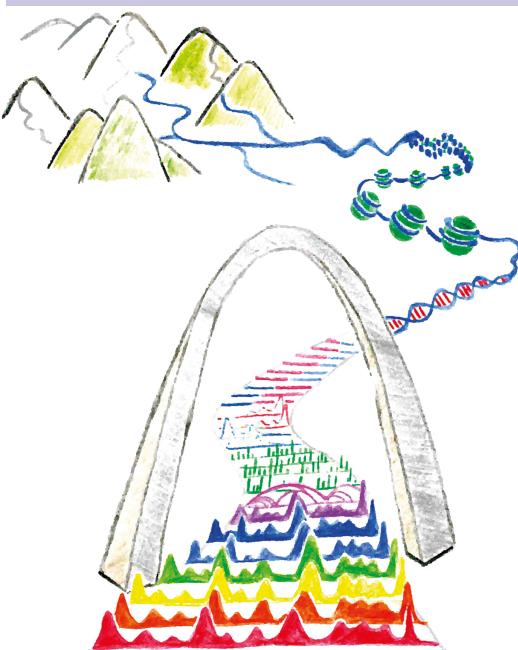


系统生物学与生物信息学
海外学者短期讲学系列课程

Current Topics in Epigenomics

表观基因组学前沿



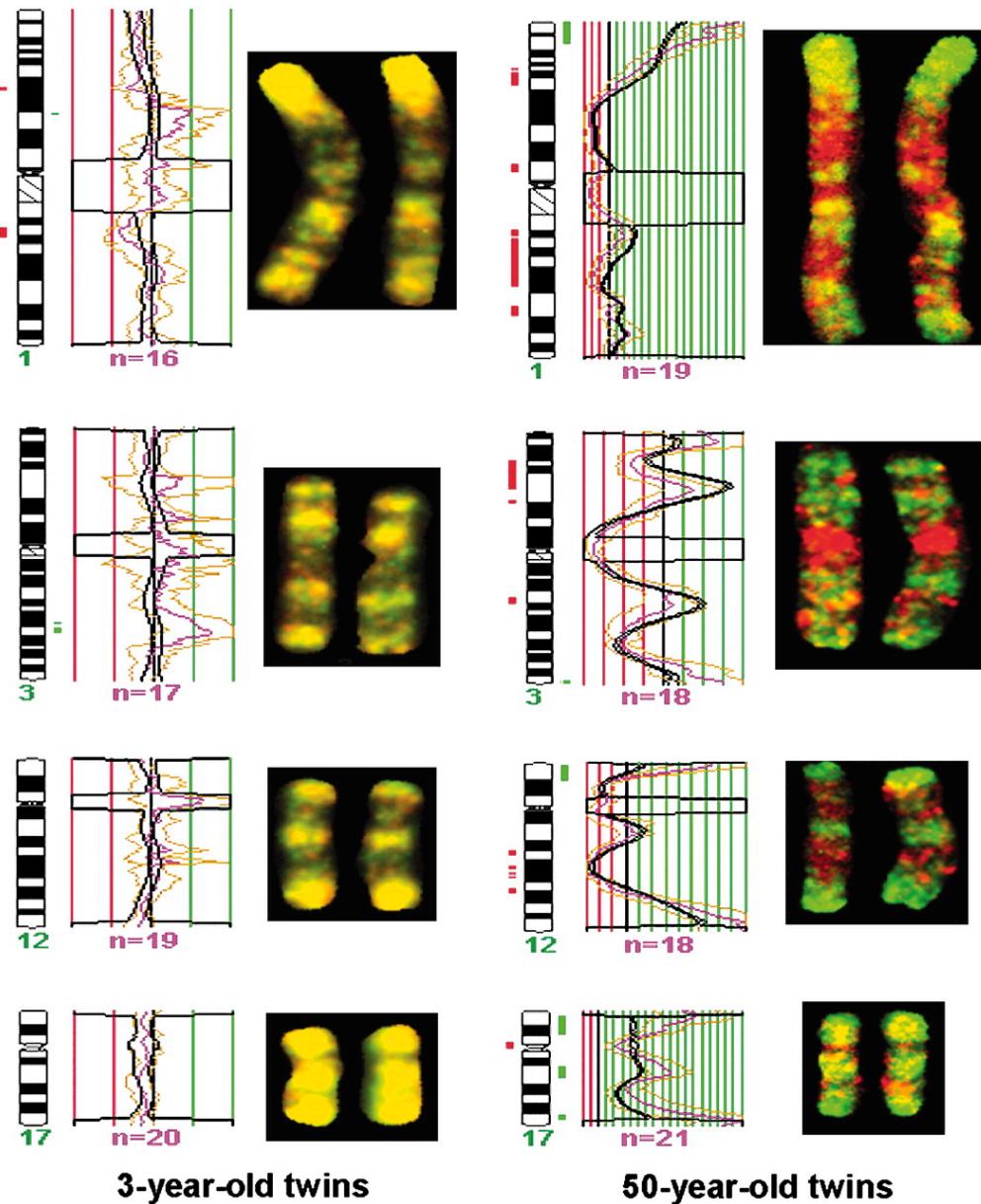
Ting Wang
Department of Genetics
Center for Genome Sciences and Systems Biology
Washington University School of Medicine

Tsinghua University
April 15-27

Epigenetic Mechanisms

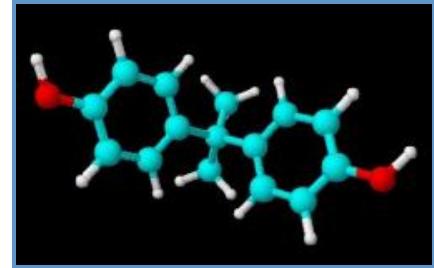
DNA Methylation with age, environment

Differences in DNA methylation patterns between identical twins increase with aging



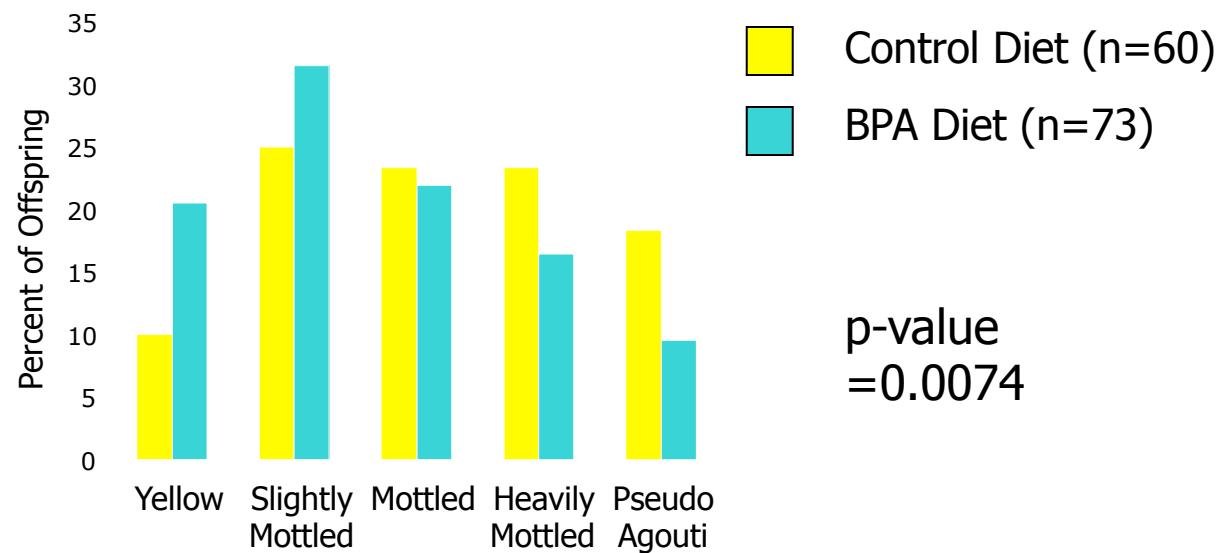
Maternal Bisphenol A (BPA) Exposure

- Monomer that makes up polycarbonate plastic
- Endocrine active compound
- Found in commonly used products
- Present in 95% of humans tested
- Some animal studies reveal negative health outcomes

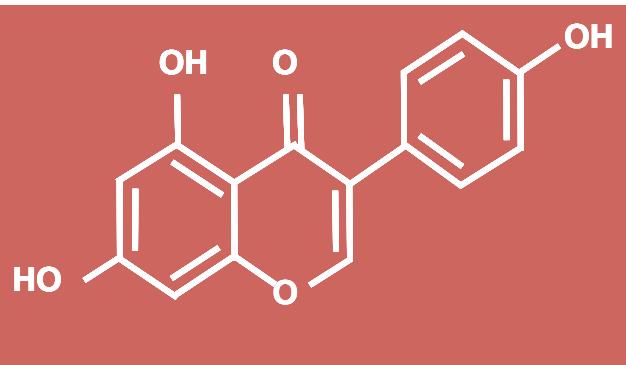


Maternal BPA Exposure

Results: Coat Color Distribution



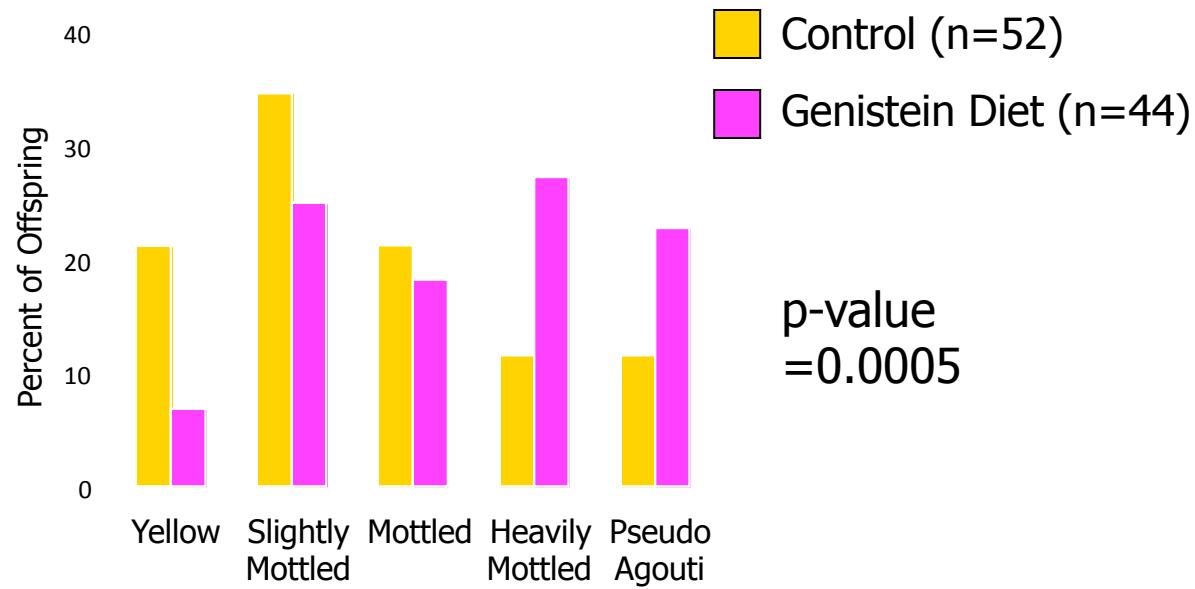
Maternal Genistein Supplementation



- Plant phytoestrogen
- Found in soy and soy products
- Selective estrogen receptor modulator
- Worldwide exposure varies by diet
- Chemoprevention and decreased adipocyte deposition

