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Histone modifications & Cancer

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Epigenetic

- Heritable changes in gene expression patterns that are not explained by DNA sequence changes.

- "Epigenetics has always been all the weird and wonderful things that can't be explained by genetics."
Denise Barlow (Vienna, Austria)

- "DNA is just a tape carrying information, and a tape is no good without a player. Epigenetics is about the tape player."
Bryan Turner (Birmingham, UK)

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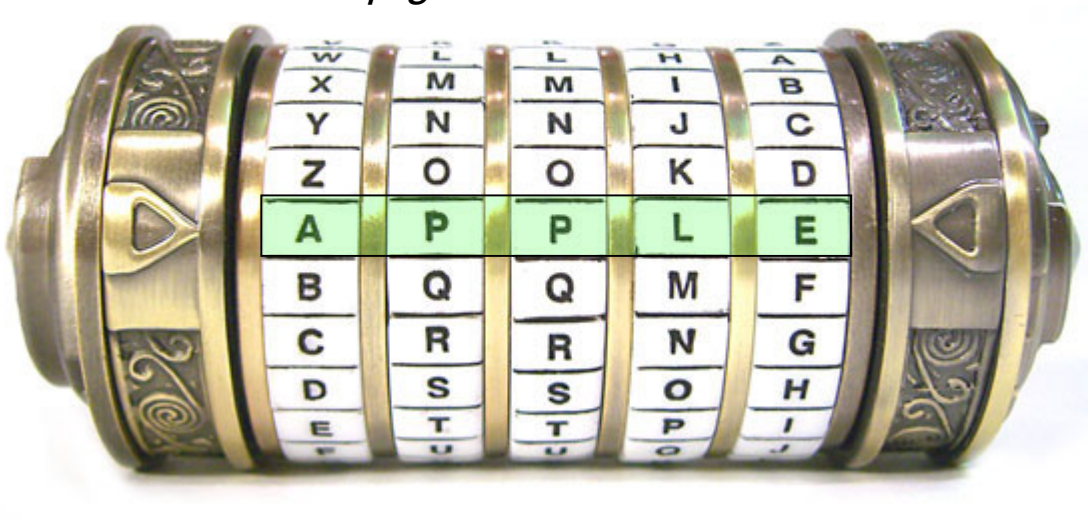
Da Vinci code
complexity is nothing compared to the
epigenetic code.



The epigenetic code is an complex language based on gene programming.

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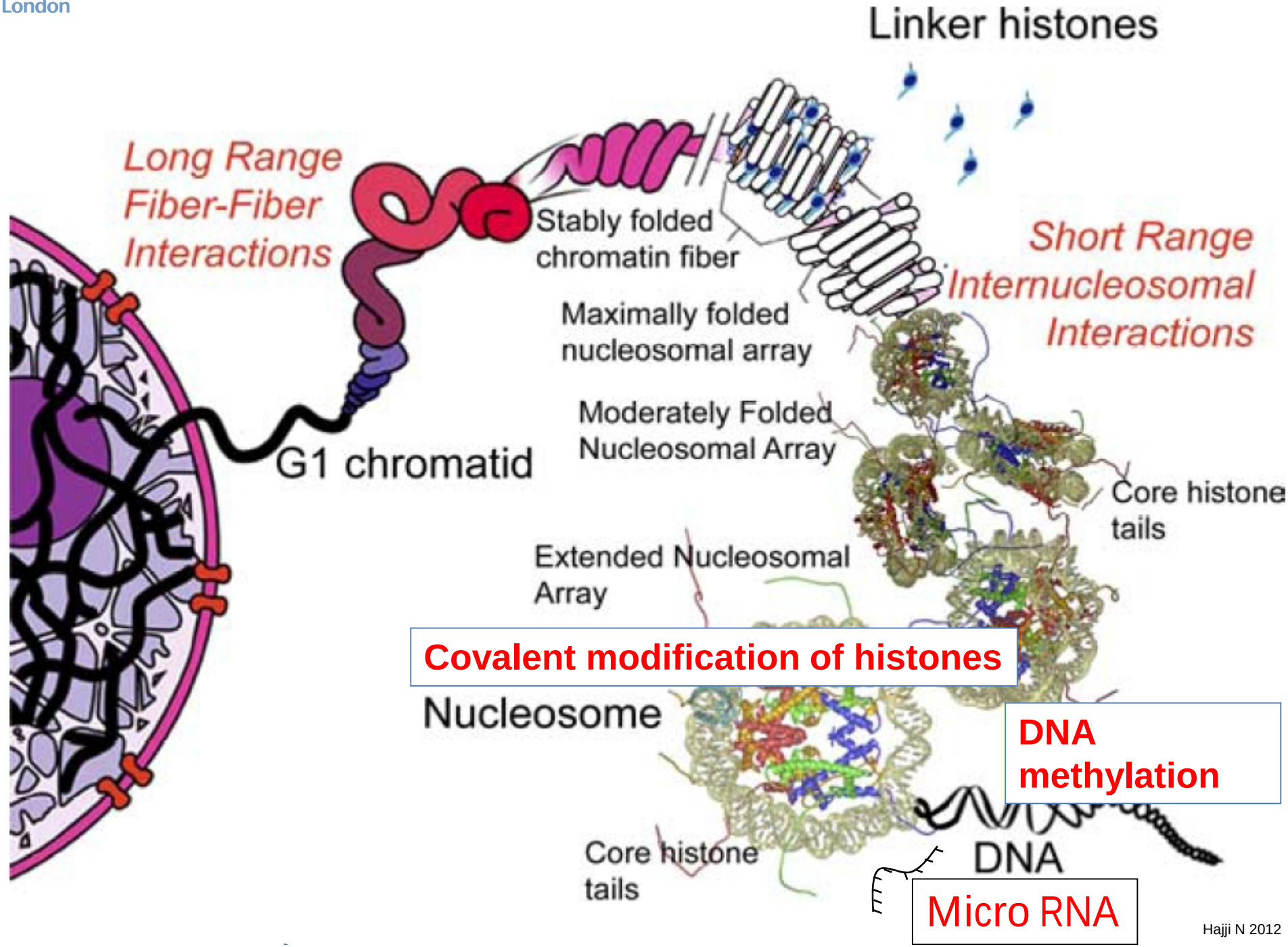

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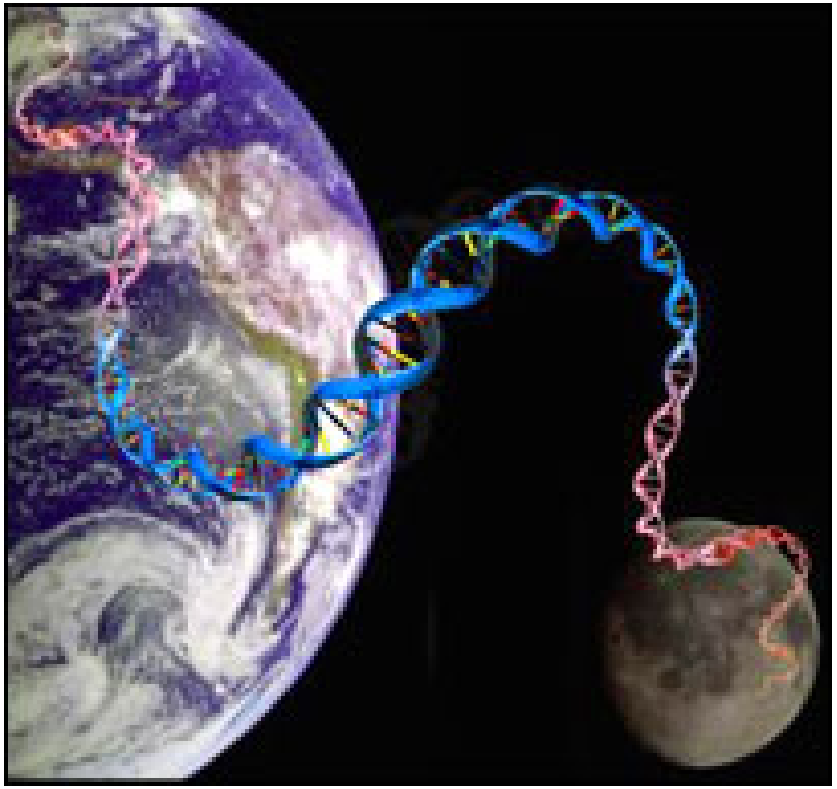
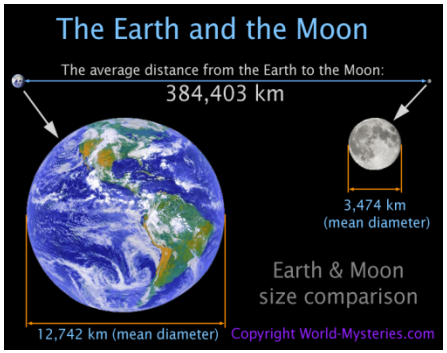
Decoded DNA would 'reach to Moon'

