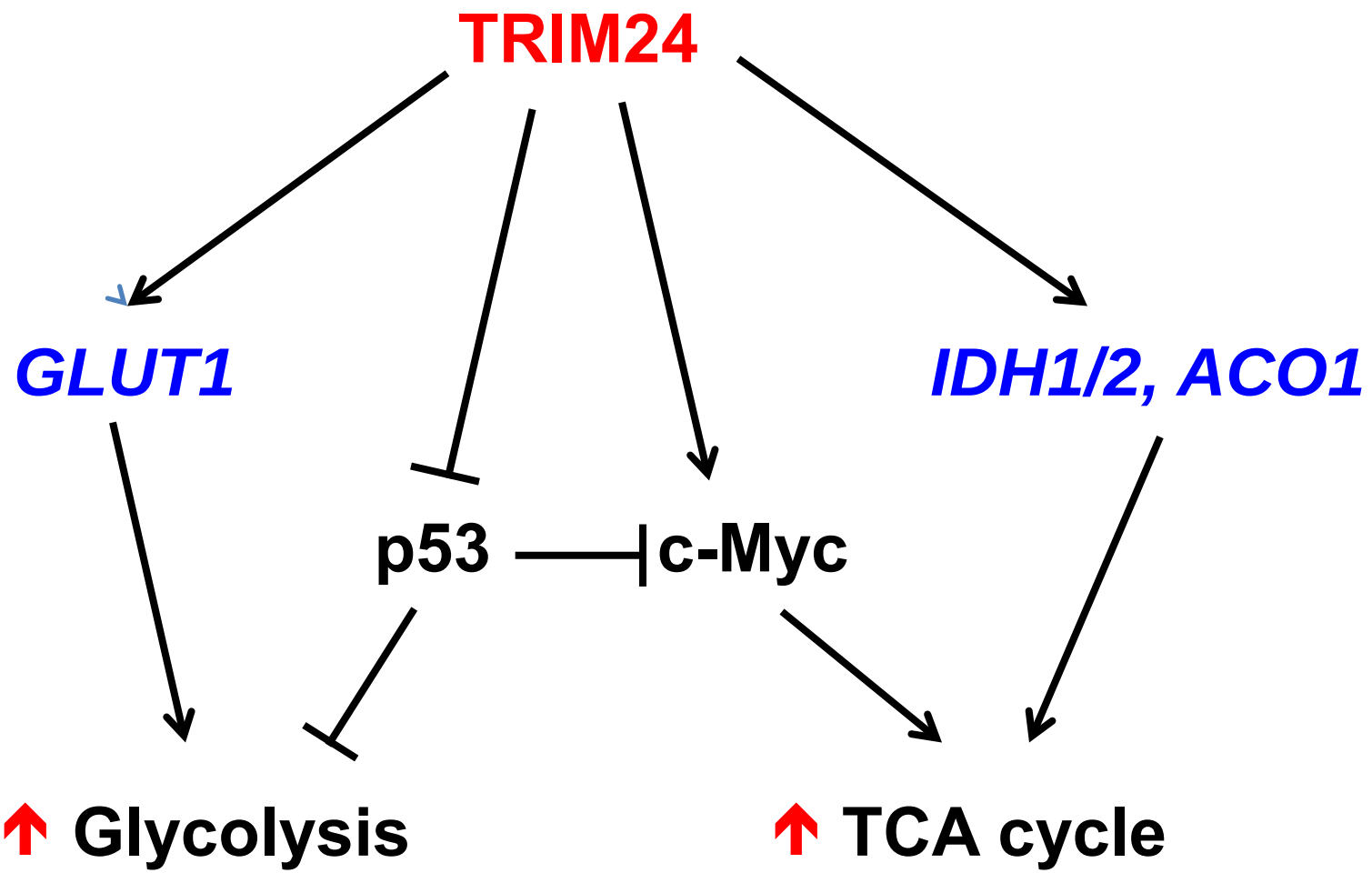
TRIM24 causes deregulation of two key players in metabolism





***p value < 0.001
**p value < 0.01
*p value < 0.05

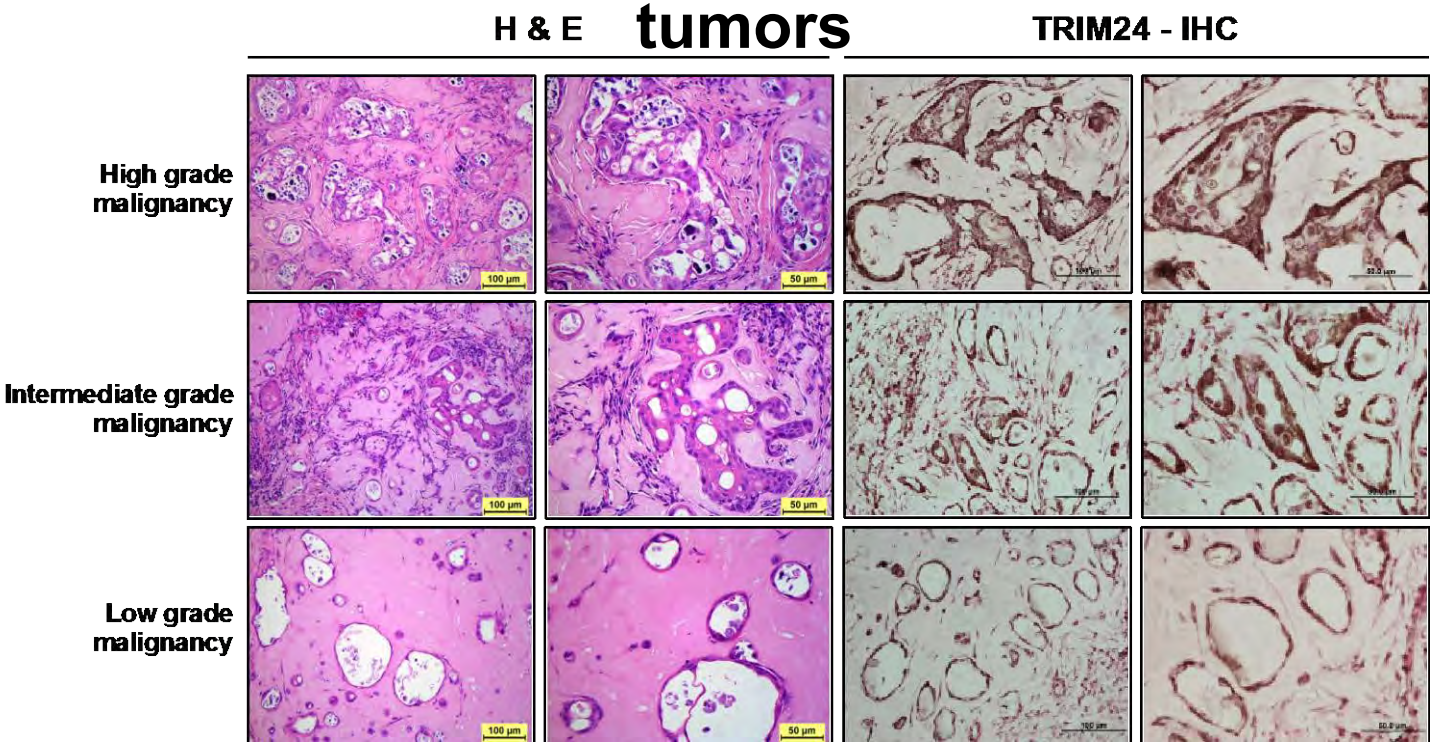
Model for TRIM24 regulation of metabolism