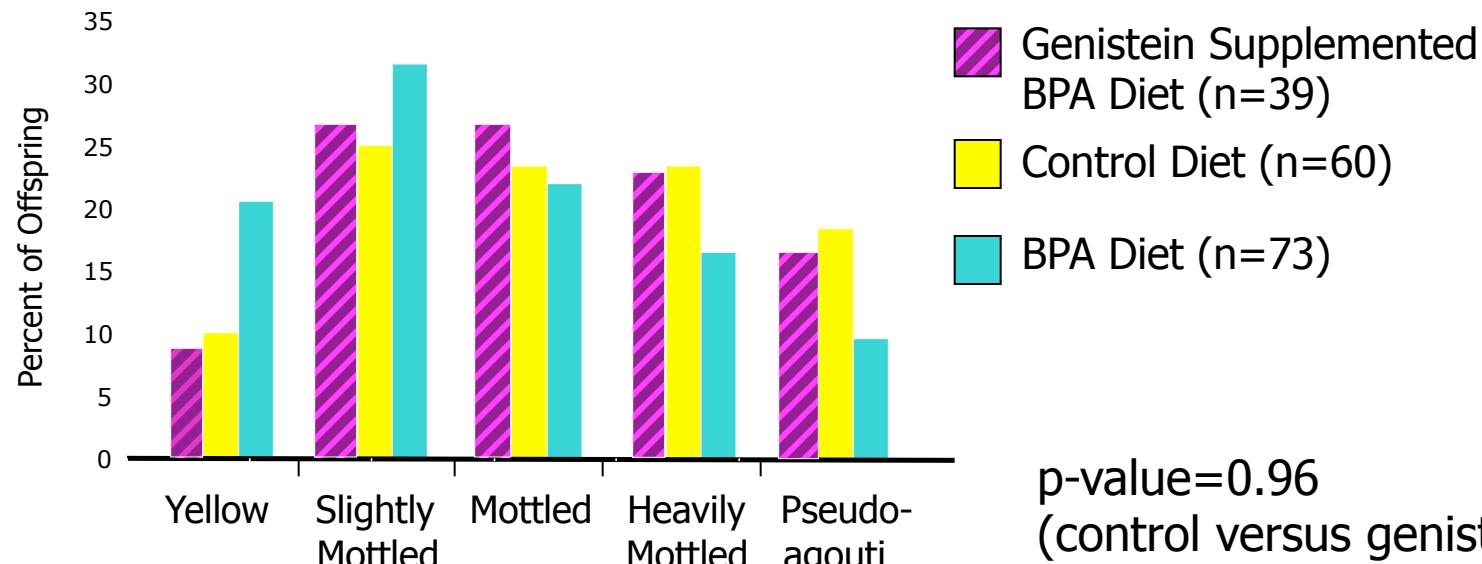
Maternal Genistein Supplementation

Results: Coat Color Distribution

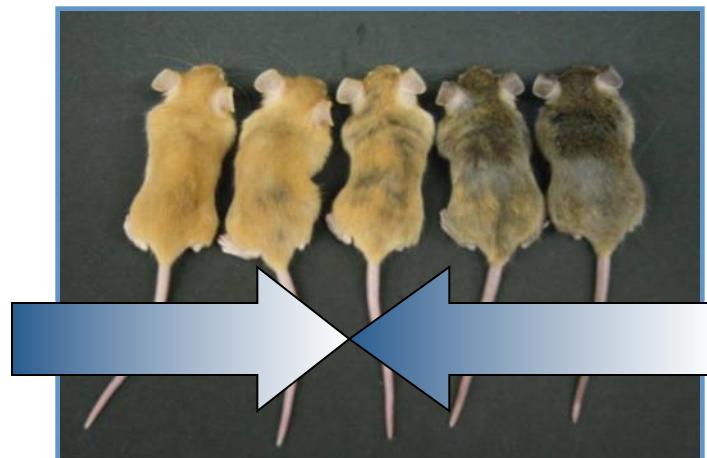


Maternal Nutritional Supplementation

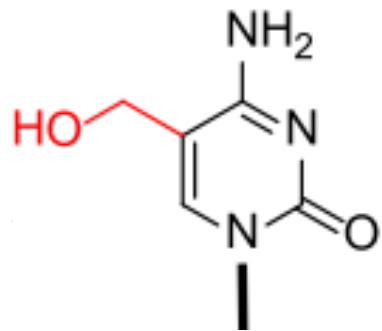
Results: Genistein – Coat Color



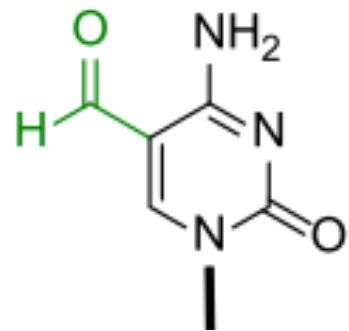
p-value=0.96
(control versus genistein supplementation)



Oxidative derivatives of DNA 5-mC



5-hydroxymethylcytosine
(5-hmC)



5-formylcytosine
(5-fC)



5-carboxylcytosine
(5-fC)

History of the oxidative derivatives of DNA 5-mC

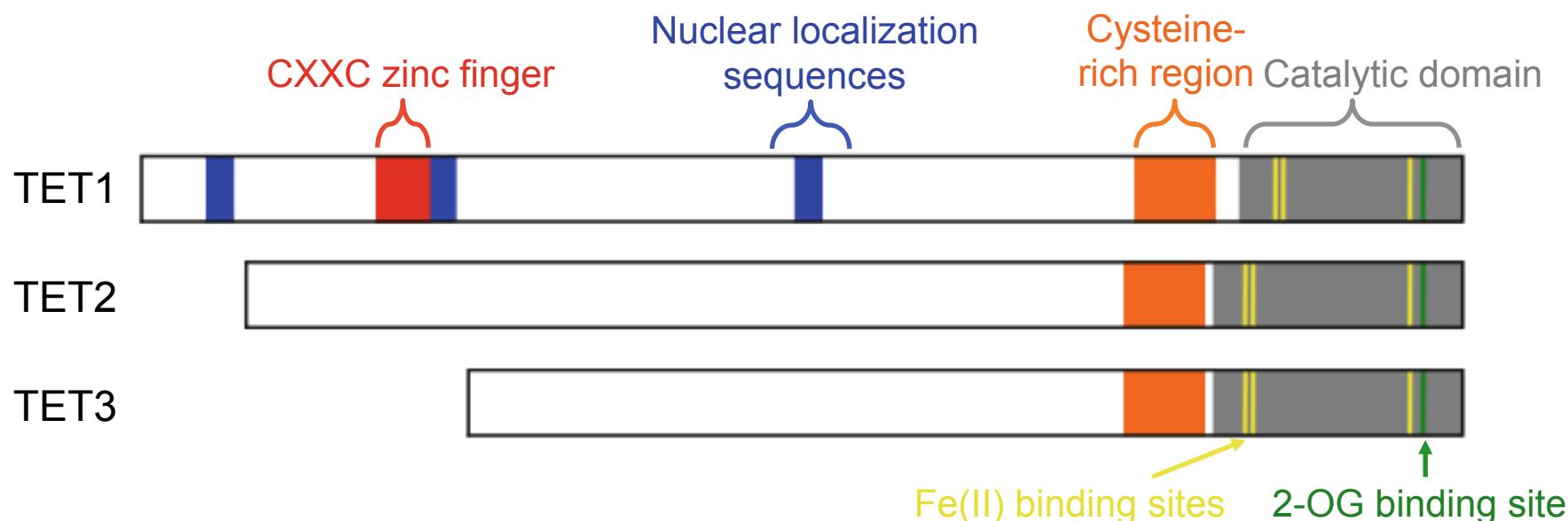
Year	Event	Scientists
1952	5-hmC first identified in T-even bacteriophages. 5-hmC protects phage DNA from bacterial restriction enzymes during infection	Wyatt
1976	5-hmC detected in several vertebrate species, but the results could not be reproduced	Penn et al.
2003	TET1 identified as a gene fusion partner of MLL in AML	Lorsbach et al.
2009	5hmC was discovered in murine ESC, Purkinje Neurons and granule cells Tet proteins convert 5-mC to 5-hmC	Tahiliani et al. Kriaucionis & Heintz
2010	Method for direct detection of modified bases using SMRT sequencing developed	Flusberg et al.
2010	Method for direct detection of modified bases using Nanopores developed	Wallace et al.
2011	Paternal DNA methylation is actively demethylated during preimplantation development	Wossidlo et al.
2011	5-fC and 5-caC discovered in murine ESC and tissues. Tet proteins convert 5-hmC to 5-fC and 5-caC	Ito et al. He et al. Pfaffeneder et al.
2012	Methods for detecting 5-hmC genome-wide developed (TAB-seq & oxBS-seq)	Booth et al. Yu et al.
2013	Methods for detecting 5-fC & 5-caC genome-wide developed (fCAB-seq & CAB-seq)	Song et al. Lu et al.

Many unanswered questions remain

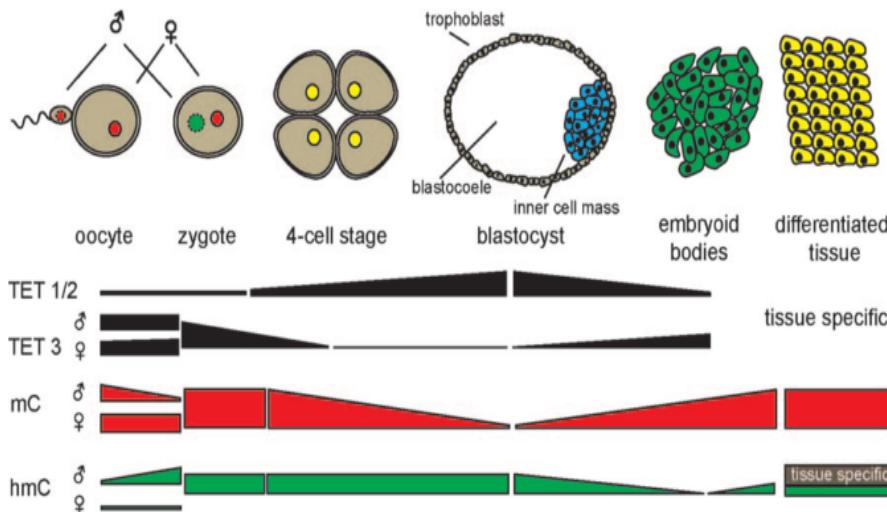
- Who are the 5-hmC readers?
- How is 5-hmC maintained during DNA replication?
- What are the physiological context and relative importance of the various pathways involving 5hmC?
- How does 5-hmC affect transcriptional regulation?
- What are the dynamics of 5-hmC during development?
- What role does 5-hmC play in cancer?

Ten-eleven translocation (TET) proteins

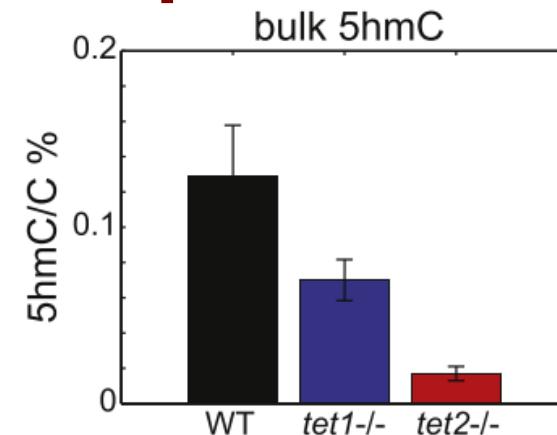
- Named after a common chromosomal translocation found in some cancers types
- Fe(II) and 2-OG are co-factors
- ~2k amino acids
- The catalytic domains of TET1-3 are ~70% similar, suggesting similar catalytic activity
- Variable regions suggest distinct binding affinities to chromatin and/or protein partners



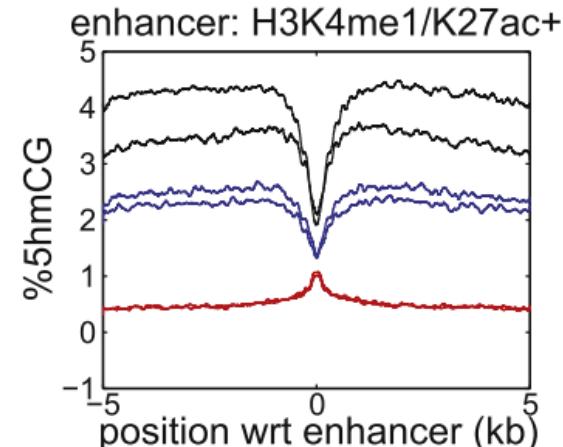
Tet proteins and 5-hmC during mouse preimplantation development



- Tet1 KO
 - Small body size
- Tet2 KO
 - Spontaneous myeloid leukemia
 - Enhancer hypermethylation
 - Reduction of enhancer activity
 - Delayed gene induction for differentiation
 - Tet2 may be a positive regulator of enhancer activity during differentiation
- Tet3 KO is neonatal lethal



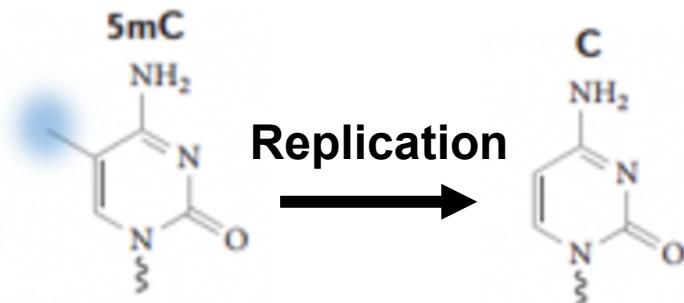
Global loss of 5-hmC in *tet1* KO & *tet2* KO