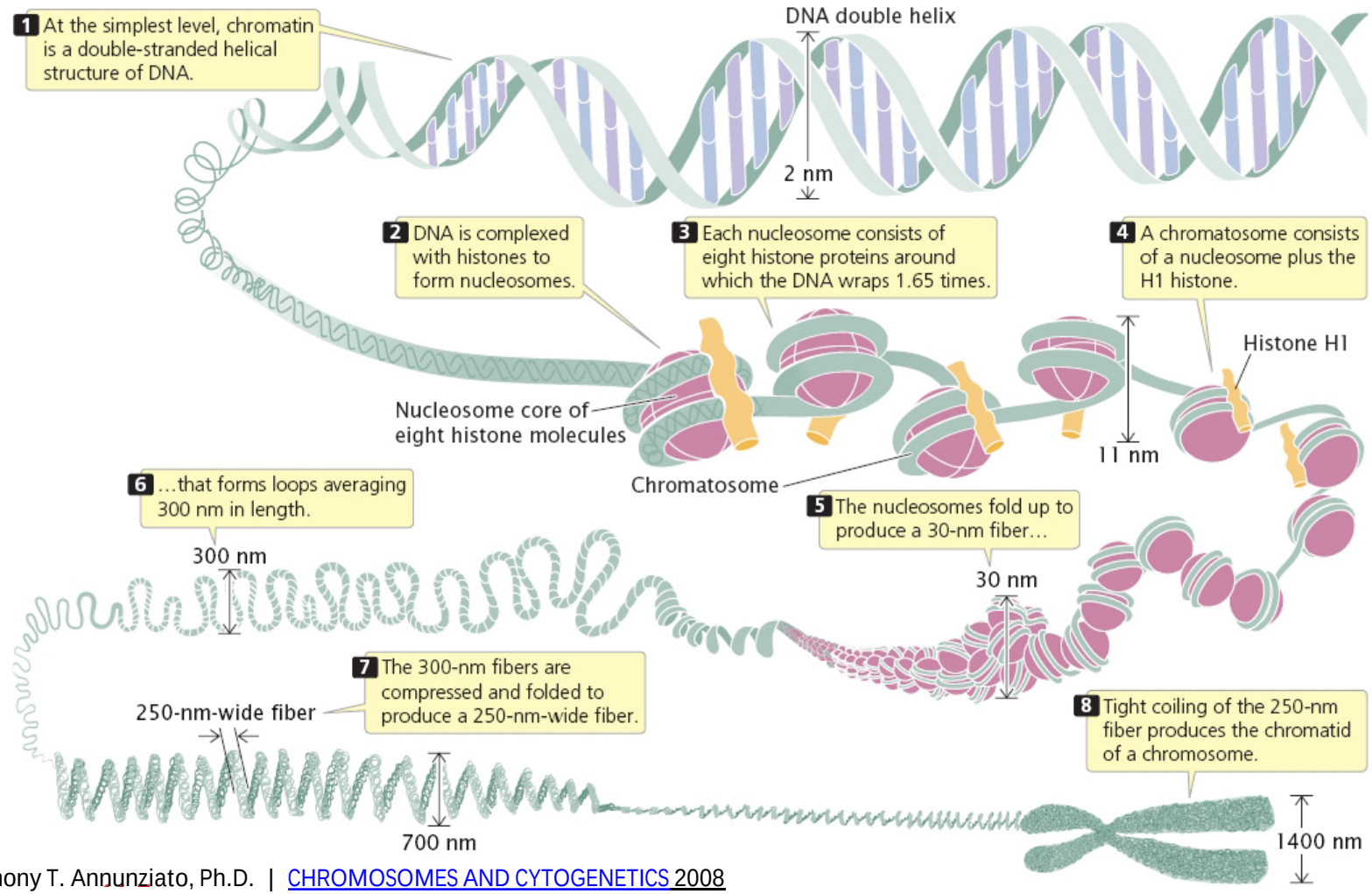


The researchers at the Wellcome Trust's Sanger Institute say that if this DNA was scaled up to the size of a spiral staircase it would stretch to the Moon. The "life code" found in cells contains information about biology, health and disease in humans and other organisms



-Every human has about 100 trillion meters of DNA coiled tightly in their cell nuclei

-Each diploid cell contains about 2 meters of DNA



Anthony T. Annunziato, Ph.D. | CHROMOSOMES AND CYTOGENETICS 2008

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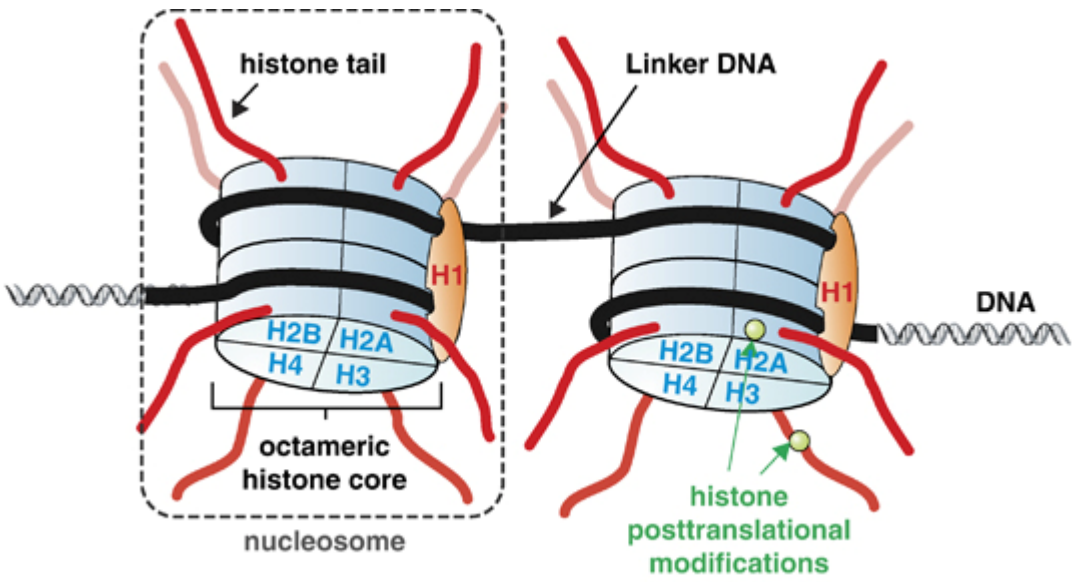
Nucleosomes = ~147 base pairs of DNA wrapped around a histone octamer.