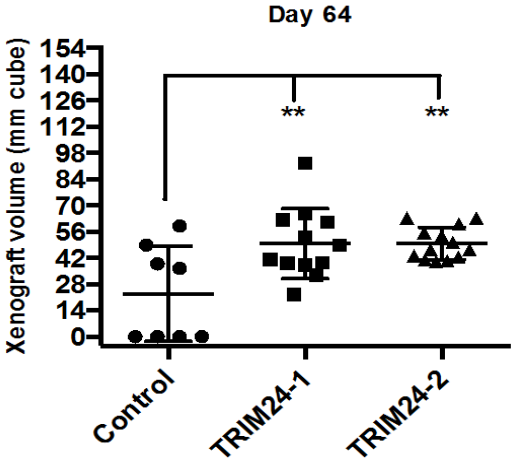


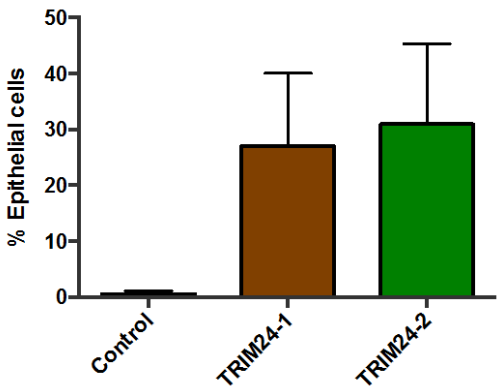
Conserved functions whether p53/ER/Myc/HER2 + or -

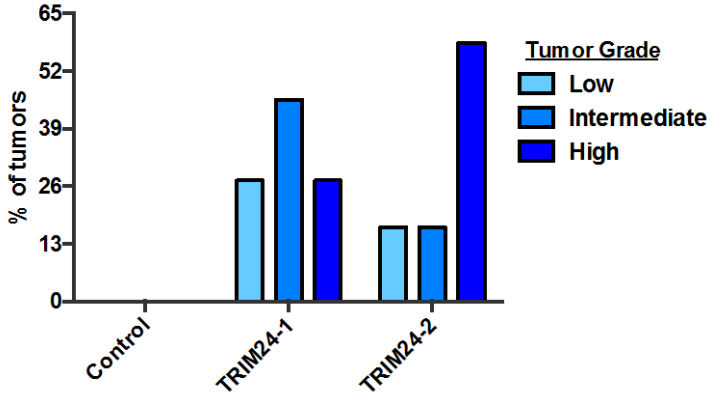
OVER EXPRESSION VERSUS LOSS OF AN ONCOGENE

Xenografts: TRIM24-HMECs form high-grade epithelial tumors



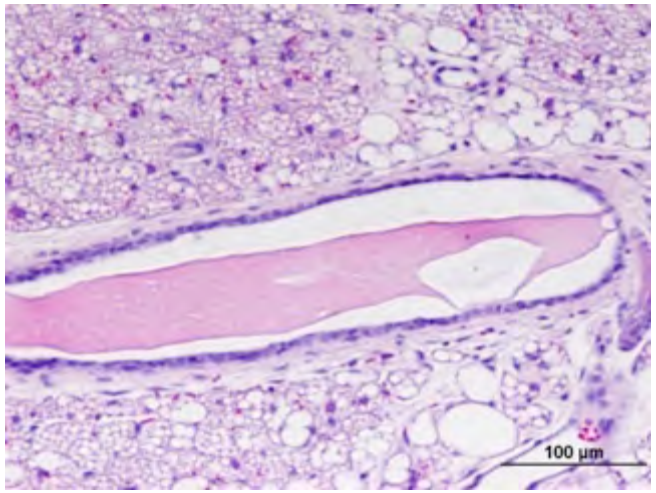


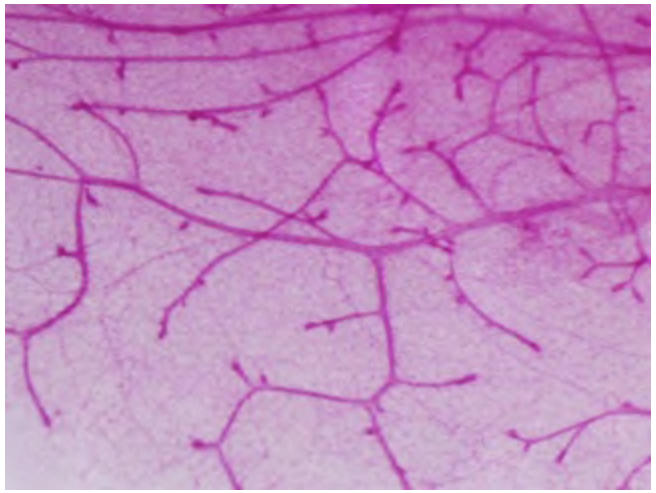




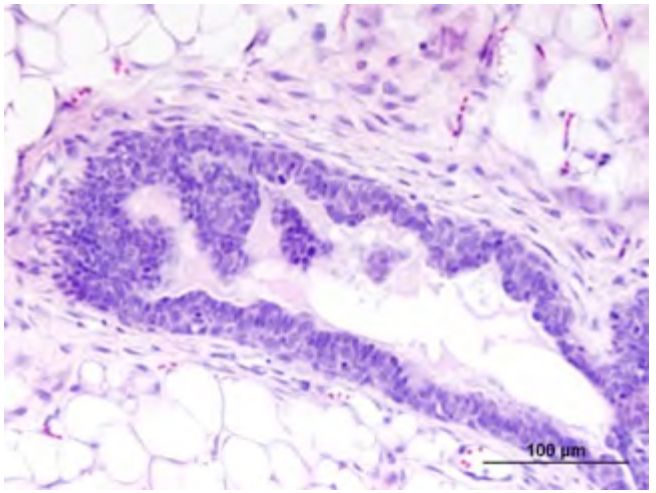
Trim24 over-expressing mice exhibit hyperplasia and increased branching in the mammary gland at 2 months