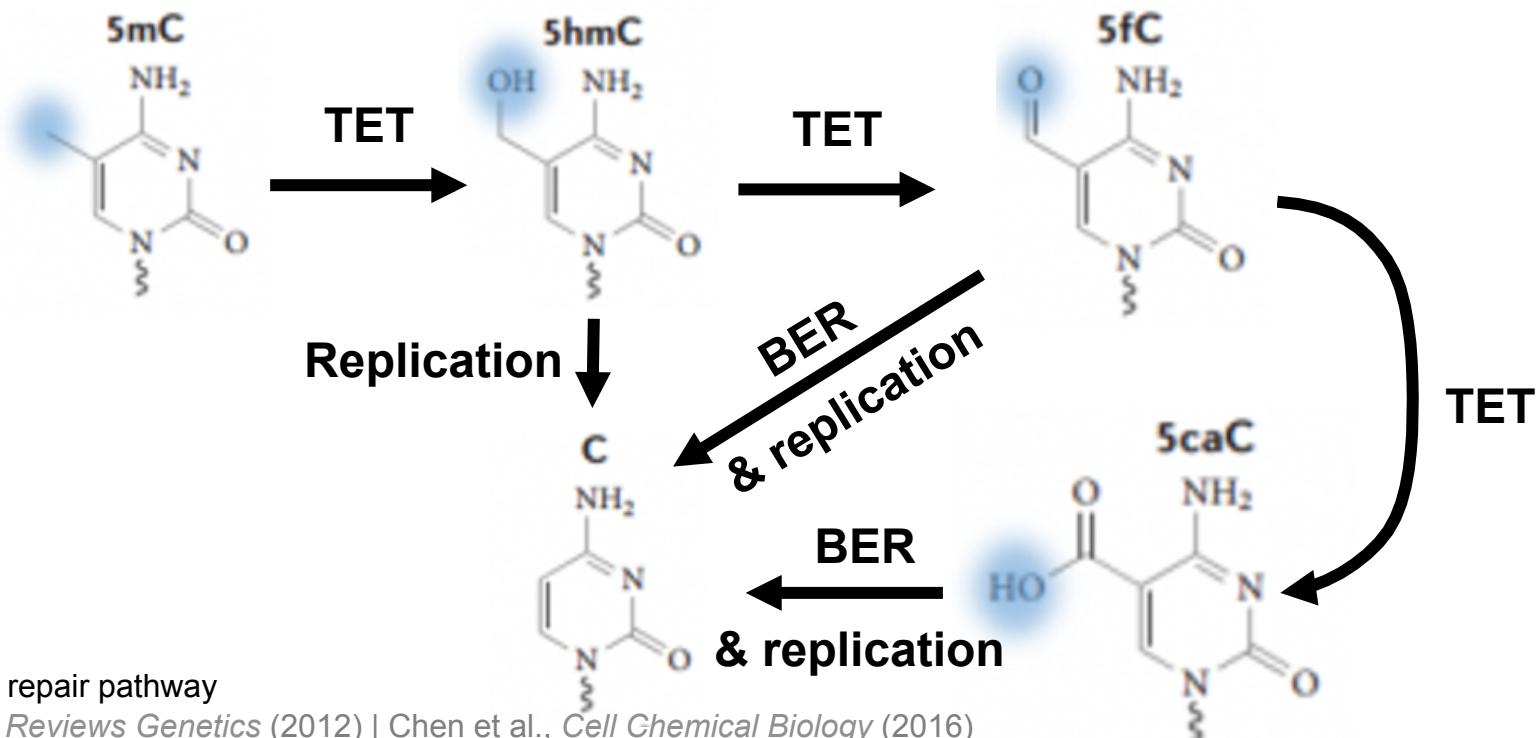
Loss of 5-hmC at enhancers in *tet1* KO & *tet2* KO

DNA methylation and demethylation pathways

Passive



Active



BER = base excision repair pathway

Branco et al., *Nature Reviews Genetics* (2012) | Chen et al., *Cell Chemical Biology* (2016)

Global 5-hmC patterns

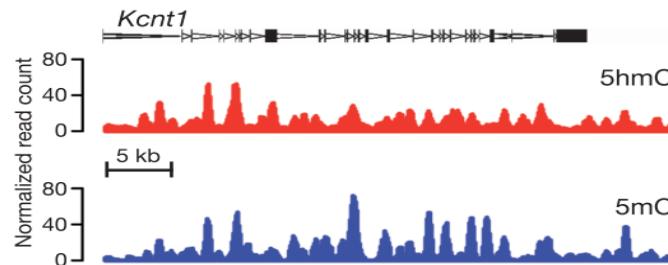
- 5-hmC is distributed nonuniformly in genome
 - Distribution differs from 5-mC
 - Is cell-type specific
 - Global 5-hmC levels vary between < 0.1% - 0.7%
 - Highest in ESC and neurons

5-hmC patterns across genomic features

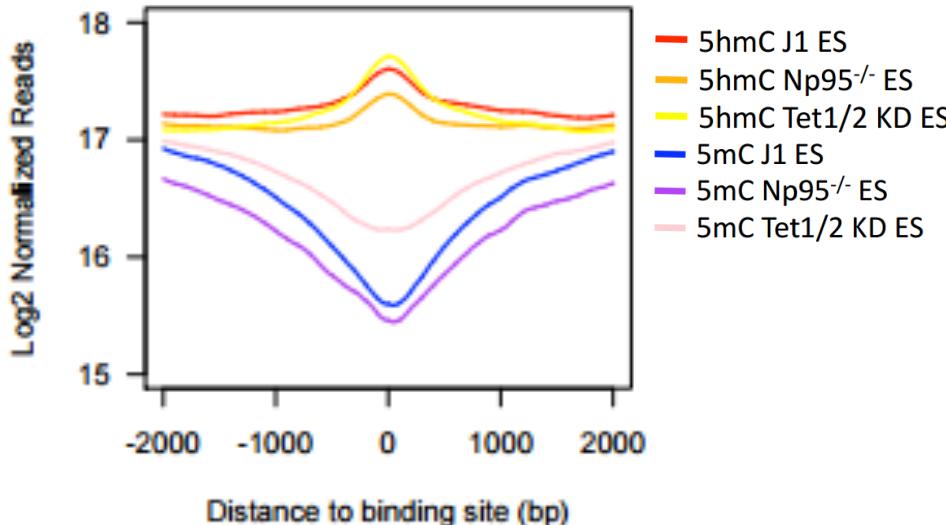
- Enriched in gene bodies, TSSs, and promoters
- Enriched in genomic regions with moderate CpG density
- Enriched in cis-regulatory elements
- Enriched in poised promoters (H3K4me3 & H3K27me3)
- Enriched in pluripotent TFBS
- Enriched in insulator BS

5-hmC patterns across genomic features

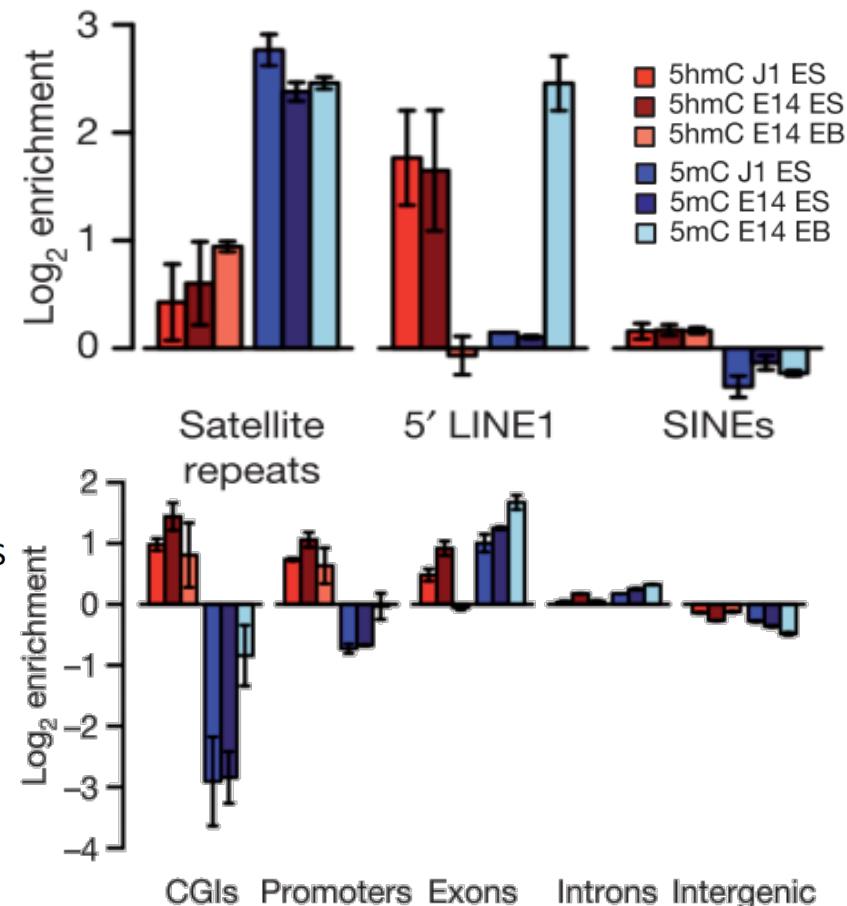
Ficz et al., used hMeDIP- and MeDIP-seq to profile 5-mC and 5-hmC genome wide in murine embryonic stem (ES) cell lines (J1 & E14) and differentiating embryoid bodies (EB)



Example hMeDIP-seq and MeDIP-seq profiles at a genomic locus



5-hmC enriched in pluripotent TFBS

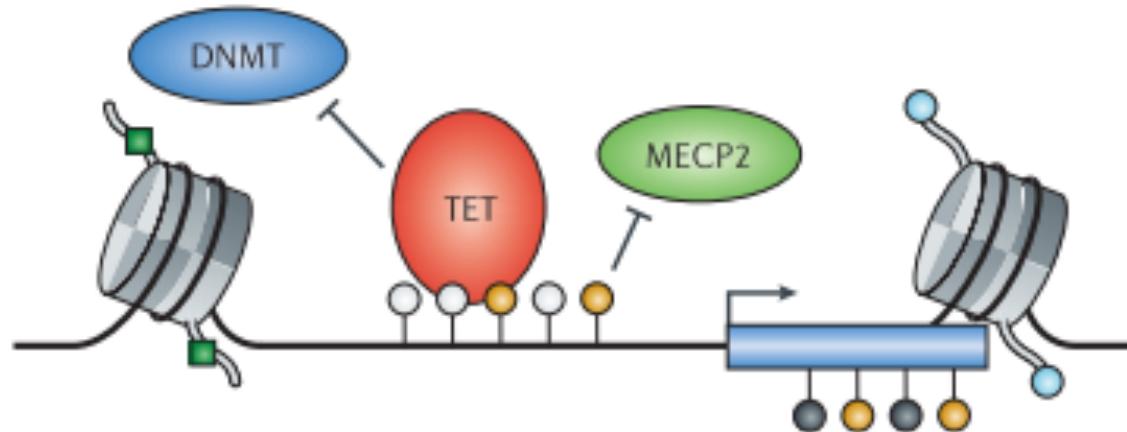


5-hmC enriched in CGIs, promoters, exons, & several repetitive elements

Functions of 5-hmC in mammals

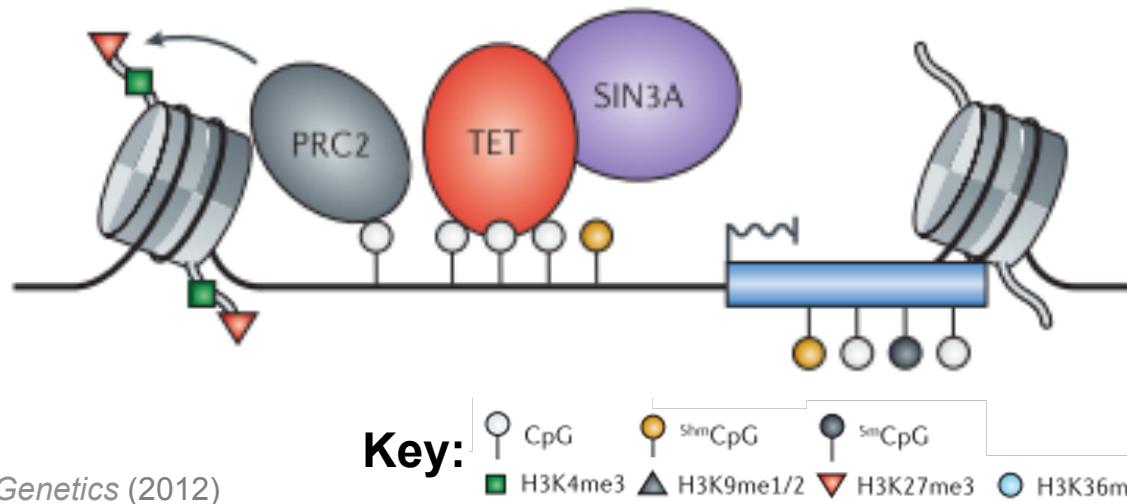
5-hmC is associated with active promoters

- 5-hmC readers exist
- 5-hmC impairs binding of 5-mC binding proteins



5-hmC also associated with repressed genes

- TET interacts with the SIN3A co-repressor complex
- Removal of 5-mC promotes recruitment of PRC2 and H3K27me3 at bivalent genes



Functions of 5-hmC (cont.)