Histone post-translational modifications

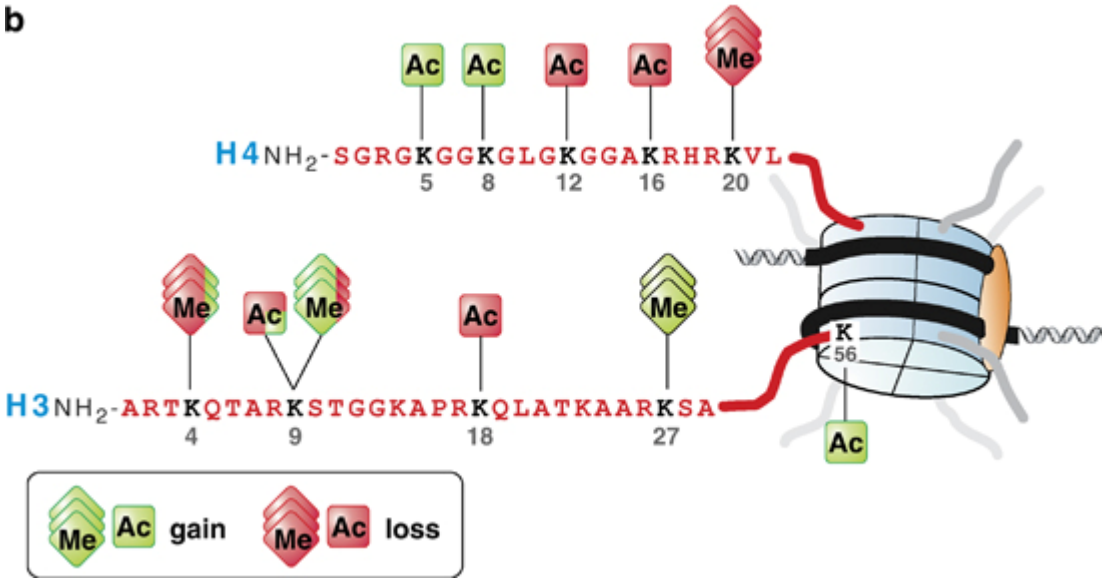
Acetylation,

Phosphorylation,

Methylation,

Ubiquitination,

Sumoylation



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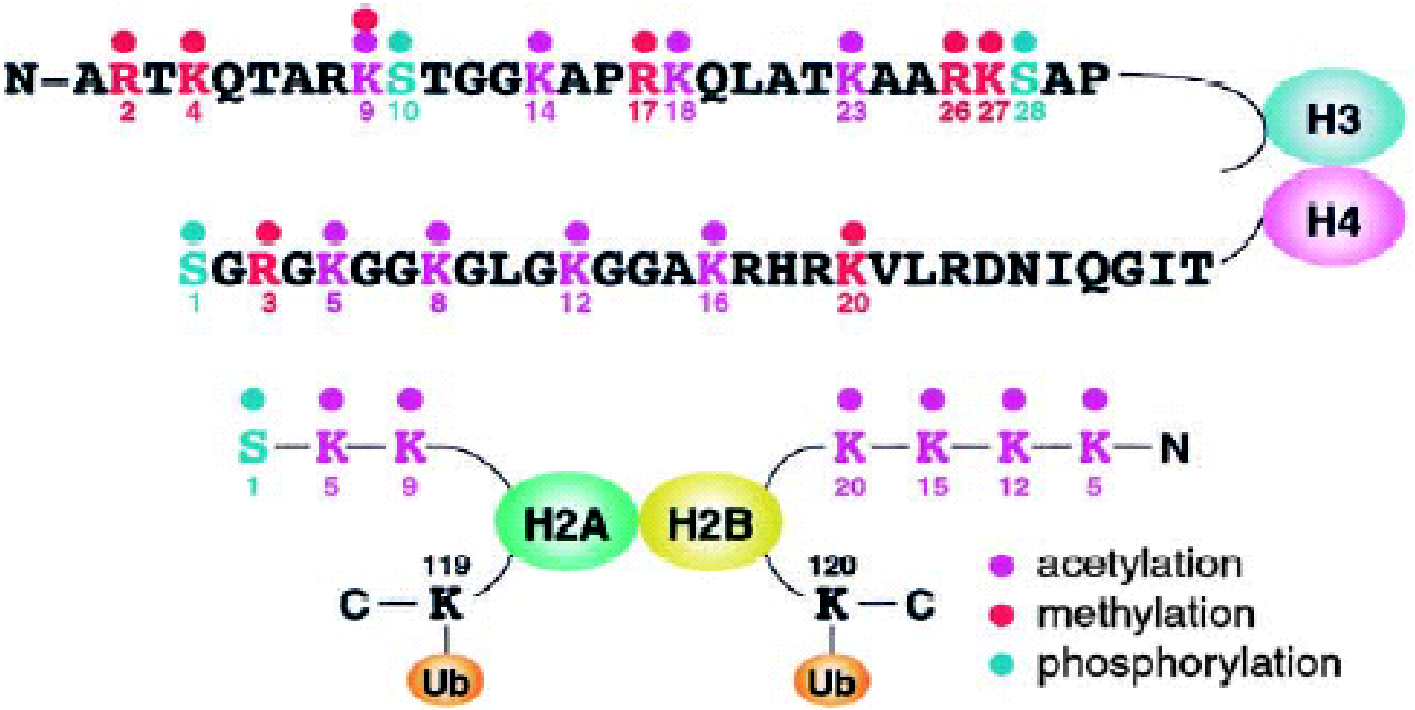
J Füllgrabe, N Hajji and B Joseph, CDD 2010

Existence of an Histone code?



Zhang, Y. and Reinberg, D. (2001)
Genes Dev. **15**: 2343-2360

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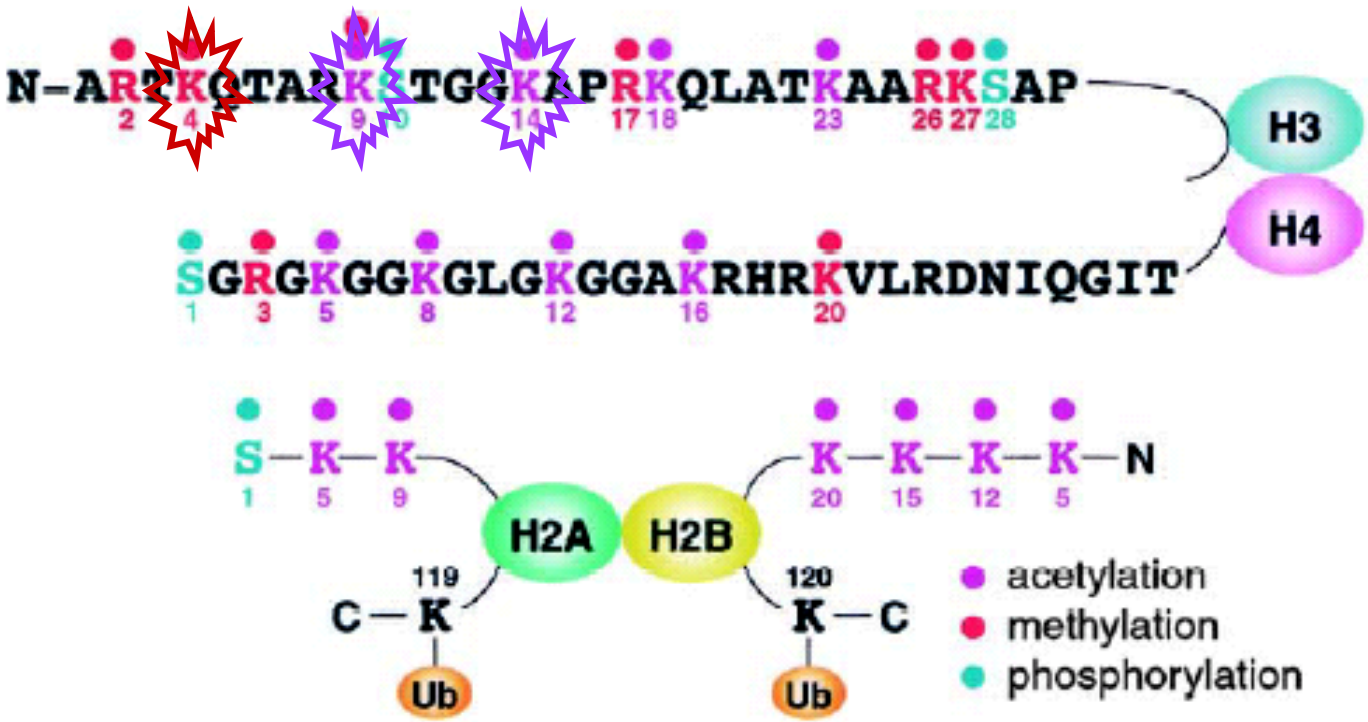
Key Aspects of the Histone Code

Transcriptionally Active Chromatin

Acetylated K9 and K14 of histone H3

Methylated K4 of histone H3



Euchromatin /Expressed genes

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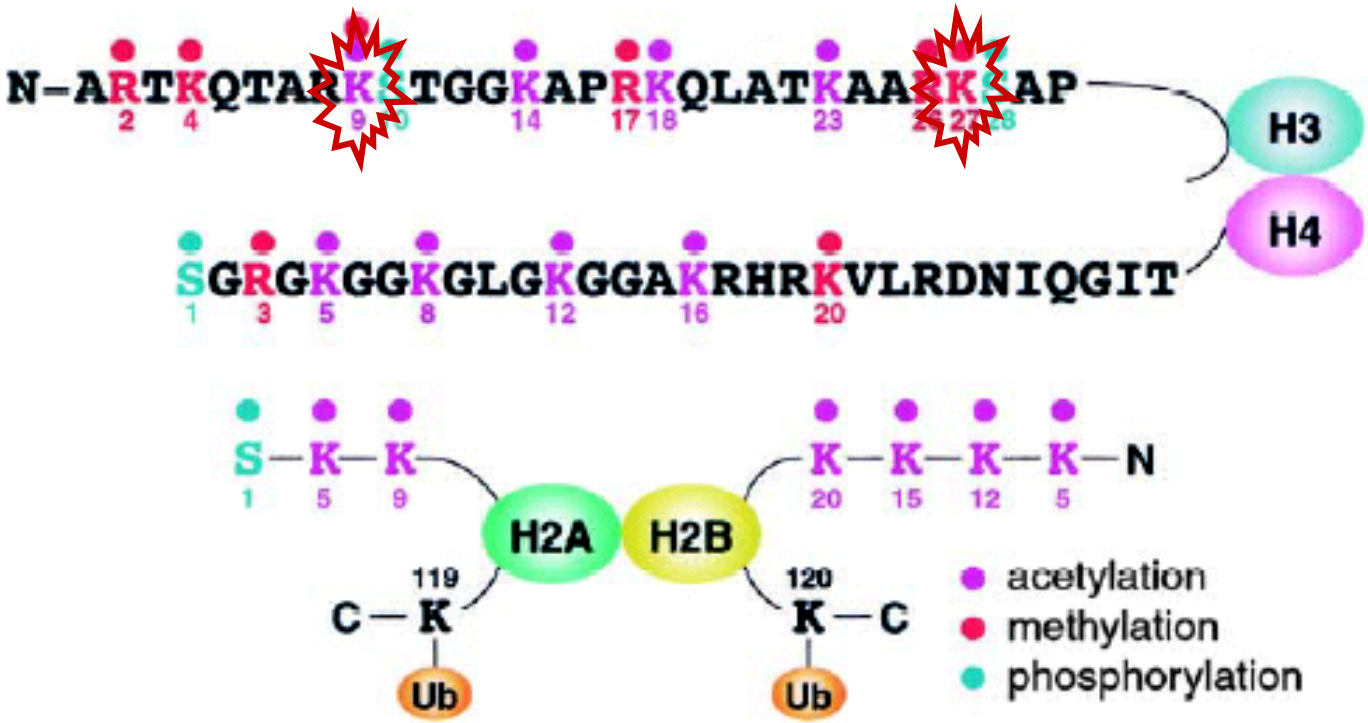
Key Aspects of the Histone Code