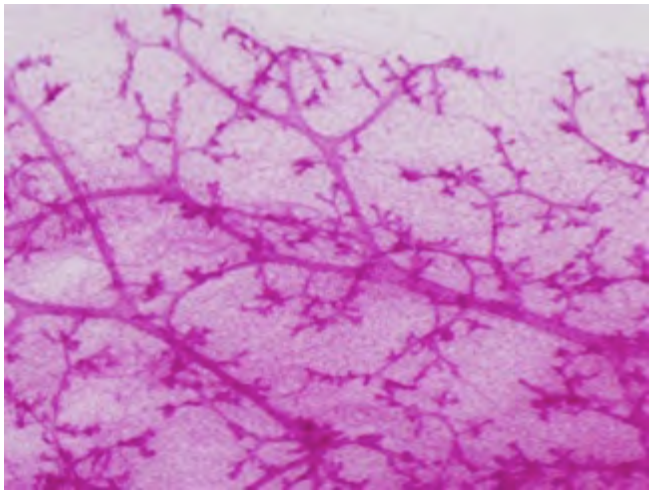
WT





MMTV-Cre$^{Tg/+}$; Flag-TRIM24 $^{Tg/+}$





Aundrietta Duncan

Shiming Jiang

What if we had twice as much Trim24?

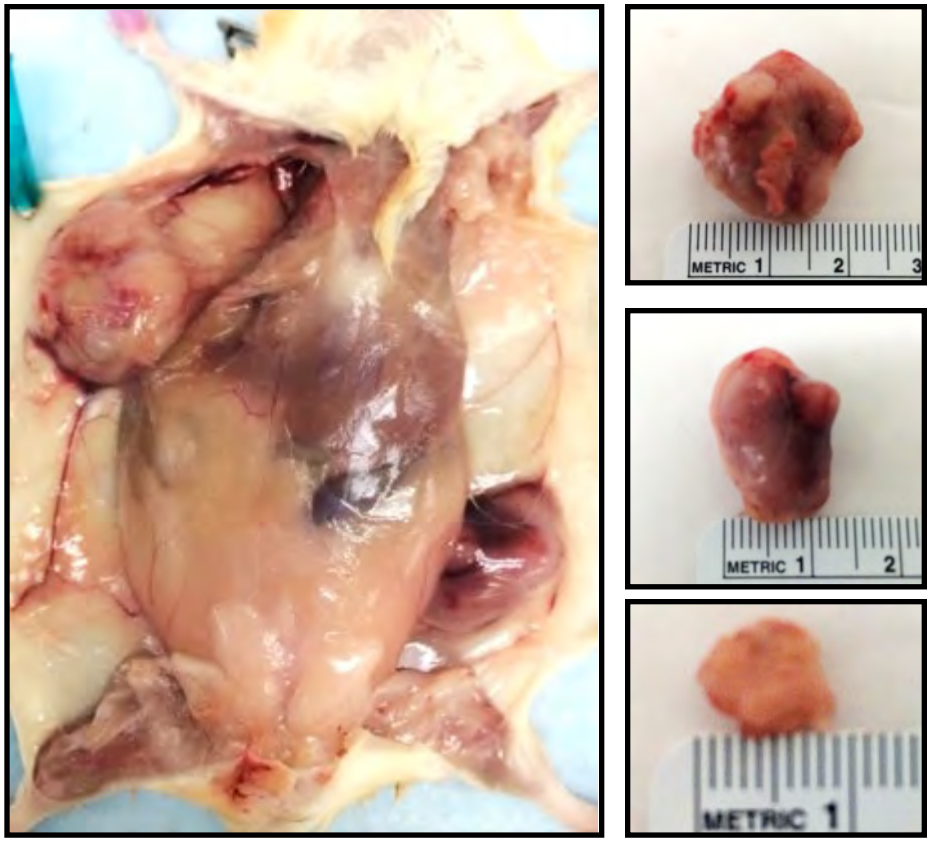
Flag-Trim24^{Tg};
MMTV-Cre^{Tg};

X

Flag-Trim24^{Tg};
MMTV-Cre^{Tg};

;Flag-Trim24$^{Tg/Tg}$;MMTV-Cre$^{Tg/Tg}$

Higher Doses of TRIM24 Lead to Mammary Tumorigenesis



8 mos

What if we had less p53?

Flag-Trim24^{Tg};
MMTV-Cre^{Tg}

X

p53$^{-/-}$

p53$^{+/-}$;Flag-Trim24^{Tg};MMTV-Cre^{Tg}

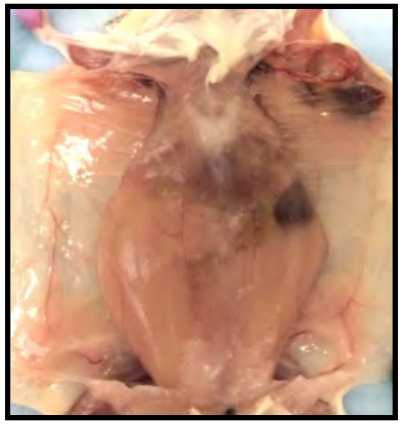
%

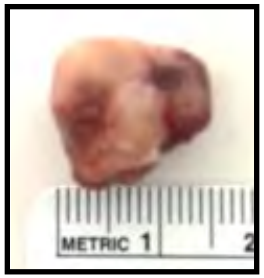
p53 depletion coupled with *Trim24* over-expression drives tumorigenesis by 8 months

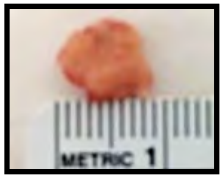
$p53^{+/-}$;FlagTrim24^{Tg};MMTV-Cre^{Tg}