- Active demethylation associated with embryonic development
 - Paternal 5-mC methylation is lost through active demethylation during fertilization
- 5-mC oxidation derivatives may play critical roles in regulating pluripotency and differentiation potential

5-hmC and cancer

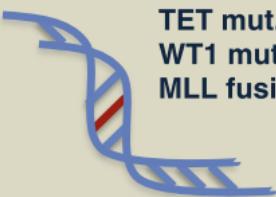
- Several human cancers are associated with aberrant TET activity
- Global 5-hmC abundance
 - Many hematological and solid cancers have hypo-5-hmC
 - Several cases of hyper-5-hmC
 - In colon cancer, global 5-hmC levels unchanged
- Few genome-wide 5-hmC mapping studies
- TET proteins appear to be both suppressors and promoters of cancer
- 5mC oxidation derivatives may be potential diagnostic and prognostic biomarkers
- The active demethylation pathway may be a potential target for therapy

Mechanisms that underlie altered 5-hmC levels in cancer

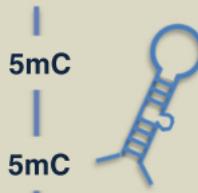
TET regulation in cancer

Genetics

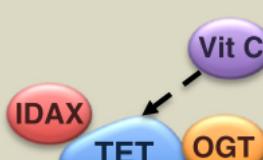
TET mut.
WT1 mut.
MLL fusion



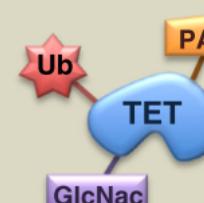
Epigenetics



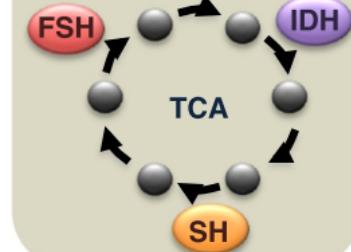
Interactors and cofactors



Post-translational modifications



Metabolism

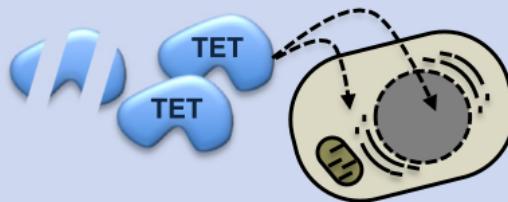


Impact on TET function

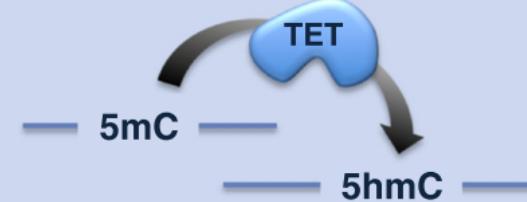
Transcription



Protein (stability and localization)



5hmC activity

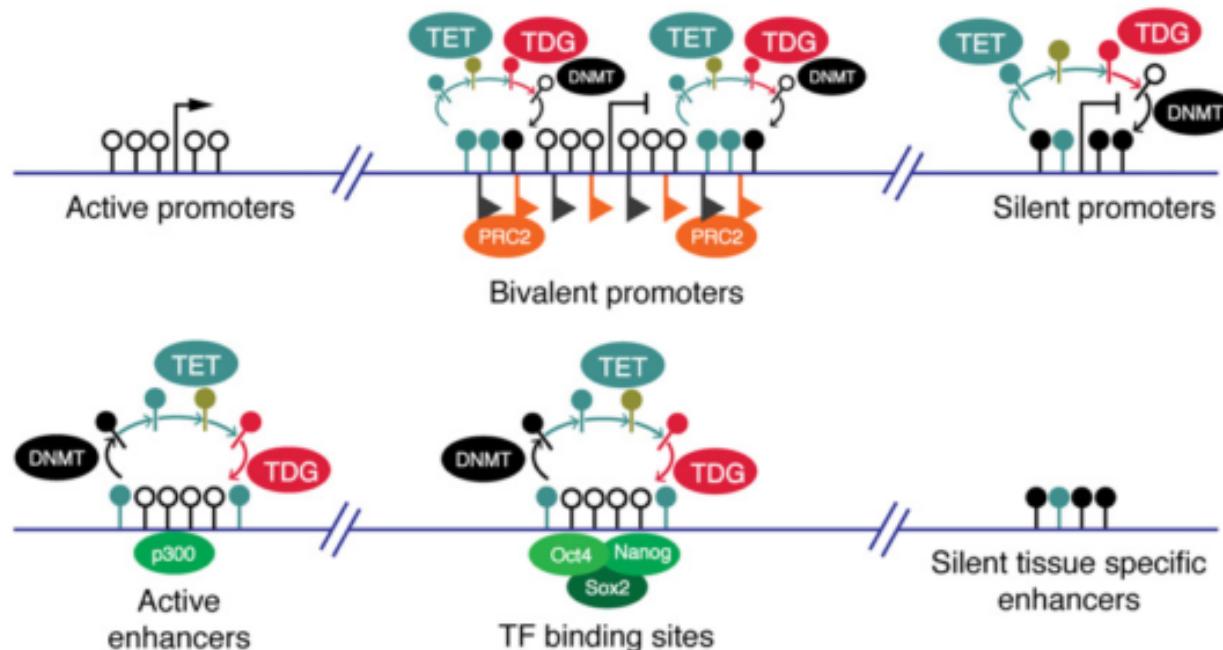


5-fC and 5-caC

- 5-fC and 5-caC are rare in mammalian DNA
 - Levels are highest in ESCs
 - < 0.002% of Cs in mESCs and several adult mouse tissues
- Removed via TDG-mediated excision
- Functions
 - Intermediates of active DNA methylation
 - Controversial if the modifications are stable or transient
 - May alter the structure of the DNA double helix

Genome-wide profiling of 5-fC and 5-caC

- Genomic profiles are distinct from 5-mC, 5-hmC, 5-caC, & each other
- Show strand asymmetry in palindromic CpGs
- TDG-mediated excision of 5-fC and 5-caC occur preferentially at active enhancers and inactive gene promoters
- Enriched at pericentric heterochromatin