Transcriptionally Repressive Chromatin

Deacetylated K9 and K14 of histone H3

Methylated K9 and K27 of histone H3



Heterochromatin/Silenced genes

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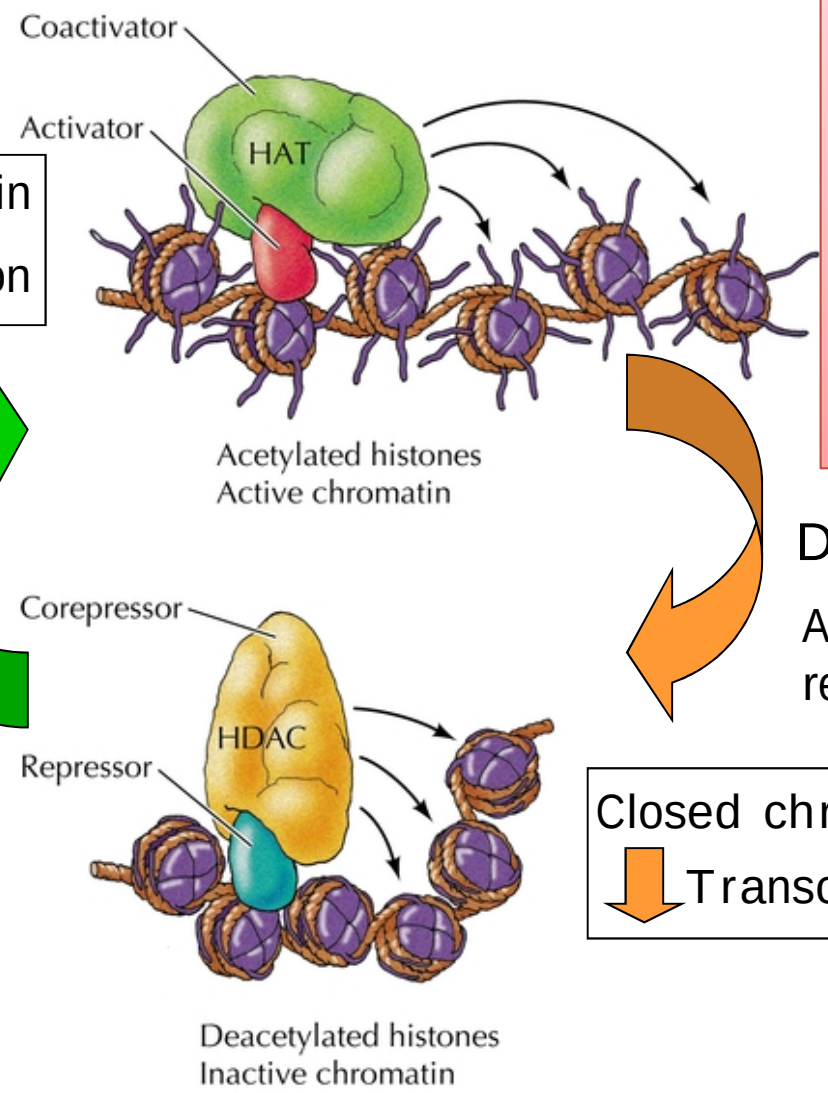
Histone acetylation machinery

Histone deacetylases

Class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8)
Class II HDACs (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10)
Class III HDACs (SIRT1–7) Sir2-like (yeast transcriptional repressor)
Class IV HDACs (HDAC11)

Open chromatin
Transcription



Acetyl groups are added
Acetylation

Deacetylation
Acetyl groups are removed

Closed chromatin
Transcription

Histone acetyltransferases

GNAT (Gcn5-related N-acetyltransferase , PCAF, Hat1, Elp3, Hpa2)
MYST (Esa1, MOF, MOZ, MORF, Ybf2/Sas3, Sas2 , Hbo1 and Tip60)
p300/CBP (p300, CBP)

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Histone methylation machinery

v Histones are methylated by histone lysine methyltransferases (HKMT)

- SUV39
- SET1
- SET2
- RIZ

Family	SUV39	SET1	SET2	RIZ
Members	Suv39h1	ySET1	ySET2	RIZ
	Suv39h2	ALR	NSD1	MDS–EVI1
	EuHMTaseI	MLL3	NSD2	MDL1
	G9a	MLL2	NSD3	BLIMP1
	ESET	MLL1	HIF1	PFM1
	CLLL8	EZH2	ASH1	MEL1
		EZH1		
		SET7		

v Histones are methylated by histone arginine methyltransferases (HRMT)

•PRMT1-3, CARM1

v Histones are demethylated by histone demethylases (HDM)

- PADI Peptidylarginine deiminases
- LSD1 lysine-specific demethylase-1
- JmjC-domain containing proteins (JHDM1, PHF2/PHF8, JARID, JHMD3/JMJD2, UTX/UTY, JHDM2, JmjC only) *lysine* or *arginine* residues

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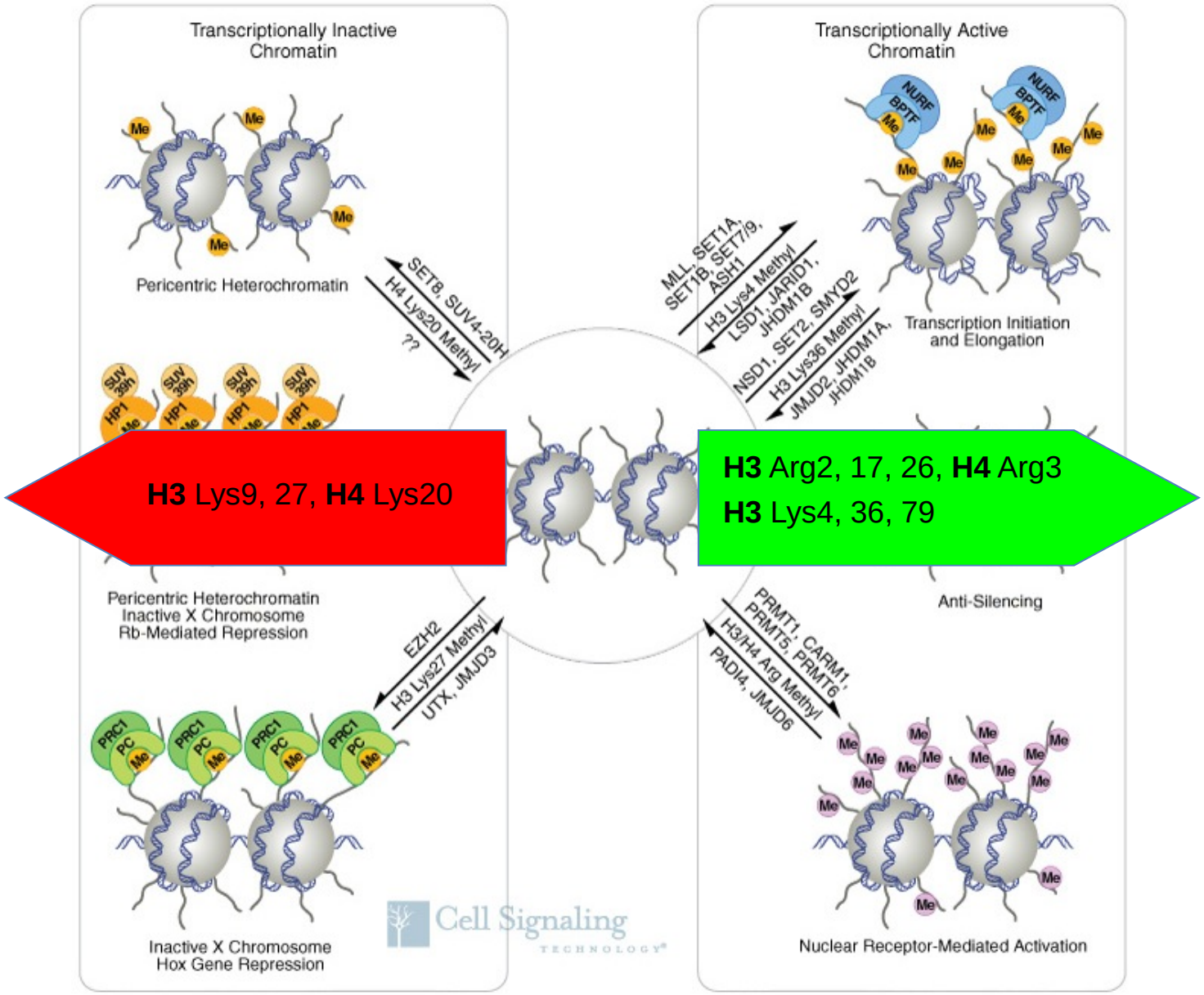
Histone methylation