11 months - pregnant

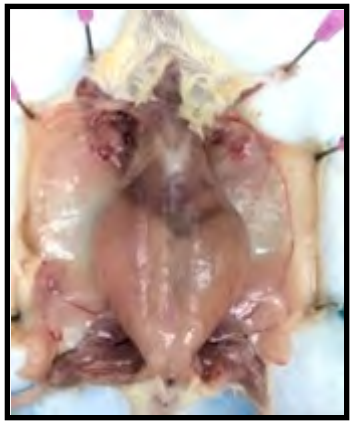
8 months - virgin

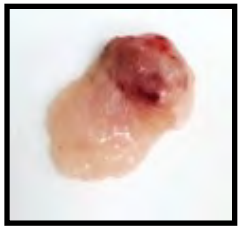


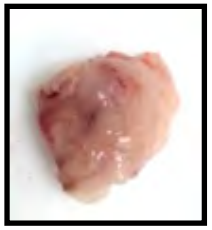




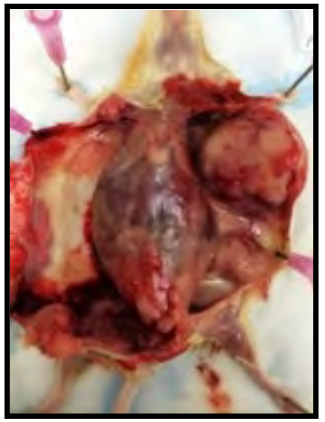
8 months - virgin

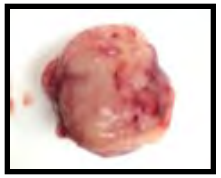


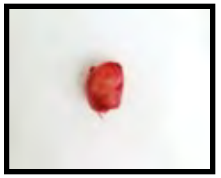




10 months - virgin

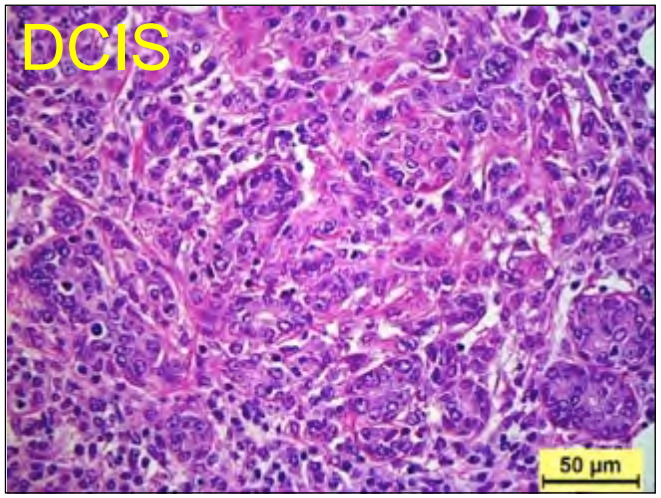


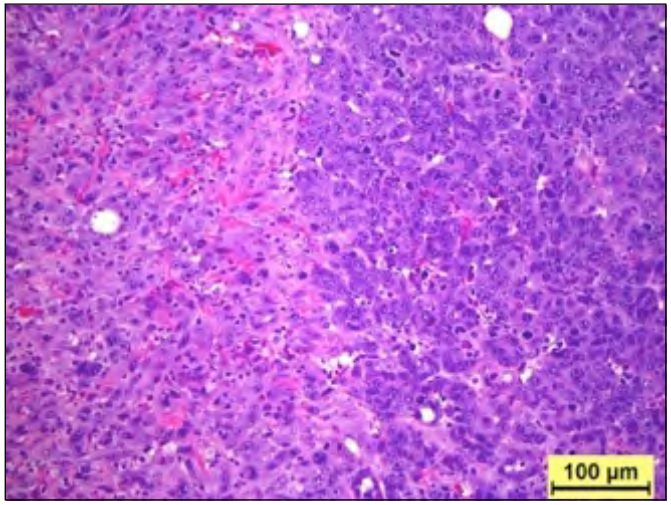




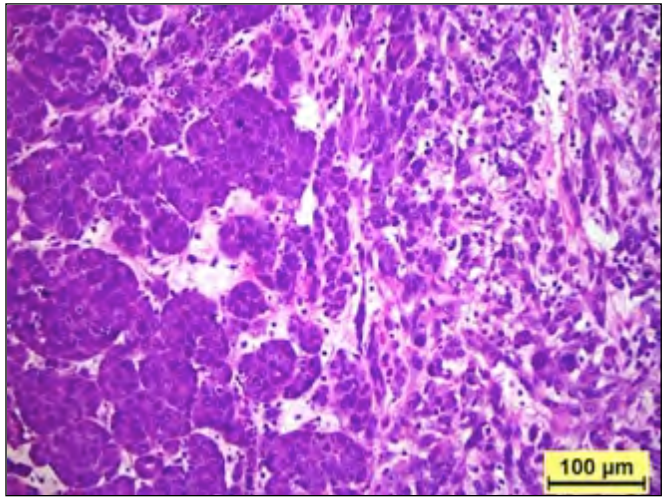
p53 depletion coupled with *Trim24* over-expression drives tumorigenesis by 8 months

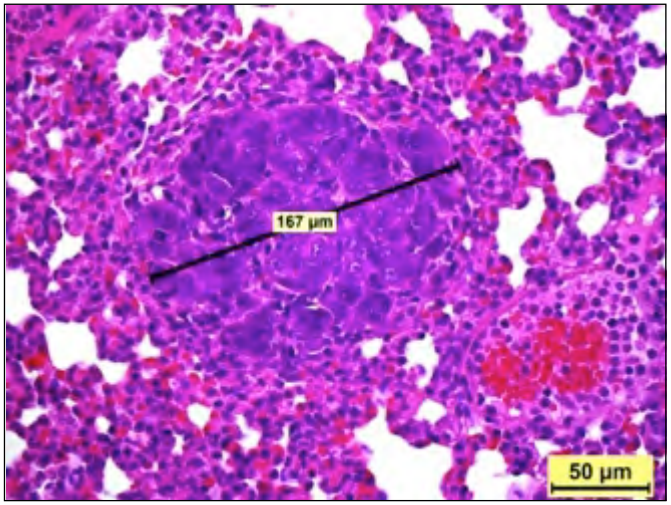
$p53^{+/-}$;$FlagTrim24^{Tg}$;$MMTV\text{-}Cre^{Tg}$





Carcinoma





Lung Metastasis

TARGETING EPIGENETIC CHANGES IN BREAST CANCER

- WHAT DO WE NEED TO KNOW?
  - Expression correlates
  - Function
- HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
  - Specific pathway interactions
  - Effective combinatorial approaches
- HOW CAN THERAPEUTICS BE DEVELOPED?
- WHERE DO WE GO FROM HERE?