DNA methylation

+

Histone modification

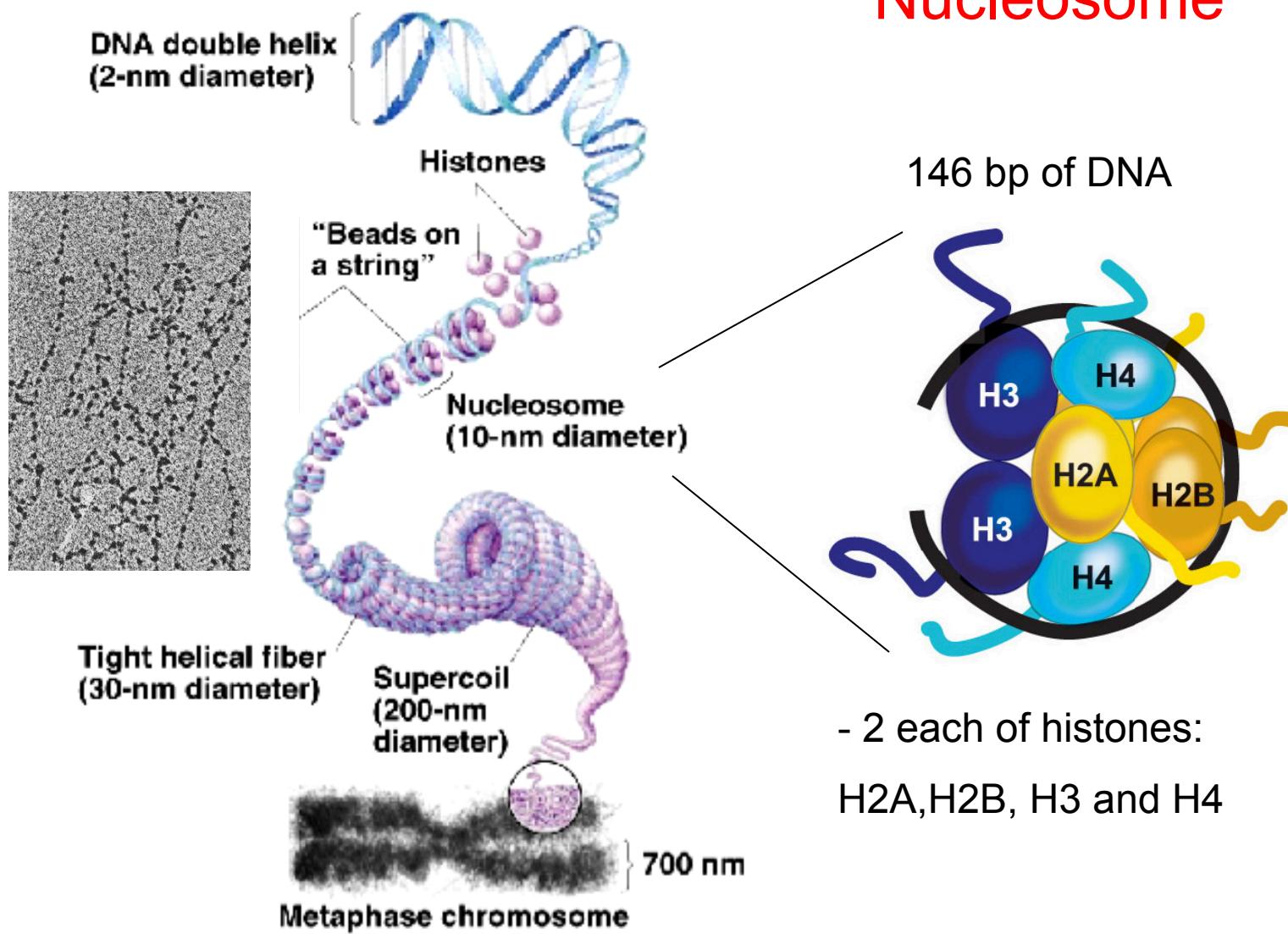
→

Chromatin

Chromatin

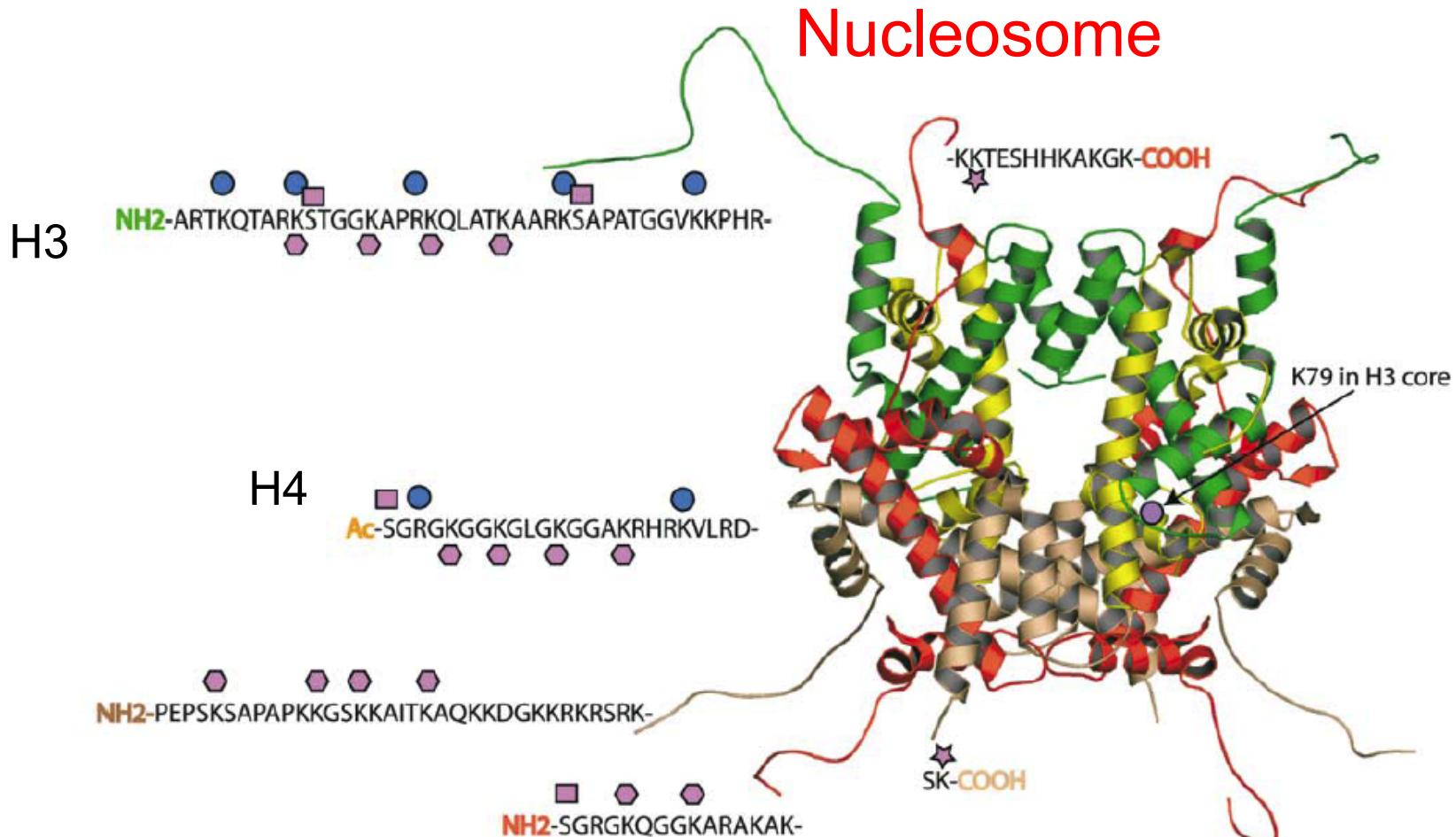
DNA plus Protein in cells with nuclei

Nucleosome



The Nucleosome core particle

A



SnapShot: Histone Modifications

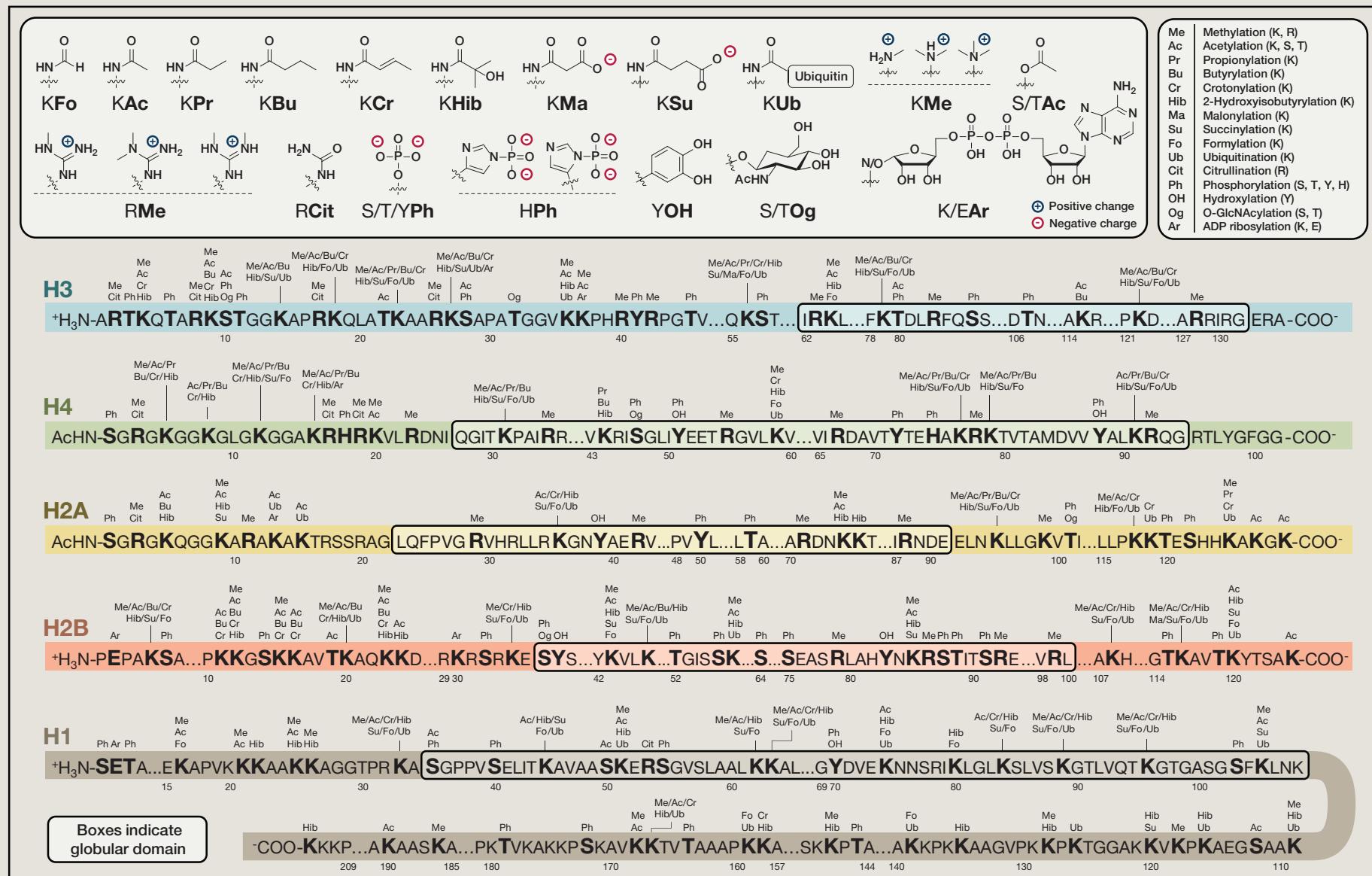
He Huang,¹ Benjamin R. Sabari,² Benjamin A. Garcia,³ C. David Allis,² and Yingming Zhao¹

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

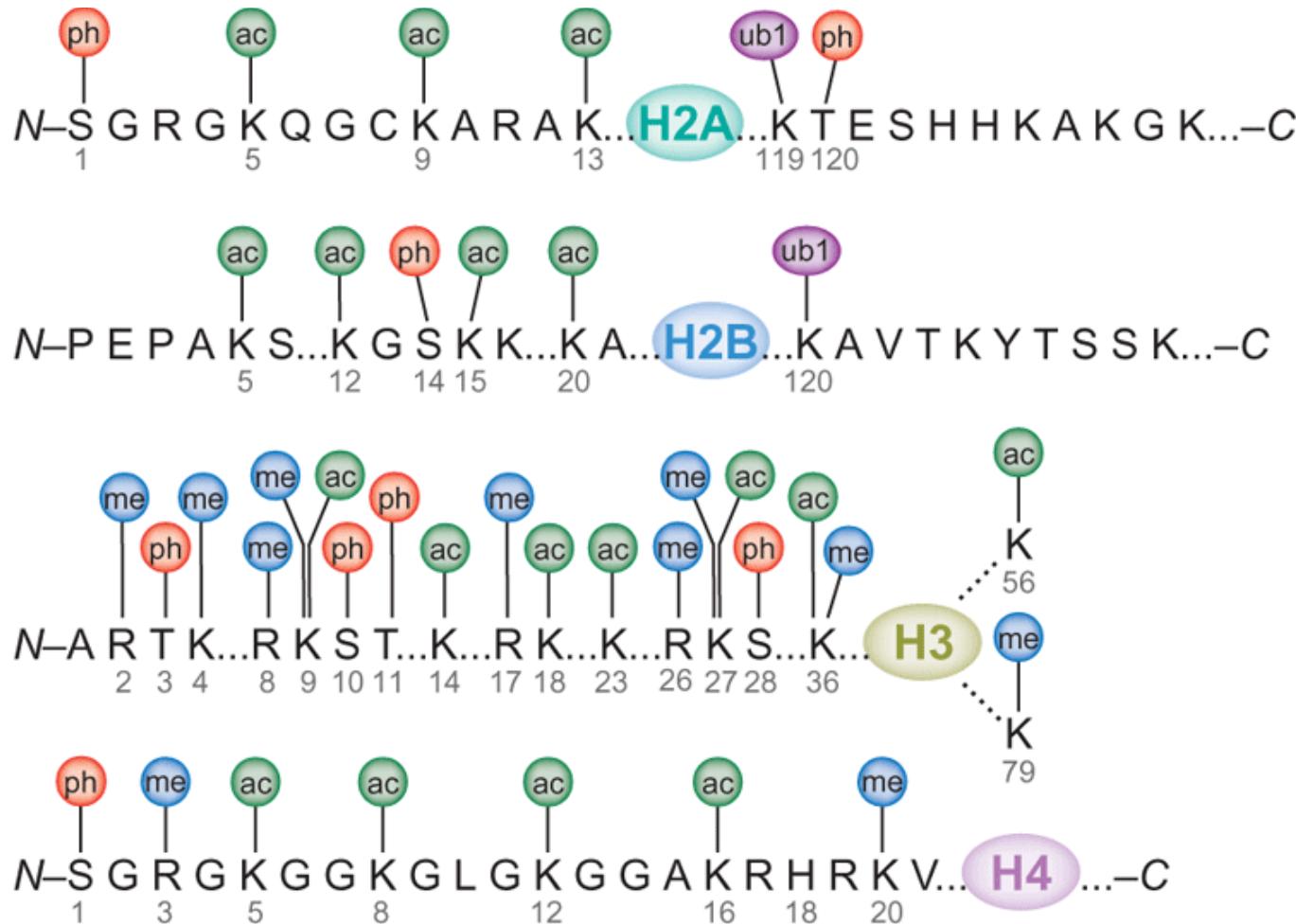
²Laboratory of Chromatin Biology and Epigenetics, The Rockefeller University, New York, NY 10021, USA

³Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104, USA

Cell

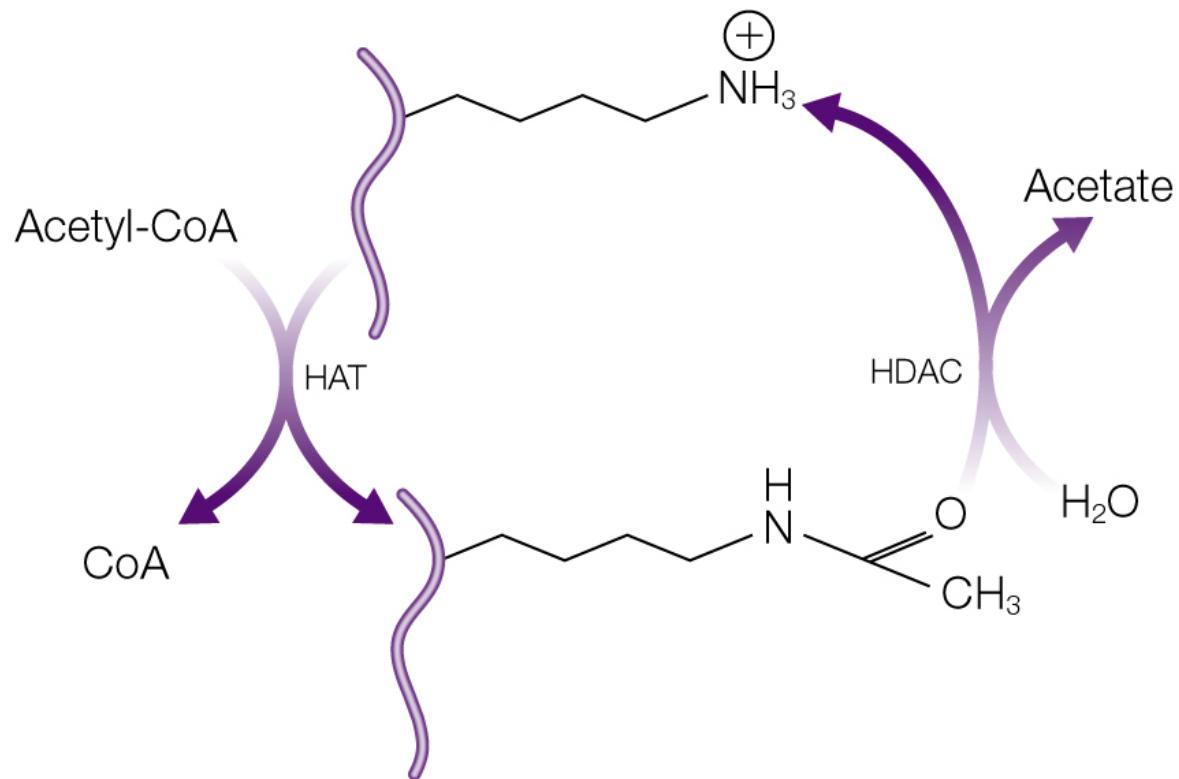


Post-translational Histone Modifications



Histone acetylation reaction

A



HAT Characteristics

Table 5.1

Histone acetyltransferase (HAT) complexes

Complex	Catalytic subunit ¹	Substrate specificity	Tra1-related subunit
GNAT family complexes²			
Yeast SAGA	yGcn5 (BrD)	H3/H2B	Tra1
Human STAGA	hGcn5 (BrD)	H3/H2B	TRRAP
Human PCAF	PCAF (BrD)	H3/H4	PAF400
MYST family complexes³			
Yeast NuA4	Esa1 (ChD)	H4/H2A	Tra1
Human Tip60	Tip60 (ChD)	H4/H2A	TRRAP
Fly MSL	MOF (ChD)	H4 K16	?
Yeast SAS	Sas2 (ChD)	H4 K16	?

¹In addition to a HAT domain, each catalytic subunit contains either a bromodomain (BrD) or a chromodomain (ChD) as indicated.

²All the GNAT (for Gcn5-related acetyltransferase) family complexes contain a catalytic subunit with homology to yeast GCN5.

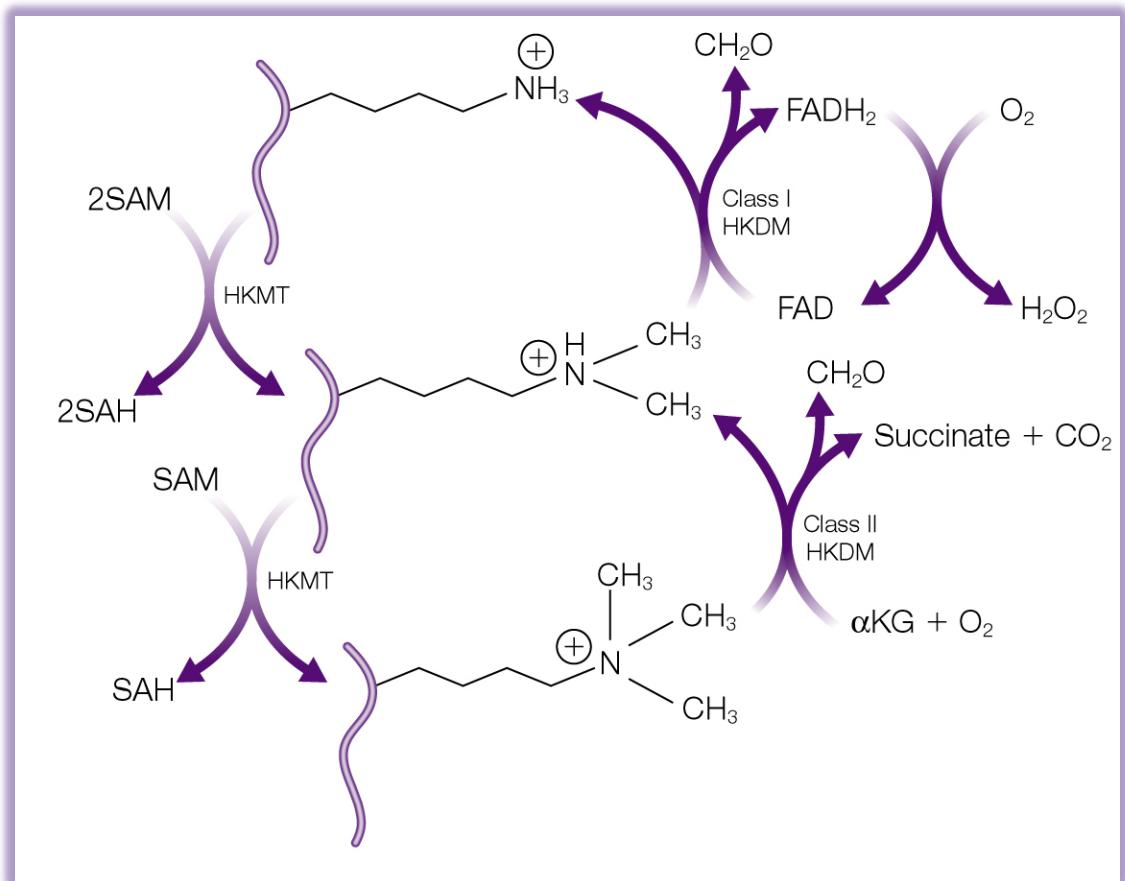
³All the MYST (for MOF, Ybf2, Sas2, Tip60) family complexes contain a catalytic subunit with homology to yeast Esa1.