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⊏nzyme families involved in Histone PTMs regulation

- Histone acetylation.
  histone acetylases (HAT)
  histone deacetylases (HDAC)

- Histone methylation.
  histone lysine methyltransferases (HMT)
  histone demethylases (HDM)

Histone modifications and DNA methylation are coupled

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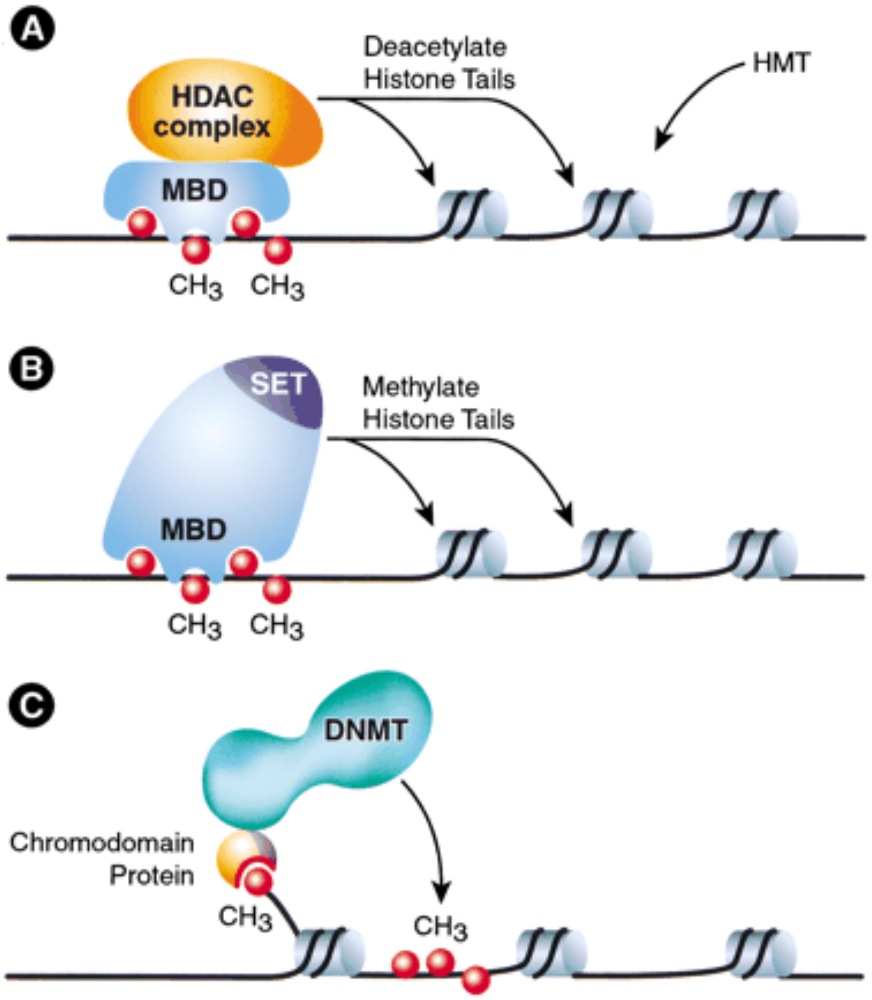
(A) Methyl-CpG-binding proteins recruit HDAC complex to deacetylate histone so that the histone tails will be suitable for subsequent methylation by HMTs.

(B) In chromatin domains where histones are hypoacetylated, the MBD domain-containing HMTs may bind directly and methylate the histones.

(C) Methylated histone tails may recruit DNMTs to methylate DNA for long-term gene silencing.



Zhang, Y. and Reinberg, D. (2001)
Genes Dev. 15: 2343-2360

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Histone post-translational modifications (PTMs) involvement in cellular functions and cancer

Post-translational modification of histones provides an important regulatory platform for multiple cell biological processes.

Disruption

- **gene expression**
- **DNA replication**
- **DNA repair**
- **DNA stability**
- **Chromosome condensation**
- **Chromosome segregation**
- **Apoptosis.**

Process of carcinogenesis

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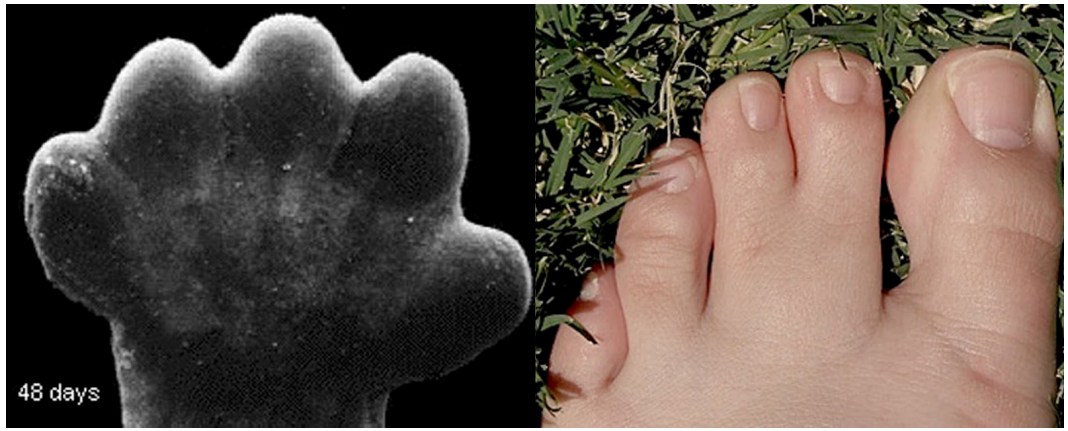
For every cell, there is a time to live and a time to die.

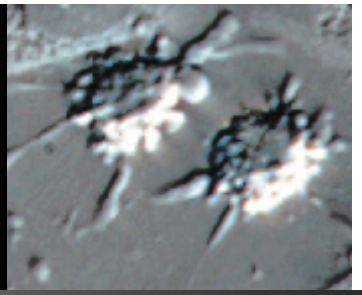
Apoptosis or programmed cell death (PCD)

PCD is a key regulator of physiological growth control and regulation of tissue homeostasis.



Hands or feet develop with individual fingers, but covered by tissue all over.
-Apoptosis divides them into individual fingers or toes. The apoptosis alteration could lead to fused Digits (Syndactyly). http://biologicalexceptions.blogspot.co.uk/2012/06/cellular-self-sacrifice.html

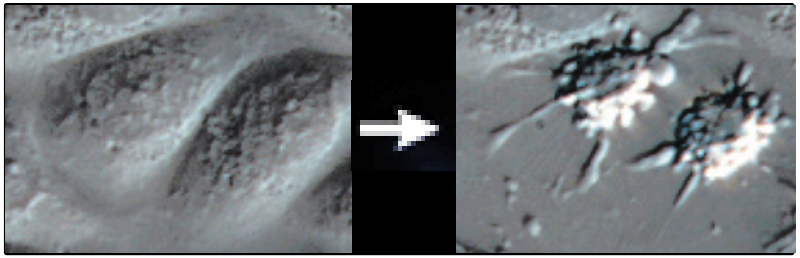
APOPTOSIS



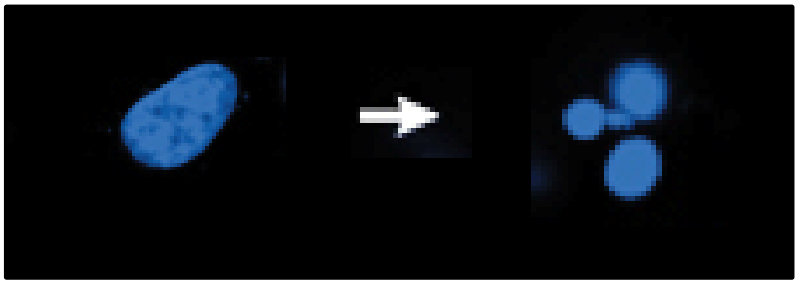
“Evolutionary strictly conserved suicidal mechanism of the cell”

“Active” Cell Death:

- **cell shrinkage**
- **membrane blebbing**



- **chromatin condensation and nuclear fragmentation**



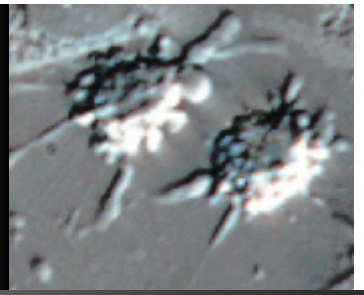
Apoptosis or programmed cell death (PCD)

PCD alteration

Cancer

Apoptosis is one of the most important advances in cancer research in recent years is the recognition that cell death alteration mostly by apoptosis is crucially involved in the regulation of tumor formation and also critically determines treatment response.