QUESTIONS OF SELECTIVITY – IS IT POSSIBLE?

ARTICLE

doi:10.1038/nature09504

LETTER

doi:10.1038/nature10334

Cell

Cell

www.impactjournals.com/oncotarget/ Oncotarget, December, Vol.3, No 12

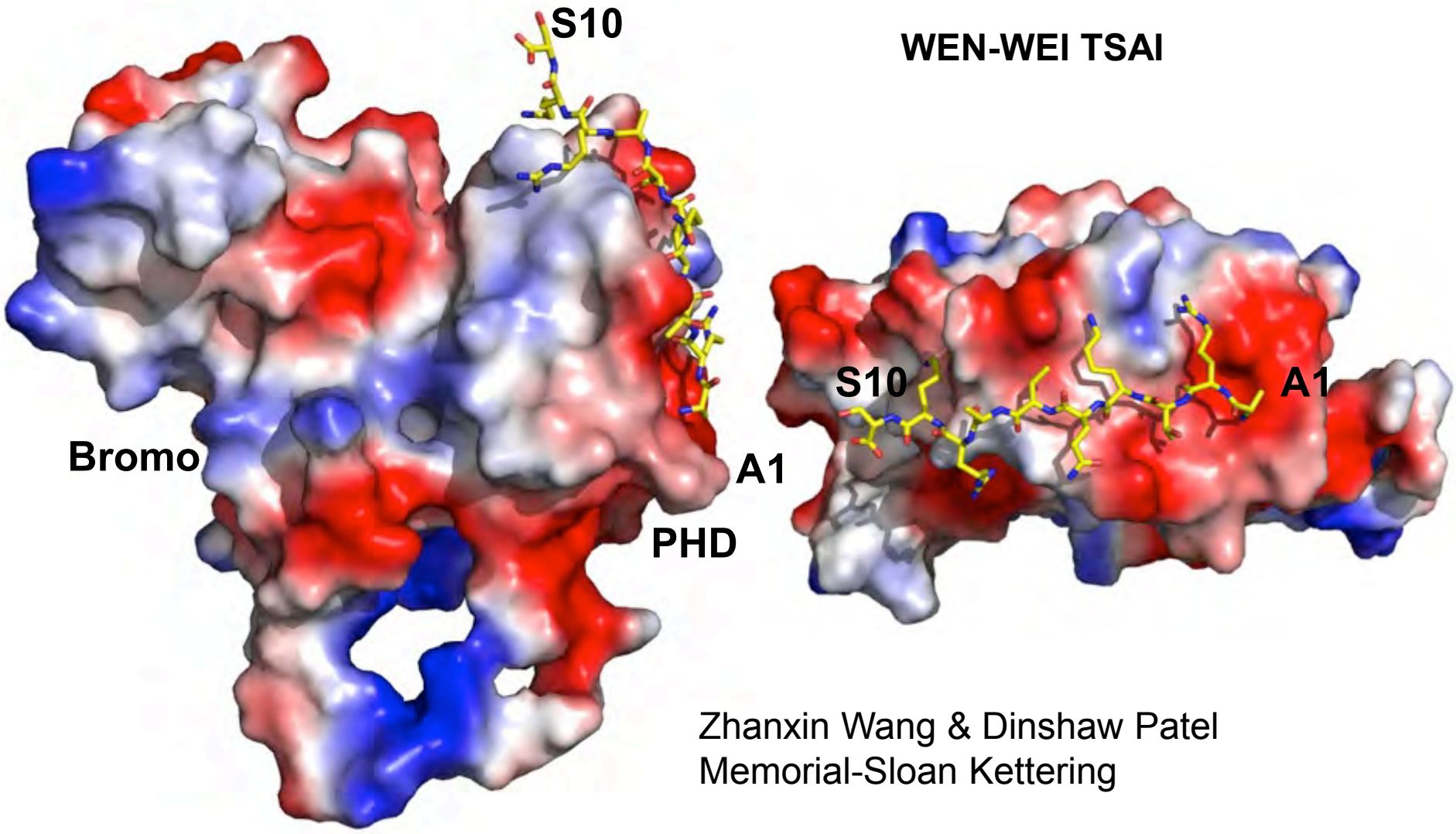
Small-molecule inhibition of BRD4 as a new potent approach to eliminate leukemic stem- and progenitor cells in acute myeloid leukemia (AML)

Harald Herrmann[1], Katharina Blatt[2], Junwei Shi[3], Karoline V. Gleixner[2], Sabine Cerny-Reiterer[1], Leonhard Müllauer[4], Christopher R. Vakoc[3], Wolfgang R. Sperr[1,2], Hans-Peter Horny[6], James E. Bradner[5], Johannes Zuber[3,7], Peter Valent[1,2]

TARGETING EPIGENETIC CHANGES IN BREAST CANCER

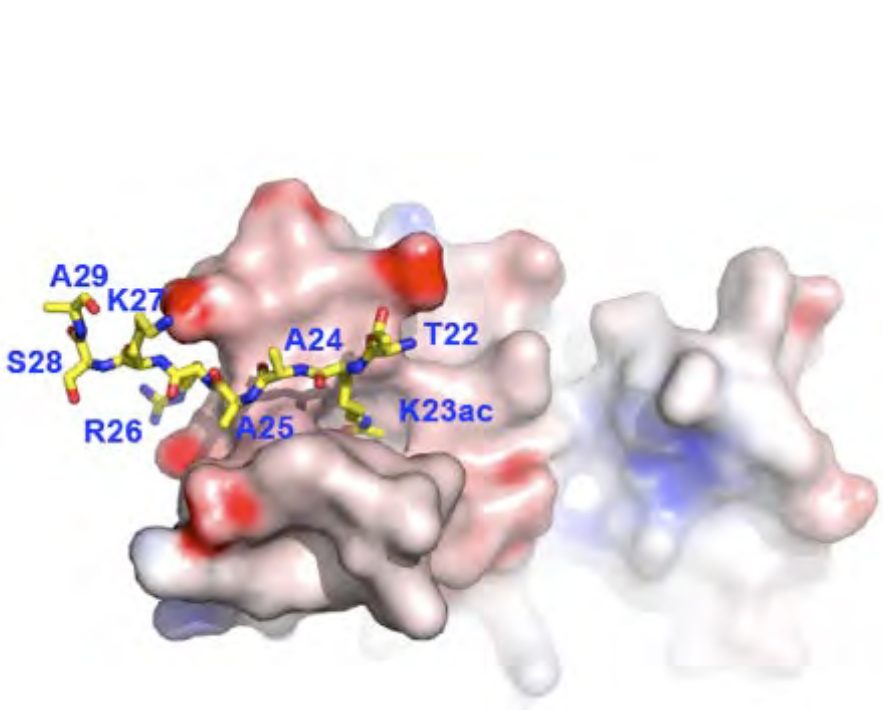
- WHAT DO WE NEED TO KNOW?
  - Expression correlates
  - Function
- HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
  - Global substrate
  - Specific pathway interactions
- HOW CAN THERAPEUTICS BE DEVELOPED?
  - Structure and biophysics
- WHERE DO WE GO FROM HERE?