HDAC Complexes

Complex	HDAC1 & HDAC2 (16-19,39,47,56-59,95,117,118)			HDAC3 (36,41,50,55,65-67)	HDAC4	HDAC5 (32,36,36,46,50,66,73)	HDAC7 (32,40,48-50,54)	HDAC9 (43,46,67)	* Nuclear hormone receptors (e.g. ER, GR, TR and RAR) function as transcriptional activators, directly binding the respective responsive element, when ligand is present. In the case of SMRT / N-CoR binding and function no ligand is bound to the receptors, thus functioning as transcriptional repressors.
Histone binding	RbAp48	RbAp48		RbAp48					
Recruiters** (bind DNA, sequence specific, methylation specific or are intermediates between DNA and HDAC complex)	Ikaros YY1 ² , RBP-1, Sp1, BRCA1, Rb ¹ , HDRP heterochromatin protein-1, NF-κB, MeCP2, HDAC10	MBD3		HDAC4, HDAC5, HDAC7, HDRP, YY1 ² , Rb ¹ , GATA-2, HDAC9, HDRP, HDAC10	MEF2	MEF2, GATA-2	MEF2	MEF2	** From the group of recruiters only one is usually present, all the other groups represent necessary co-repressors for HDAC activity.
Nuclear hormone receptor* binding	mSin3A, N-CoR, SMRT***			SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR	SMRT, N-CoR	*** SMRT does not interact directly with HDAC1
Remodelling (helicase/ ATP-ase family members)		Mi-2							
Necessary/ modulates HDAC activity		MTA2, p70, p32,	Co-REST	SMRT/ N-CoR	SMRT/ N-CoR, HDAC3	SMRT/ N-CoR, HDAC3	SMRT/ N-CoR, HDAC3		
Unknown	RbAp46, SAP18, SAP30	RbAp46		TBL1					
Association as intermediate	MAD/MAX, Mxi/MAX, MeCP-1, ER, GR, TR, RPX homeodomain proteins, c-Ski, Sno, Aiolos, p53, NY-F, REST, Suv39H1, PML-RAR α , DNMT			CRM1, HDAC3	ERK1/2, CtBP, HDAC10	CtBP, HDAC10	CRM1, CtBP, HDAC10	HDAC1, HDAC3, SMRT, N-CoR,	

Histone Methylation (lysine)

- Both activation and repression
- More stable
- HKMT
- HKDM



Post-translational Histone Modifications

H3 tail Modifications:

 =Acetylation

 =Methylation

Active



HATs

HDACs

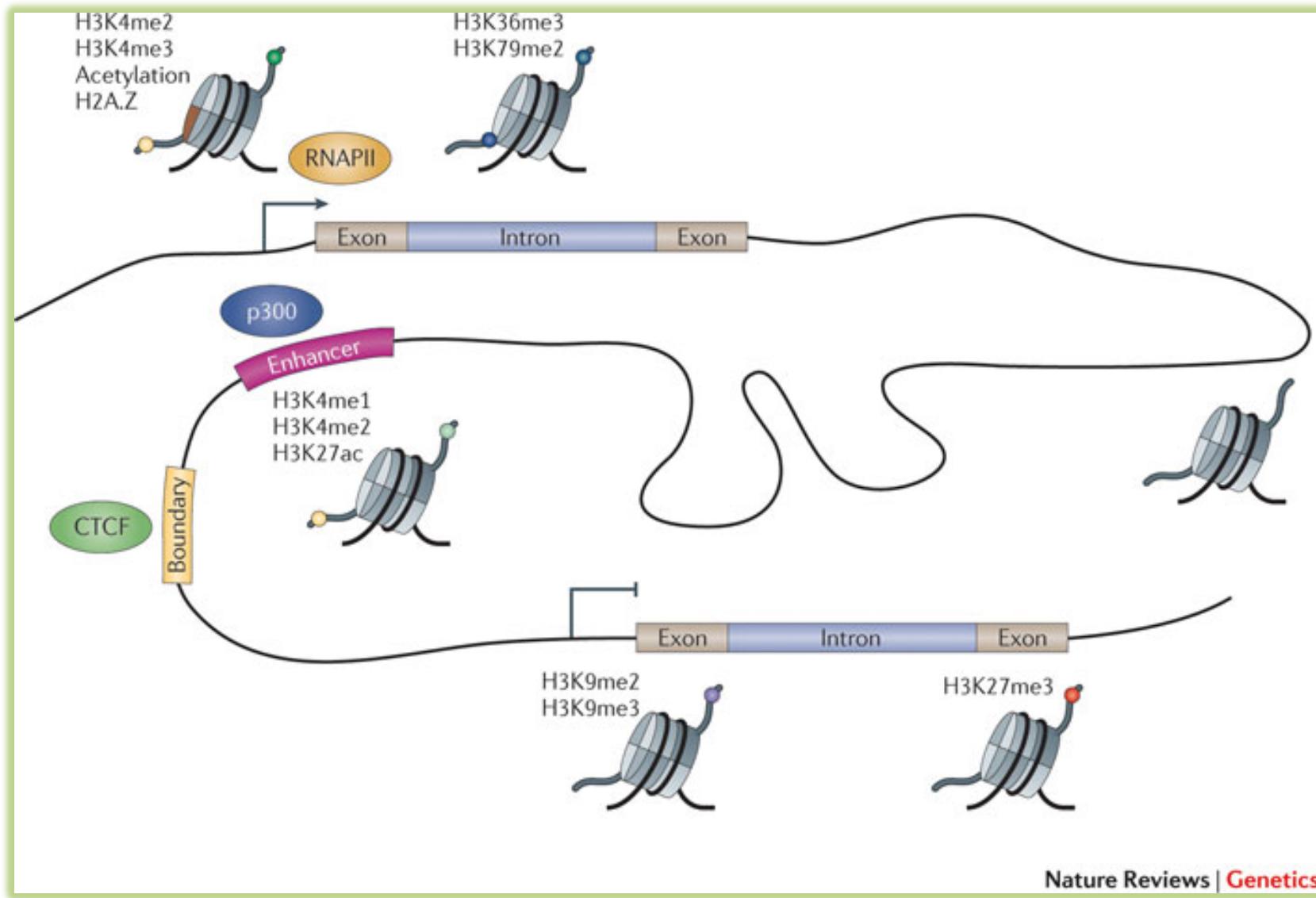
KMTases

Repressive



**Epigenome provides an annotation of
the genome**

Chromatin modifications demarcate functional elements in the genome



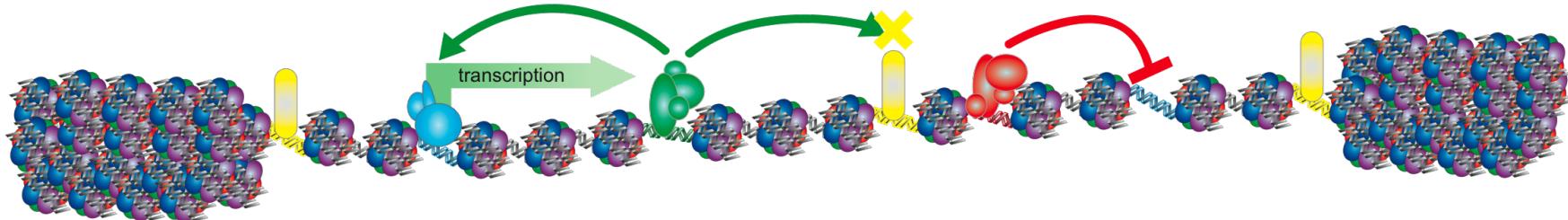
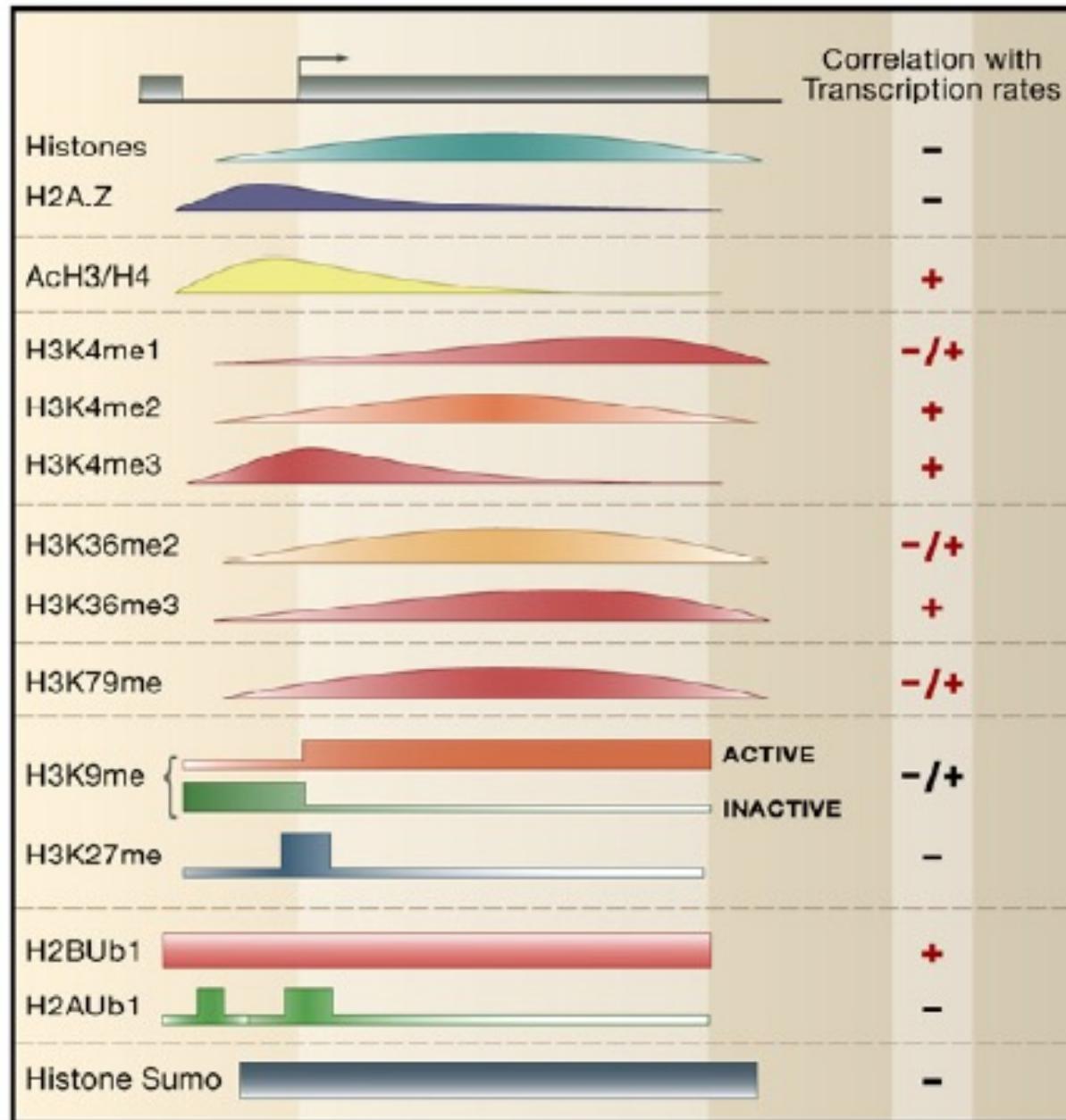