APOPTOSIS & CANCER



Apoptosis alteration

Hallmark of cancer

à Tumourigenesis

à Metastasis

à Therapy resistance

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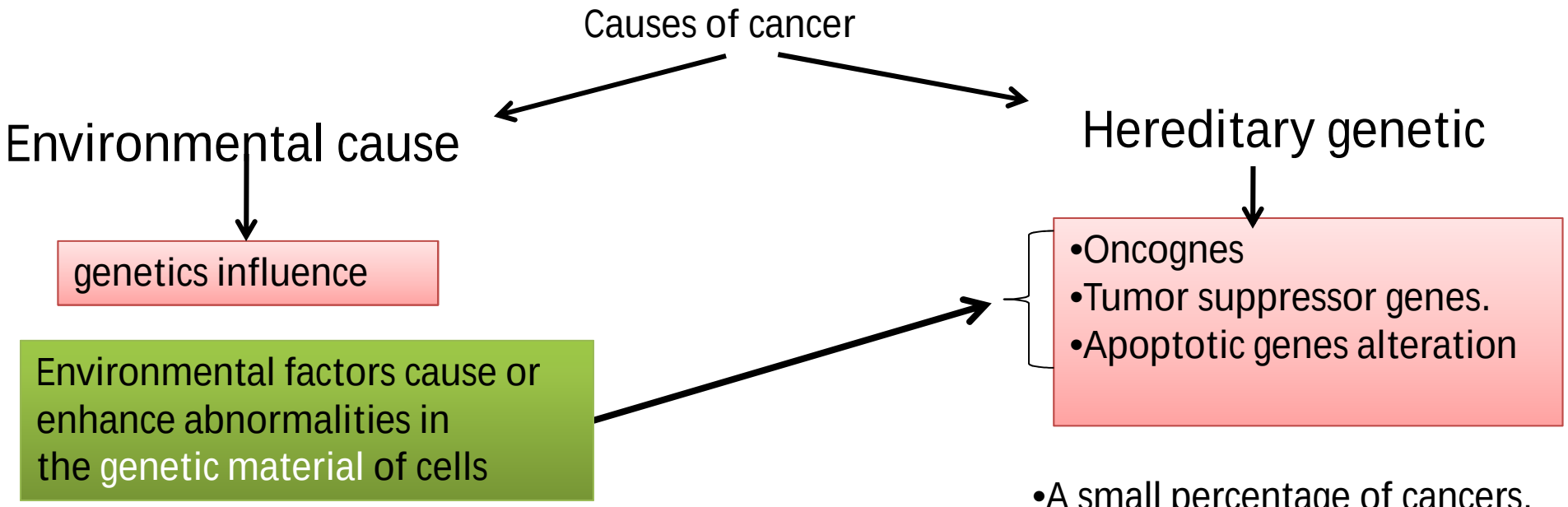
Epigenetic alteration in cancer



Tobacco/diet/obesity/infections/radiation/lack of physical activity/environmental pollutants.

•A small percentage of cancers, approximately 5 to 10 %, are entirely hereditary

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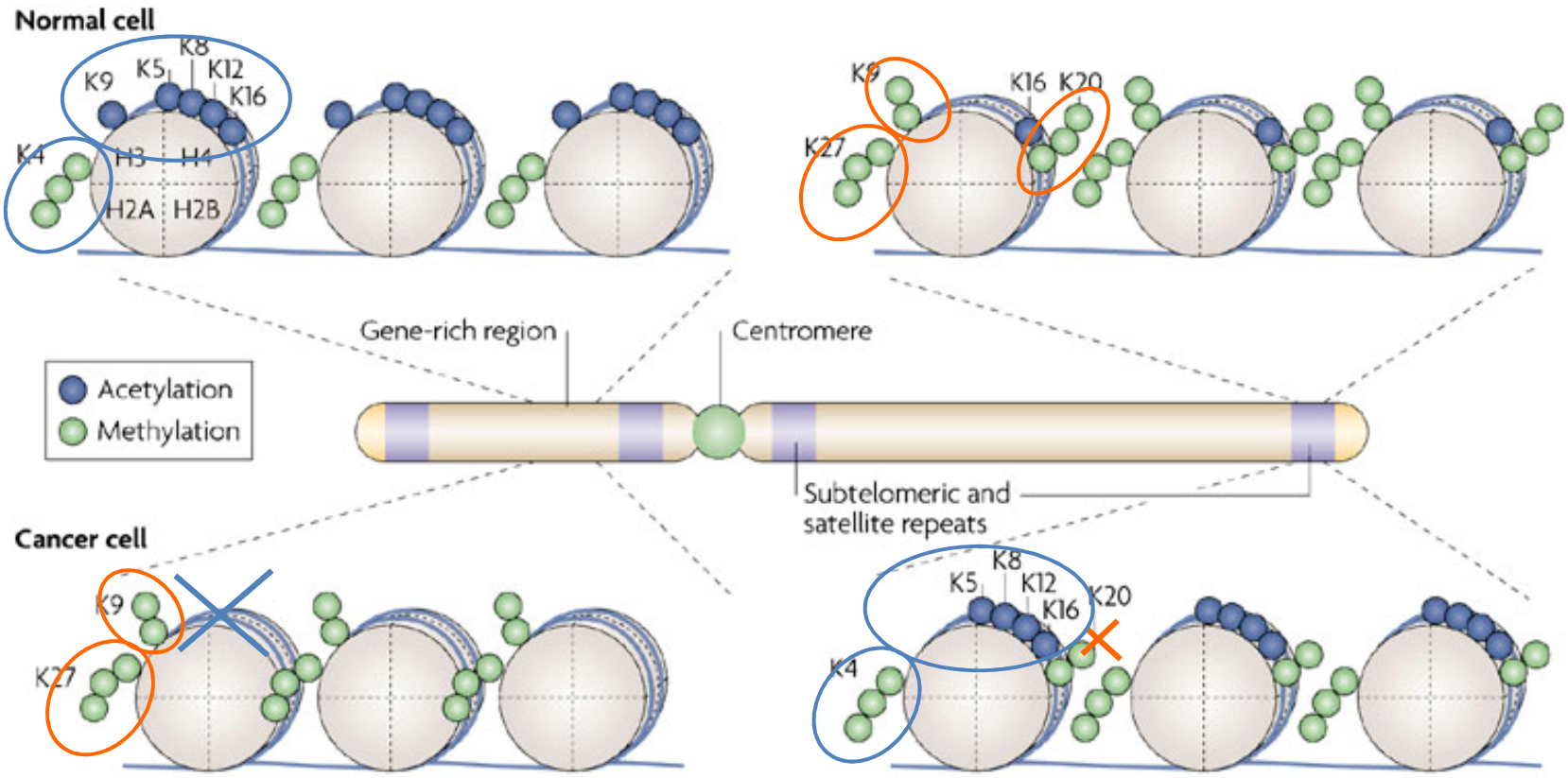
Histone-modification maps for a typical chromosome in normal and cancer cells



In 'normal' cells, genomic regions that include the promoters of tumour-suppressor genes are enriched in histone- modification marks associated with active transcription, such as acetylation of H3 and H4 lysine residues (for instance K5, K8, K9, K12 and K16) and trimethylation of K4 of H3. In the same cells, DNA repeats and other heterochromatic regions are characterized by trimethylation of K27 and dimethylation of K9 of H3, and trimethylation of K20 of H4, which function as repressive marks. In transformed cells, this scenario is disrupted by the loss of the 'active' histone-marks on tumour-suppressor gene promoters, and by the loss of repressive marks such as the trimethylation of K20 of H4 or trimethylation of K27 of histone H3 at subtelomeric DNA and other DNA repeats. This leads to a more 'relaxed' chromatin conformation in these regions.