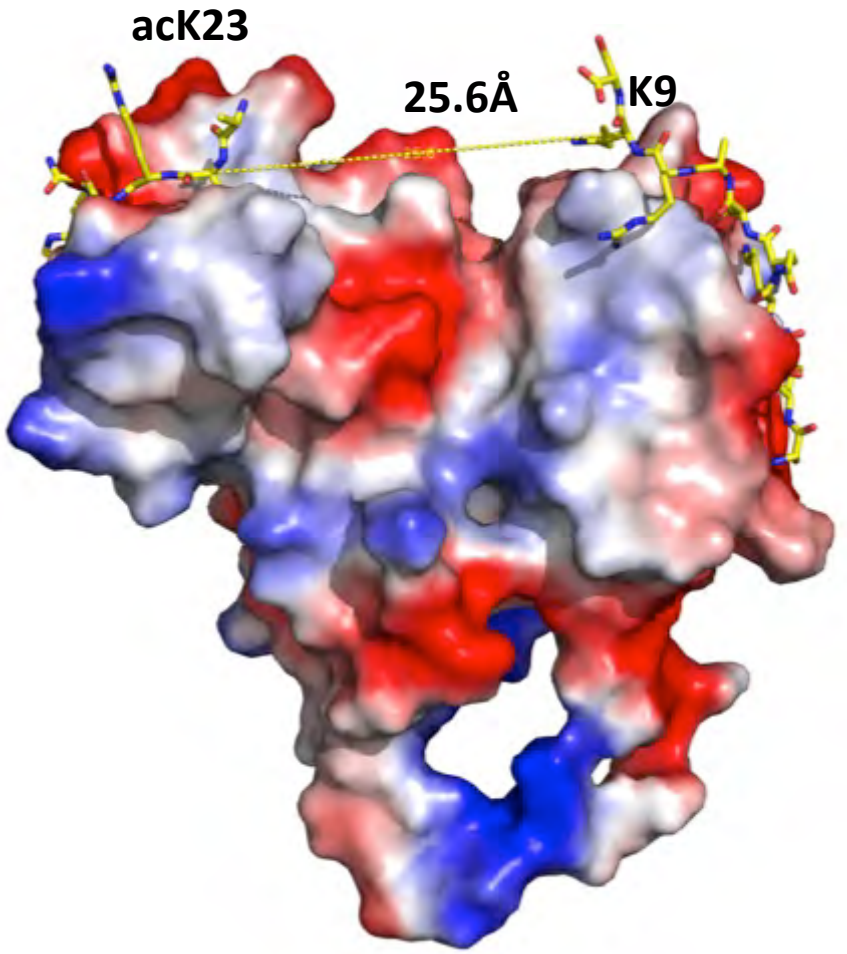
Surface view of Trim24-PHD/Bromo with H3(1-10) peptide



Zhanxin Wang & Dinshaw Patel
Memorial-Sloan Kettering

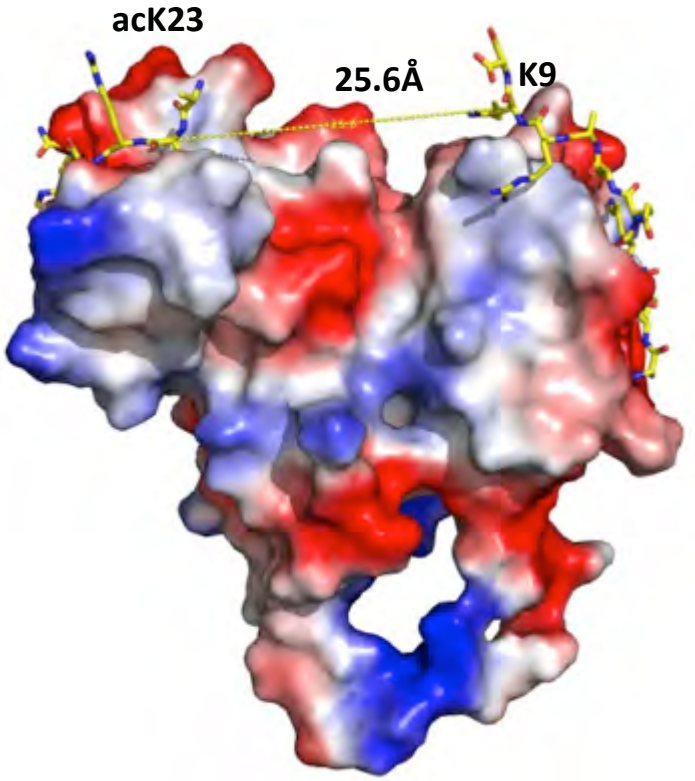
Surface view of TRIM24-PHD/Bromo with H3K23ac(14-19)