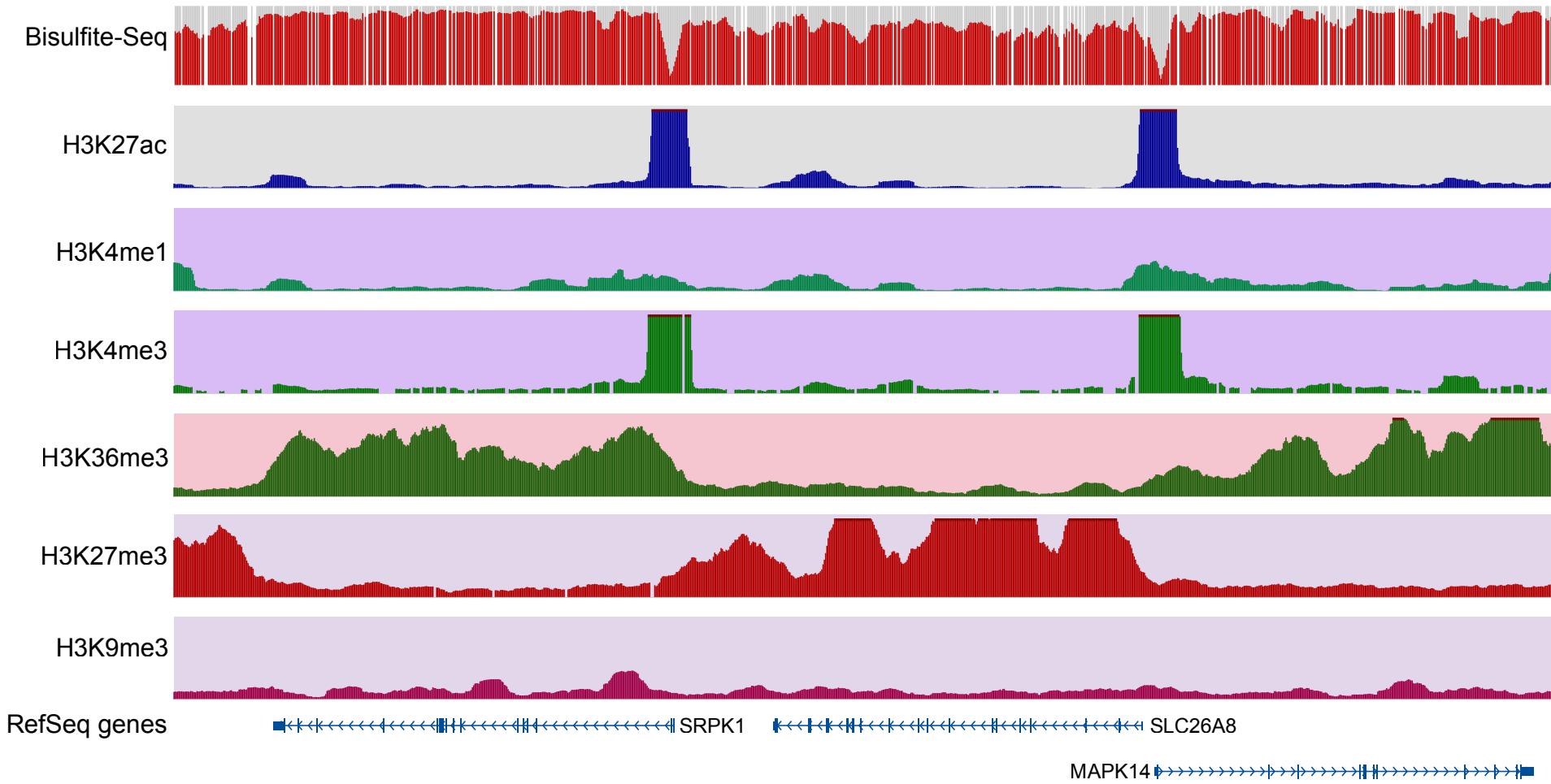
Table 3. Distinctive Chromatin Features of Genomic Elements

Functional Annotation	Histone Marks
Promoters	H3K4me3
Bivalent/Poised Promoter	H3K4me3/H3K27me3
Transcribed Gene Body	H3K36me3
Enhancer (both active and poised)	H3K4me1
Poised Developmental Enhancer	H3K4me1/H3K27me3
Active Enhancer	H3K4me1/H3K27ac
Polycomb Repressed Regions	H3K27me3
Heterochromatin	H3K9me3

Histone Modifications in Relation to Gene Transcription



Histone Modifications in Relation to Gene Transcription



Epigenome Mapping Projects

Table 1. Large-Scale National and International Epigenomic Consortia

Project Name	Start Date	Affiliations	Completed and Expected Data Contributions	Selected Publication	Access Data
Encyclopedia of DNA Elements	2003	NIH	Dnase-seq, RNA-seq, ChIP-seq, and 5C in 100s of primary human tissues and cell lines	ENCODE Project Consortium et al., 2012	http://encodeproject.org/ENCODE/
The Cancer Genome Atlas (TCGA)	2006	NIH	DNA methylomes in 1,000s of patients samples from more than 20 cancer types	Garraway and Lander, 2013	http://cancergenome.nih.gov/
Roadmap Epigenomics Project	2008	NIH	Dnase-seq, RNA-seq, ChIP-seq, and MethylC-seq in 100 s of normal primary cells, hESC, and hESC derived cells	Bernstein et al., 2010	http://www.epigenomebrowser.org/
International Cancer Genome Consortium (ICGC)	2008	15 countries, includes TCGA	DNA methylation profiles in thousands of patient samples from 50 different cancers	The International Cancer Genome Consortium, et al., 2010	http://dcc.icgc.org/web
International Human Epigenome Consortium (IHEC)	2010	7 countries, includes BLUEPRINT, Roadmap	Goal: 1,000 Epigenomes in 250 cell types	American Association for Cancer Research Human Epigenome Task Force; European Union, Network of Excellence, Scientific Advisory Board, 2008	http://ihec-epigenomes.org

ROADMAP
epigenomics
PROJECT

HOME PARTICIPANTS DATA PROTOCOLS QUALITY METRICS TOOLS PUBLICATIONS

OVERVIEW PROJECT DATA MAPPING CENTERS PROTOCOLS & STANDARDS PUBLICATIONS NEWS

UCSC Browser Mirrors

- <http://www.epigenomebrowser.org>
- <http://genomebrowser.wustl.edu/>

Data Repositories

- [NCBI Epigenomics Gateway](#)

- Producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research.
- Mapping DNA methylation, histone modifications, chromatin accessibility and RNA transcripts in stem cells and primary ex vivo tissues selected to represent the normal counterparts of tissues and organ systems frequently involved in human disease.
- Rapid release of raw sequence data, profiles of epigenomic features and higher-level integrated maps.

The consortium homepage: <http://epigenomebrowser.org>

All protocols in use can be found here, as well as information about quality metrics, news, publications, general program information, and links to other associated websites.

Predicting non-coding RNA?

- From sequence?
 - Not clear which properties can be exploited
 - Sequence features such as promoters are too weak
- Histone modifications + conservation worked

Vol 458 | 12 March 2009 | doi:10.1038/nature07672

nature

LETTERS

Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals

Mitchell Guttman^{1,2}, Ido Amit¹, Manuel Garber¹, Courtney French¹, Michael F. Lin¹, David Feldser³, Maite Huarte^{1,6}, Or Zuk¹, Bryce W. Carey^{2,8}, John P. Cassady^{2,8}, Moran N. Cabili⁷, Rudolf Jaenisch^{2,8}, Tarjei S. Mikkelsen^{1,4}, Tyler Jacks^{2,3}, Nir Hacohen^{1,9}, Bradley E. Bernstein^{1,10,11}, Manolis Kellis^{1,5}, Aviv Regev^{1,2}, John L. Rinn^{1,6,11*} & Eric S. Lander^{1,2,7,8*}

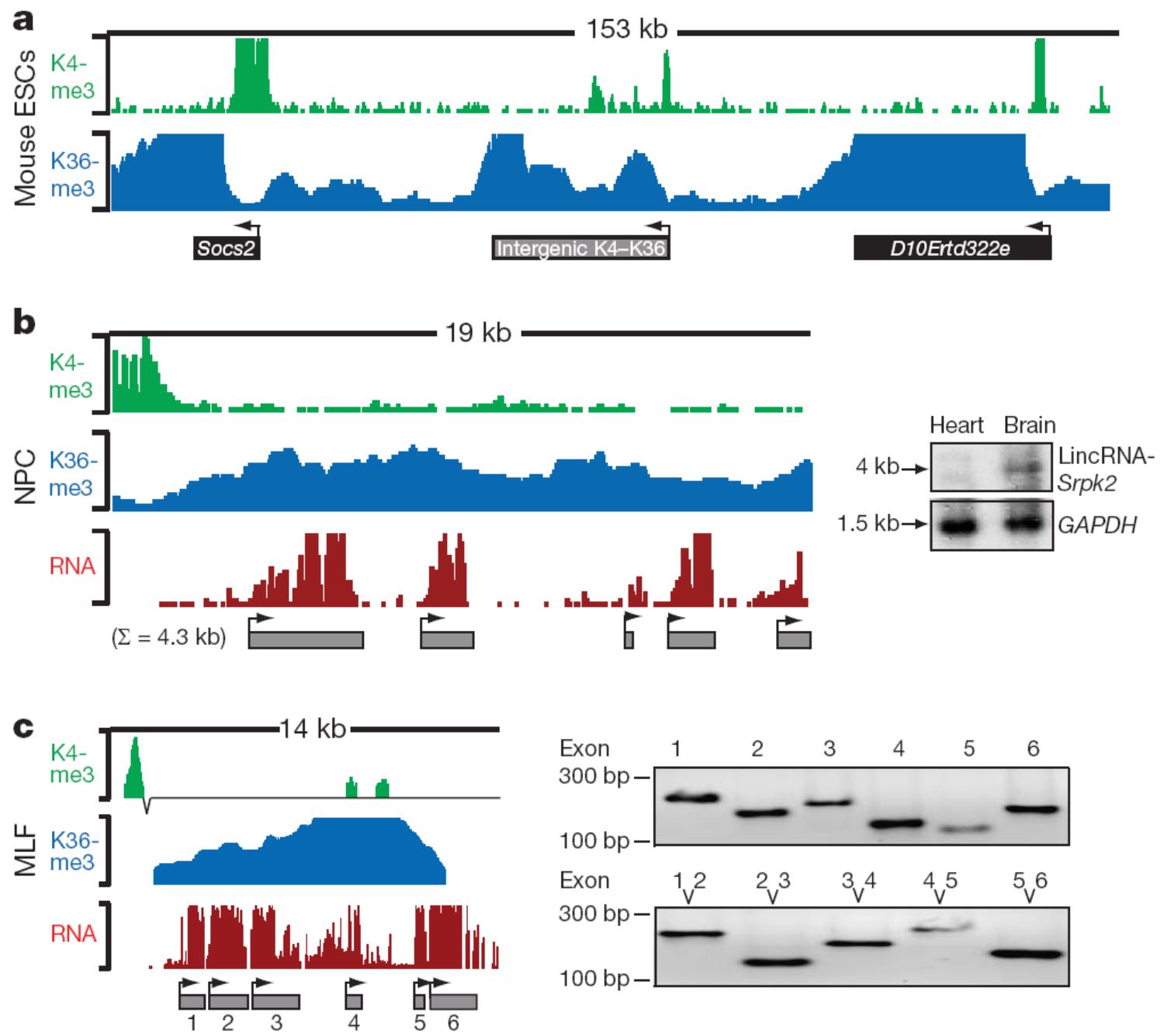
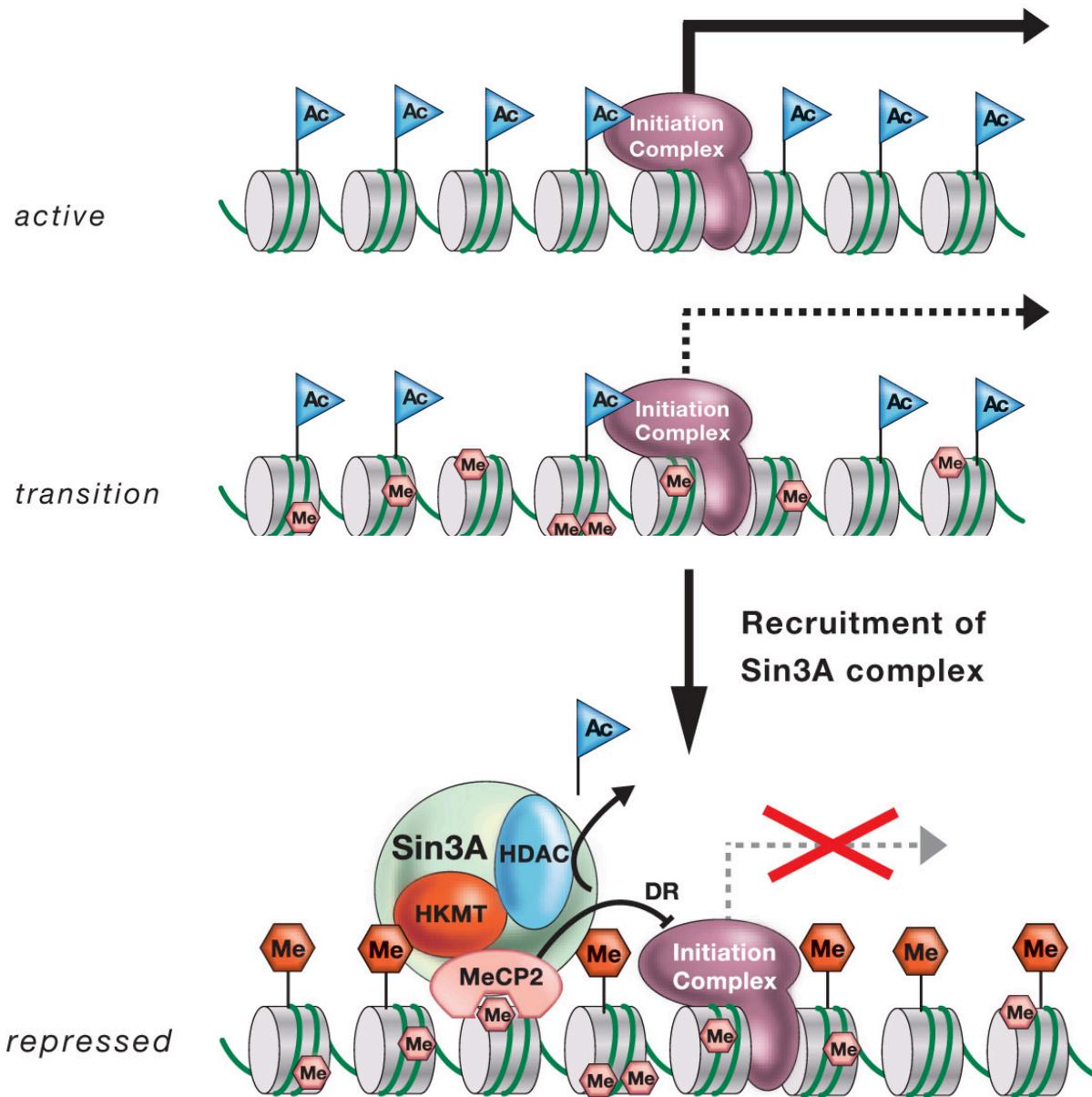
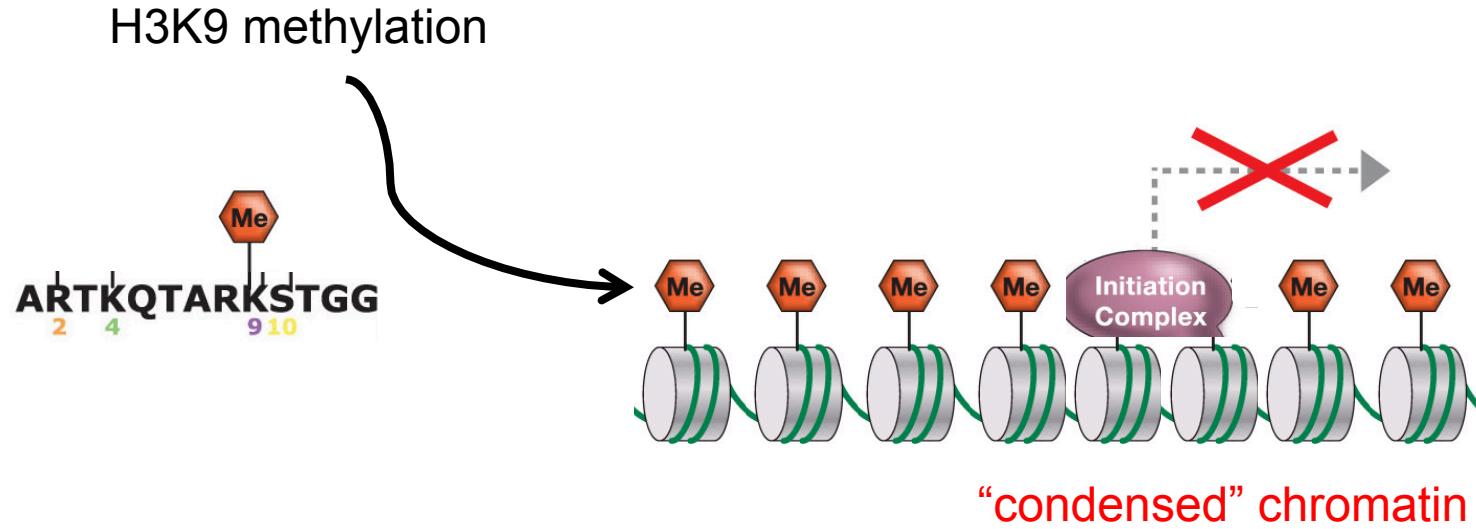