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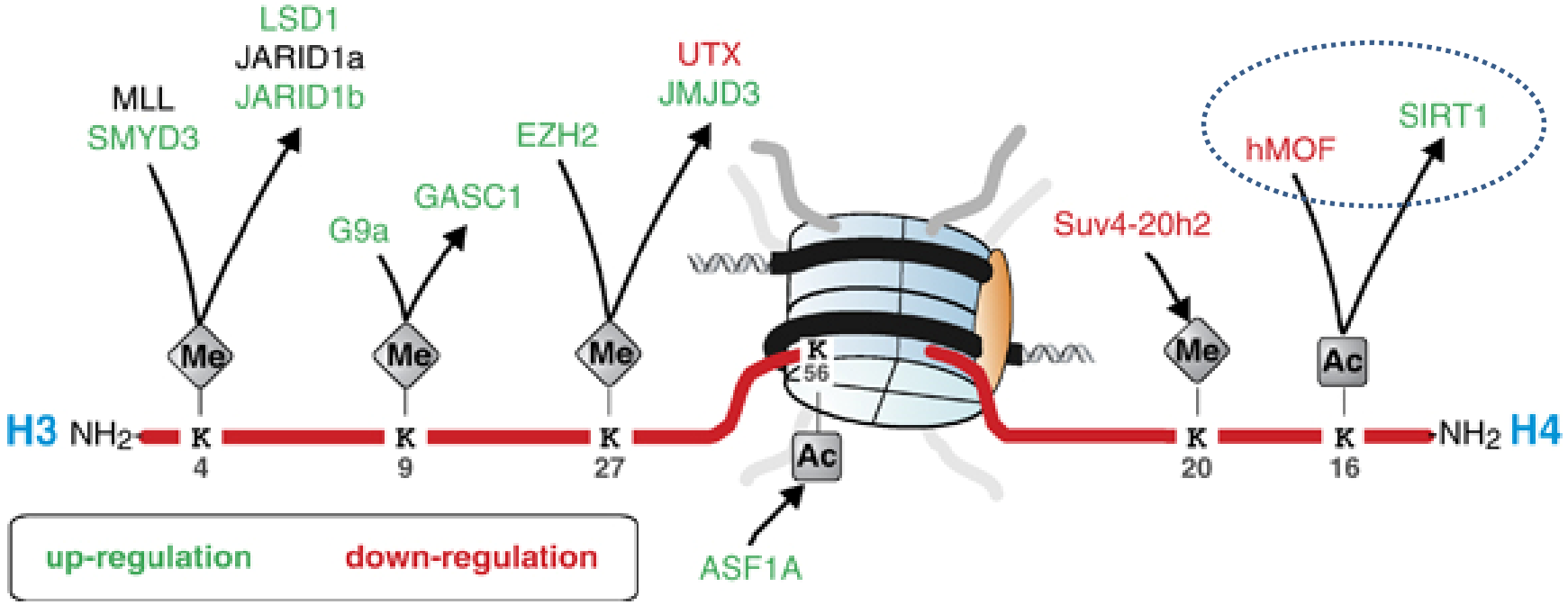
Genes deregulated by post-translational histone modifications in cancer



Histone Onco-modification	Enzymes	*Genes affected*	Gene function	References
↓ H4K16ac	hMOF, SIRT1	***TMS1/ASC***	Apoptosis/Inflammation	(Kapoor-Vazirani *et al.* 2008)

J Füllgrabe, N Hajji and B Joseph, CDD . 2010

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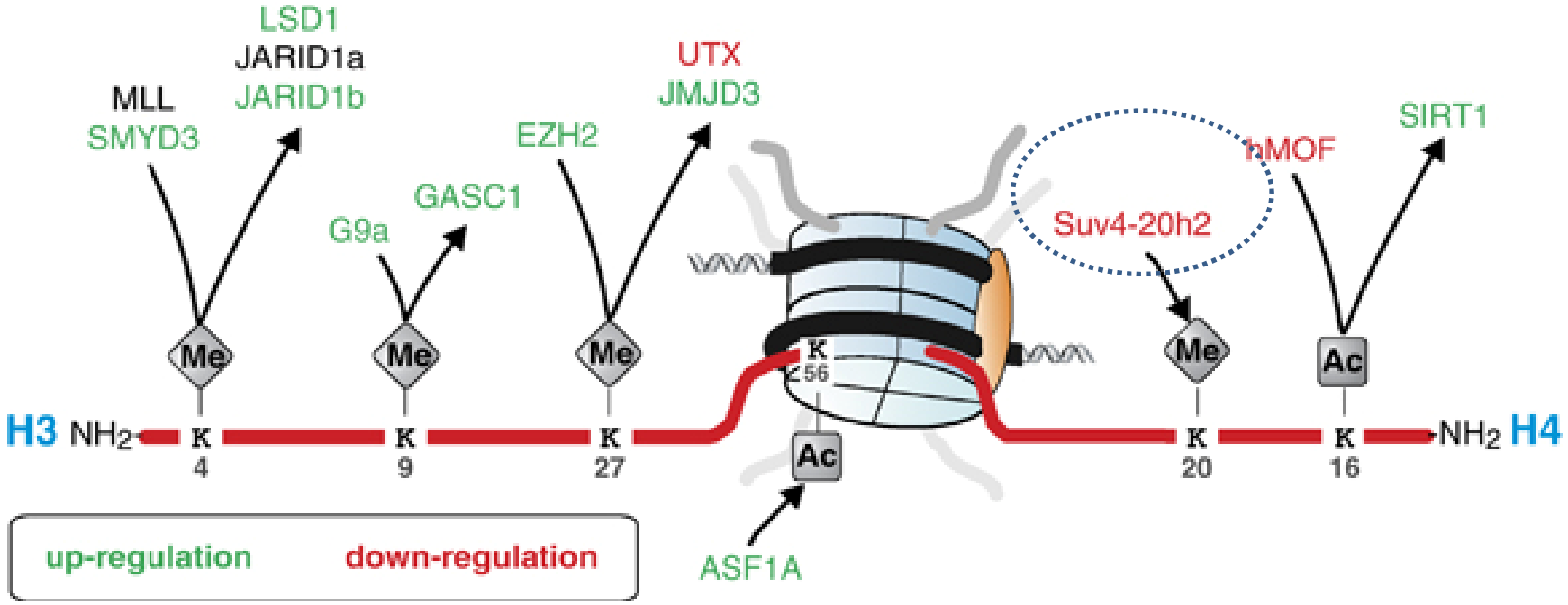
Additional support information

Genes deregulated by post-translational histone modifications in cancer



Histone Onco-modification	Enzymes	*Genes affected*	Gene function	References
↓ H4 K20me3	Pr-Set7, Suv4-20	***CLDN3/4***	Cell-cell adhesion	(Kwon *et al.* 2010)

J Füllgrabe, N Hajji and B Joseph, CDD . 2010

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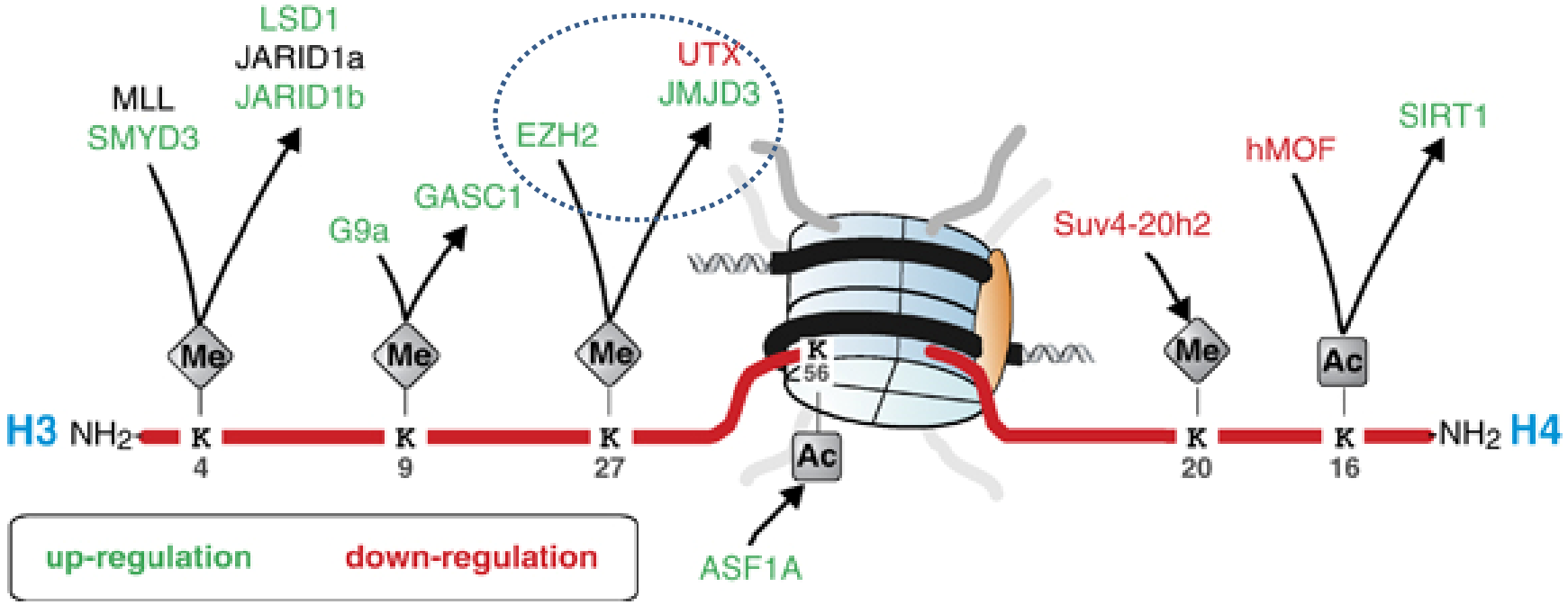
Additional support information