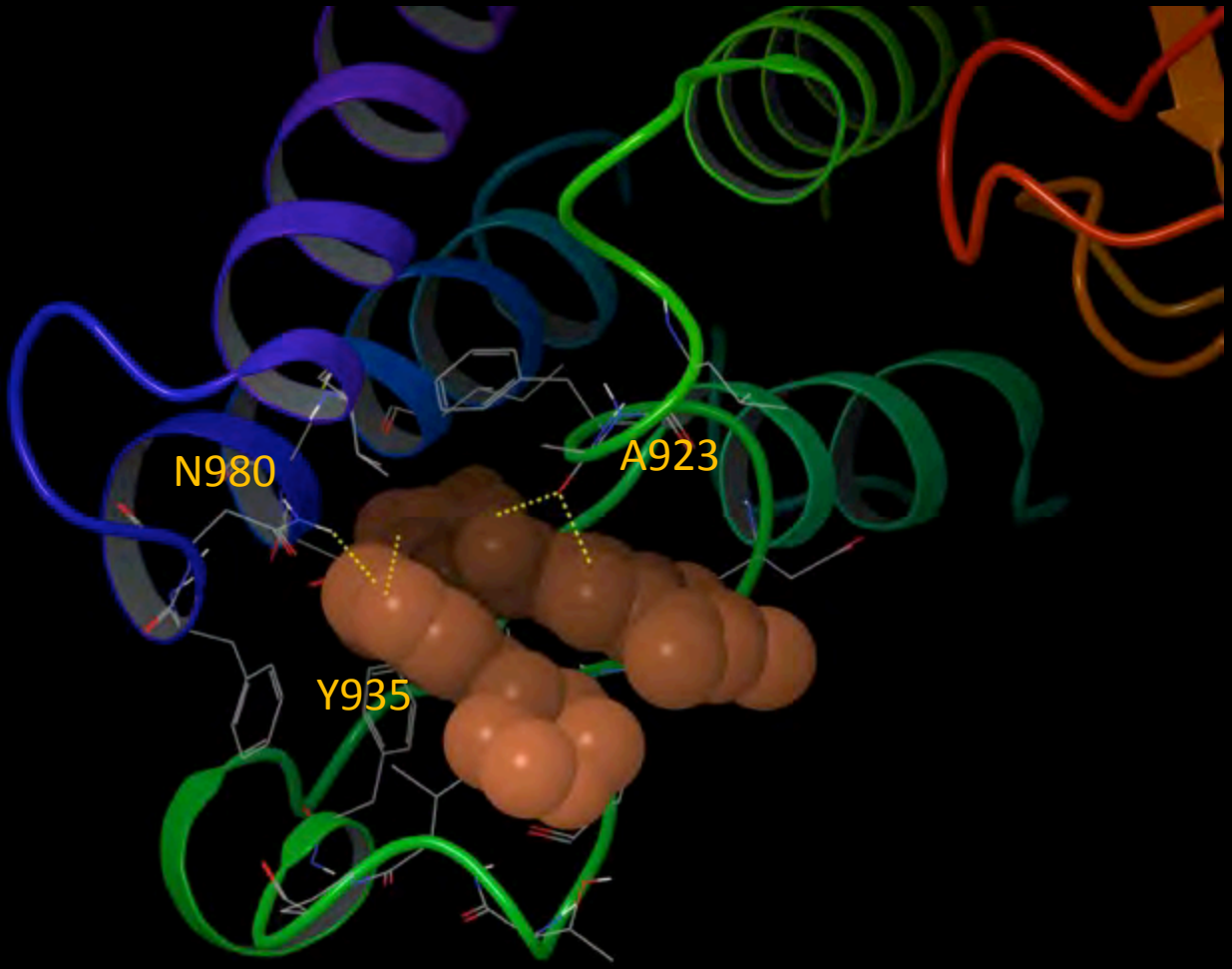
Binding parameters of TRIM24-PHD/Bromo with H3 peptides: Acetylation dominates Methylation

Peptide	Protein Sample	K_D (μM)
H3(1-15)K4	PHD-Bromo (WT)	8.6 ± 0.4
H3(1-15)K4me2	PHD-Bromo (WT)	198 ± 26
H3(1-15)K4me3	PHD-Bromo (WT)	> 400
H3(13-32)K23ac	PHD-Bromo (WT)	8.8 ± 0.1
H3(13-32)K27ac	PHD-Bromo (WT)	206 ± 44
H3(1-20)K9ac	Bromo	232 ± 33
H3(1-19)K14ac	Bromo	229 ± 32
H3(1-33)K4K23ac	PHD-Bromo (WT)	0.070± 0.010
H3(1-33)K4me3K23ac	PHD-Bromo (WT)	0.34 ± 0.04
H3(1-33)K4	PHD-Bromo (WT)	1.4 ± 0.3

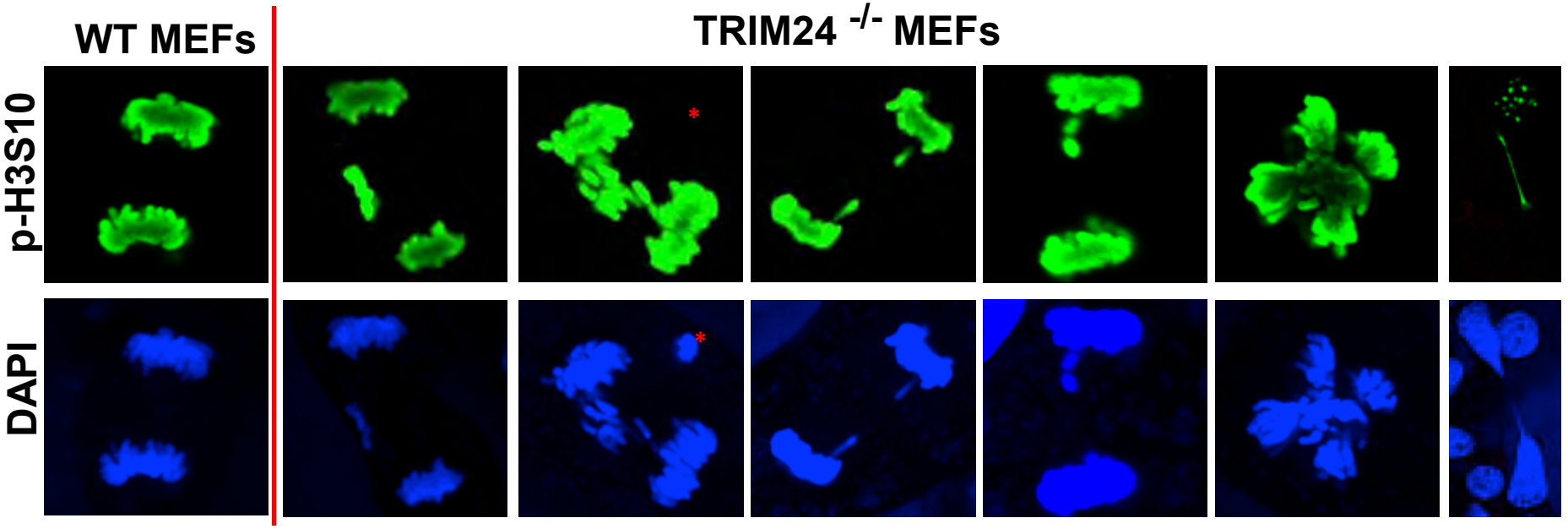


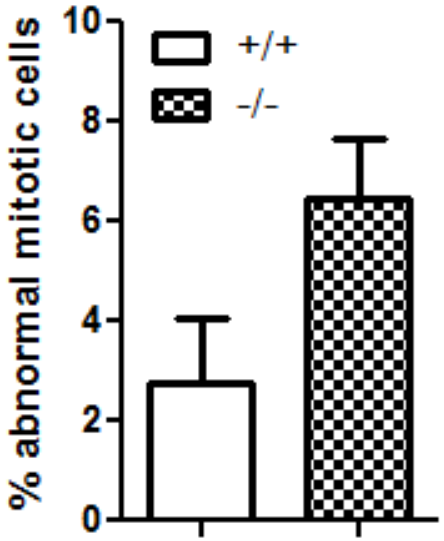
Wolfgang Fischle
Max Planck Institute for
Biophysical Chemistry