Figure 1 | Intergenic K4–K36 domains produce multi-exonic RNAs.

DNA methylation mediated repression



Repression independent of DNA methylation



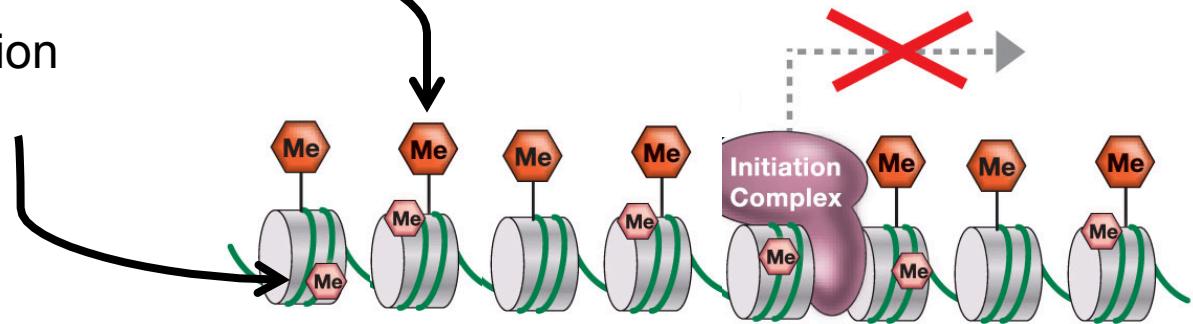
Epigenetic silencing of tumour suppressor gene *p15* by its antisense RNA

Wenqiang Yu¹, David Gius², Patrick Onyango¹, Kristi Muldoon-Jacobs², Judith Karp³, Andrew P. Feinberg^{1*}
& Hengmi Cui^{1*}

H3K27 methylation mediated repression



1. H3K27 methylation
2. DNA methylation



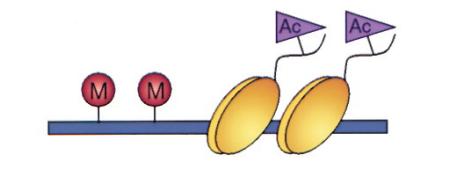
Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer

Yeshayahu Schlesinger¹, Ravid Straussman¹, Ilana Keshet¹, Shlomit Farkash², Merav Hecht¹, Joseph Zimmerman³, Eran Eden⁴, Zohar Yakhini^{4,5}, Etti Ben-Shushan⁶, Benjamin E Reubinoff⁶, Yehudit Bergman⁷, Itamar Simon² & Howard Cedar¹

Mechanisms of Epigenetic Crosstalk

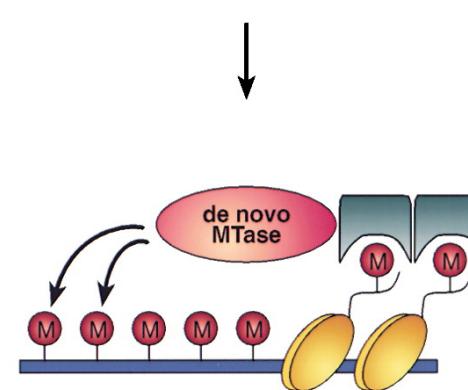
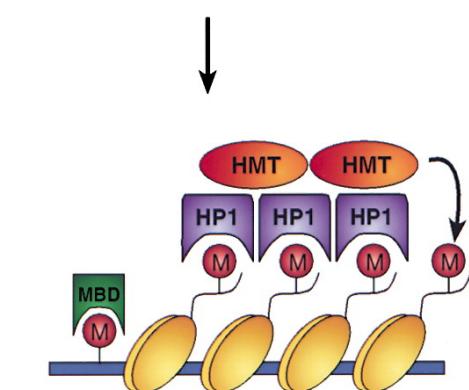
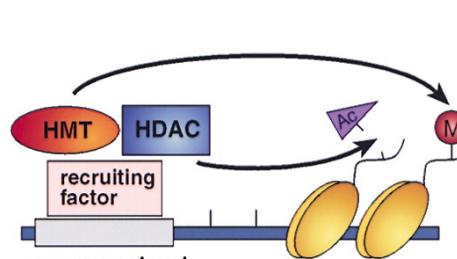
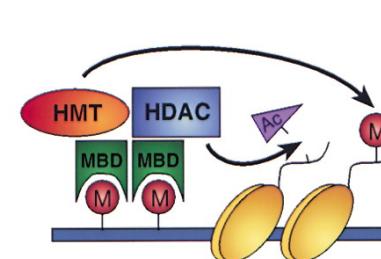
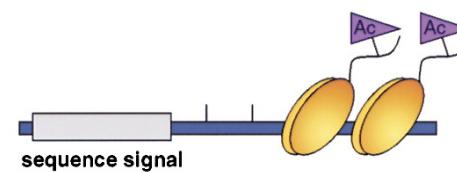
A.

Induction of silent chromatin
by DNA methylation signals

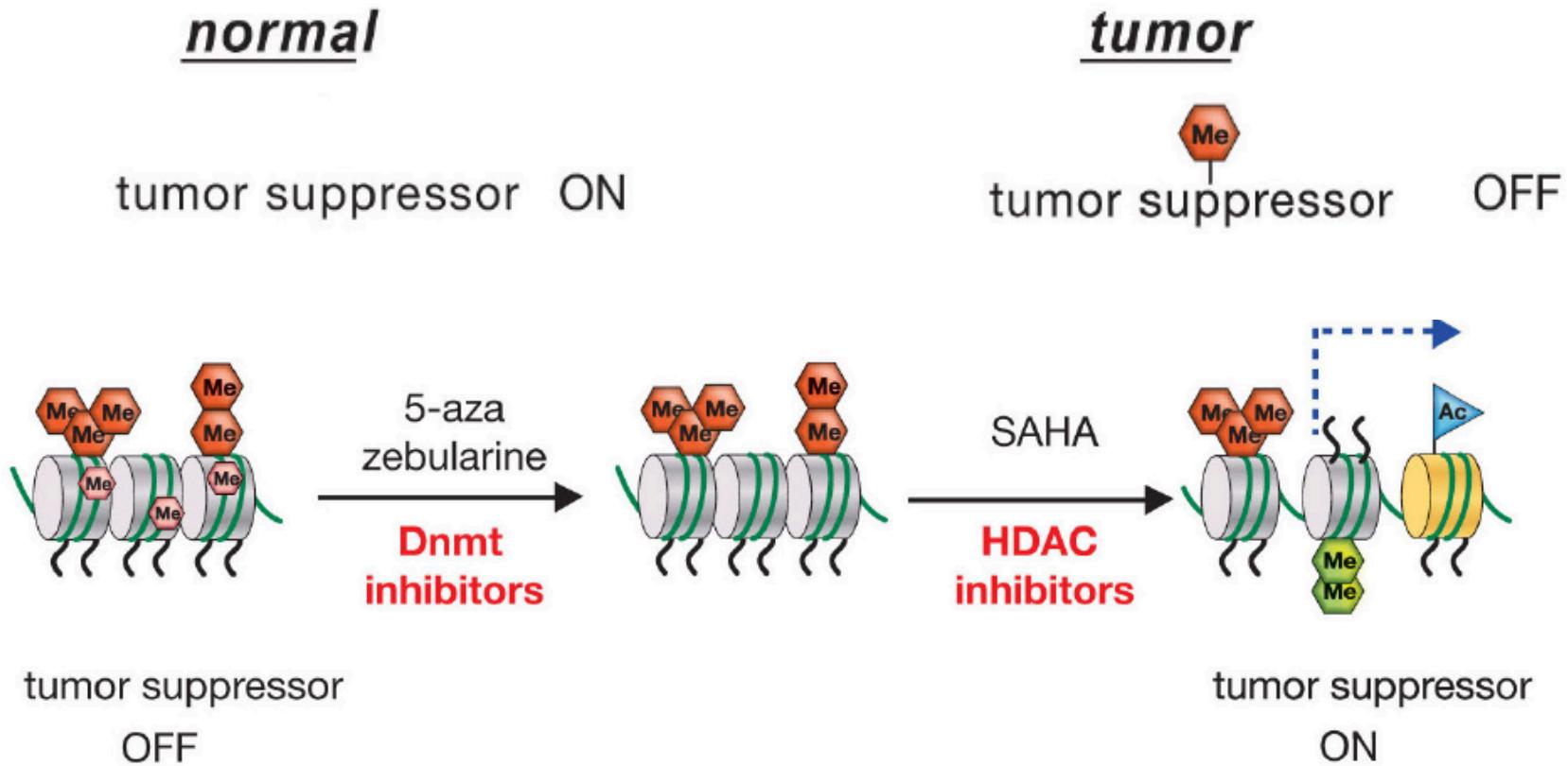


B.

Induction of DNA methylation
by chromatin signals



“Epigenetic cancer therapy”

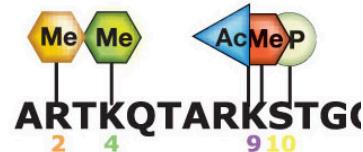


DNA-methylation and HDAC inhibitors in clinical trials

Compound	Structure	Cancer Type	Clinical Trials
DNA METHYLATION INHIBITORS			
5-Azacytidine 5-Aza-CR Vidaza		MDS; Hematologic malignancies	I, II, and III; FDA-approved for MDS
5-Aza-2'-deoxycytidine 5-Aza-CdR Dacogen		MDS; Hematologic malignancies	I, II, and III
Zebularine 1-β-D-ribofuranosyl-2(1H)-pyrimidinone		N/A	Preclinical
HISTONE DEACETYLASE INHIBITORS			
4-Phenylbutyrate (PBA)		Refractory solid tumors	I
Suberoylanilide hydroxamic acid (SAHA)		Solid tumors and hematologic malignancies	I, II
NVP-LAQ824		N/A	I

Summary

- Dnmt1, Dnmt3A, Dnmt3b - the mammalian DNMTs



- Chromatin structure is influenced by covalent modification of histone tails
- Multiple chromatin modification pathways involved in silencing of genes which may show “crosstalk” with DNA methylation