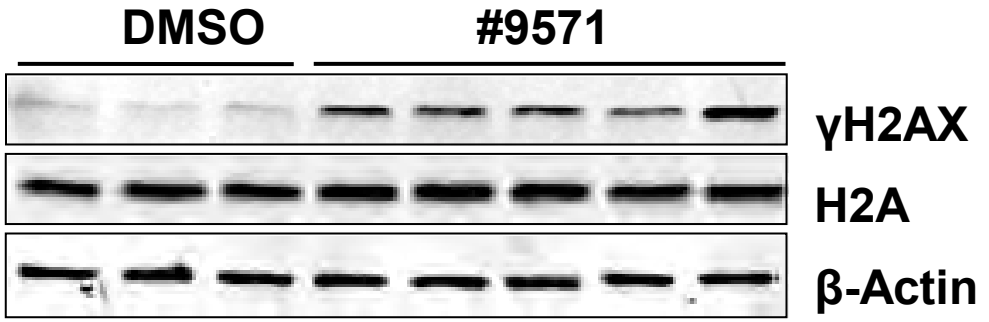
Trim24 Bromo Domain with Designed Inhibitor



Trim24 null MEFs show increased abnormal mitosis and sensitivity to antimitotic drugs



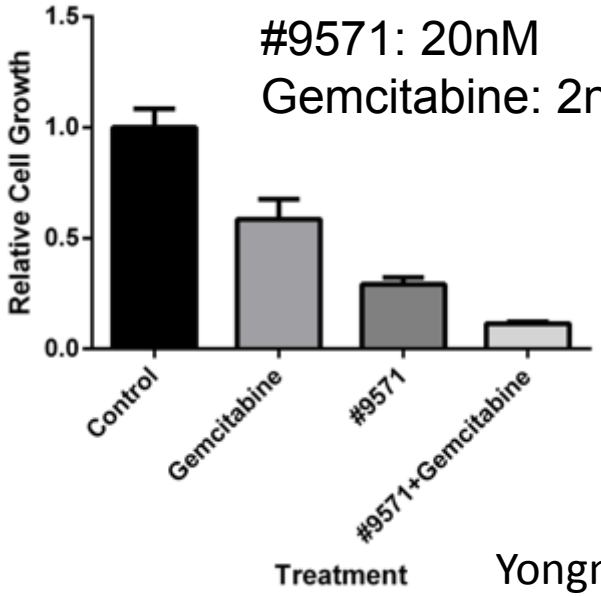


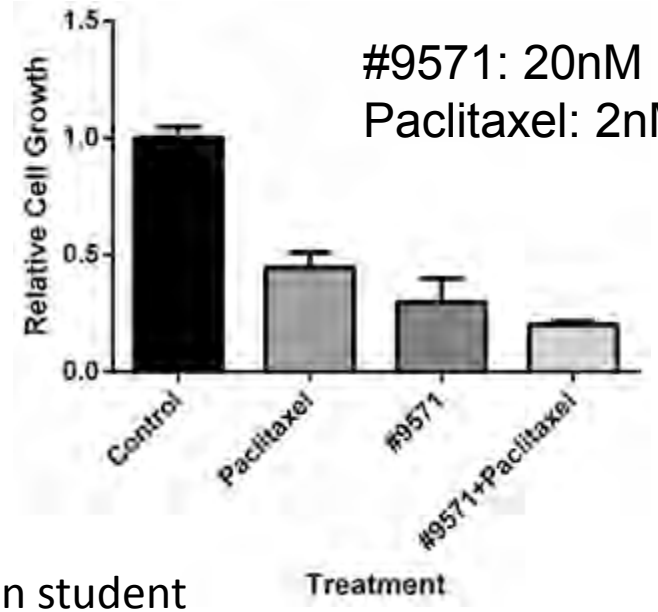


KBM5 cells

#9571 and Carboplatin/Gemcitabine/Vinorelbine/Paclitaxel work synergistically in repressing KBM5 proliferation

Drug	Pathways	Target
Carboplatin	DNA replication and DSB repair	DNA
Gemcitabine	DNA replication and DSB repair	DNA, RNR
Paclitaxel	Mitosis	Tubulin
Vinorelbine	Mitosis	Tubulin





Yongming Xue, rotation student

TARGETING EPIGENETIC CHANGES IN BREAST CANCER

- WHAT DO WE NEED TO KNOW?
  - Expression correlates
  - Function
- HOW CAN EPIGENETIC TARGETS BE SELECTIVE?
  - Global substrate
  - Specific pathway interactions
- HOW CAN THERAPEUTICS BE DEVELOPED?
  - Structure and biophysics
- WHERE DO WE GO FROM HERE?
  - Assess the appropriate targets and disease sites
  - Probe mechanism with breadth and depth
  - Throw everything you have at it: *COLLABORATE*

ACKNOWLEDGEMENTS

BARTON LAB

current

Sabrina Stratton

Kendra Allton

Kaushik Thakkar

Srikanth Appikonda

Shiming Jiang

Ryan McCarthy

Aundrietta Duncan

Abhinav Jain

Jing Li

---

former

Zeynep Coban Akdemir

Thushangi Patharaja

Kadir Akdemir

Wen-Wei Tsai

Zhaoliang Liu

Lindsey Minter

Hui Wei

MD Anderson Cancer Center

Gigi Lozano

Richard Behringer

Jannik Andersen

Phil Jones

Giulio Draetta

Parantu K. Shah

Institute of Applied Cancer Sciences

Memorial Sloan Kettering Institute

Dinshaw Patel

Zhanxin Wang

Stanford

Or Gozani

Xiaobing Shi (now MDACC)

Max Planck Inst. for Biophysical Chemistry

Wolfgang Fischle

Graduate School of Biomedical Sciences
University of Texas MD Anderson Cancer Center
Texas Medical Center, Houston, TX