Genes deregulated by post-translational histone modifications in cancer



Histone Onco-modification	Enzymes	*Genes affected*	Gene function	References
H3K27me3	EZH2	***BIM***	Apoptosis	(Wu *et al.* 2010)
		Vasohibin-1	Angiogenesis	(Lu *et al.* 2010)
		TGFβ-1	Cell growth, proliferation, differentiation, apoptosis	(Rao *et al.* 2010)
		INK4A-ARF	Cell cycle, apoptosis	(Bracken *et al.* 2007)
		CDKN1C	Cell cycle, apoptosis	(Guo *et al.* 2011)
		OCT4, NANOG	Pluripotency	(Shen *et al.* 2008)

J Füllgrabe, N Hajji and B Joseph, CDD . 2010

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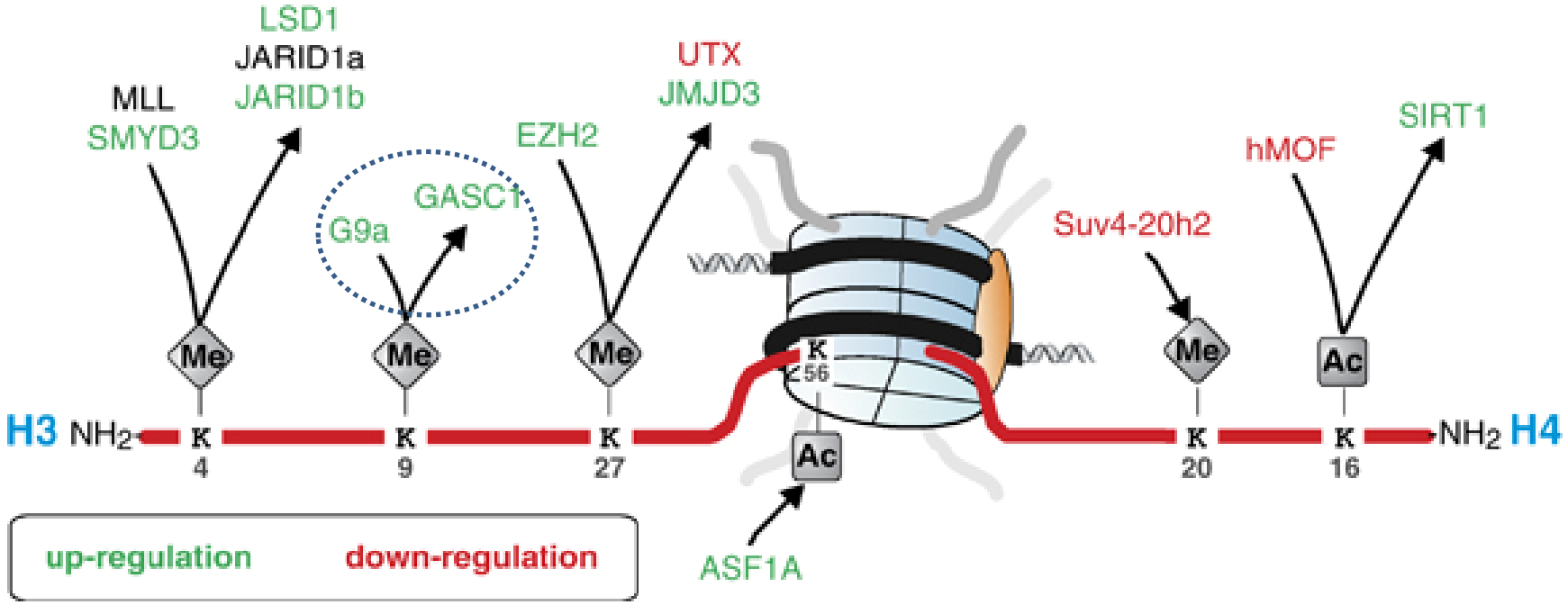
Additional support information

Genes deregulated by post-translational histone modifications in cancer



Histone Onco-modification	Enzymes	*Genes affected*	Gene function	References
H3K9me2	G9a	***EPCAM***	Cell adhesion	(Chen *et al.* 2010)
		INK4A	Cell cycle, apoptosis	(Bachman *et al.* 2003)

J Füllgrabe, N Hajji and B Joseph, CDD . 2010

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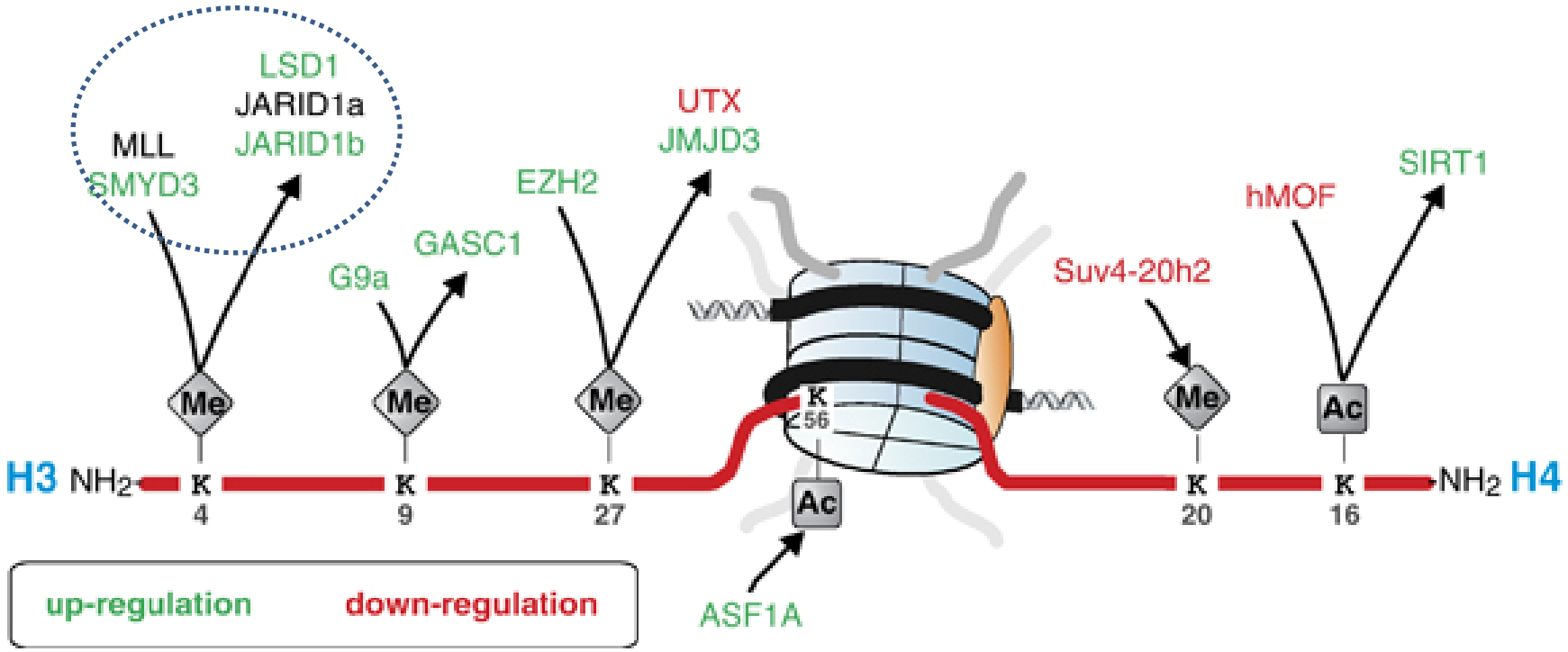
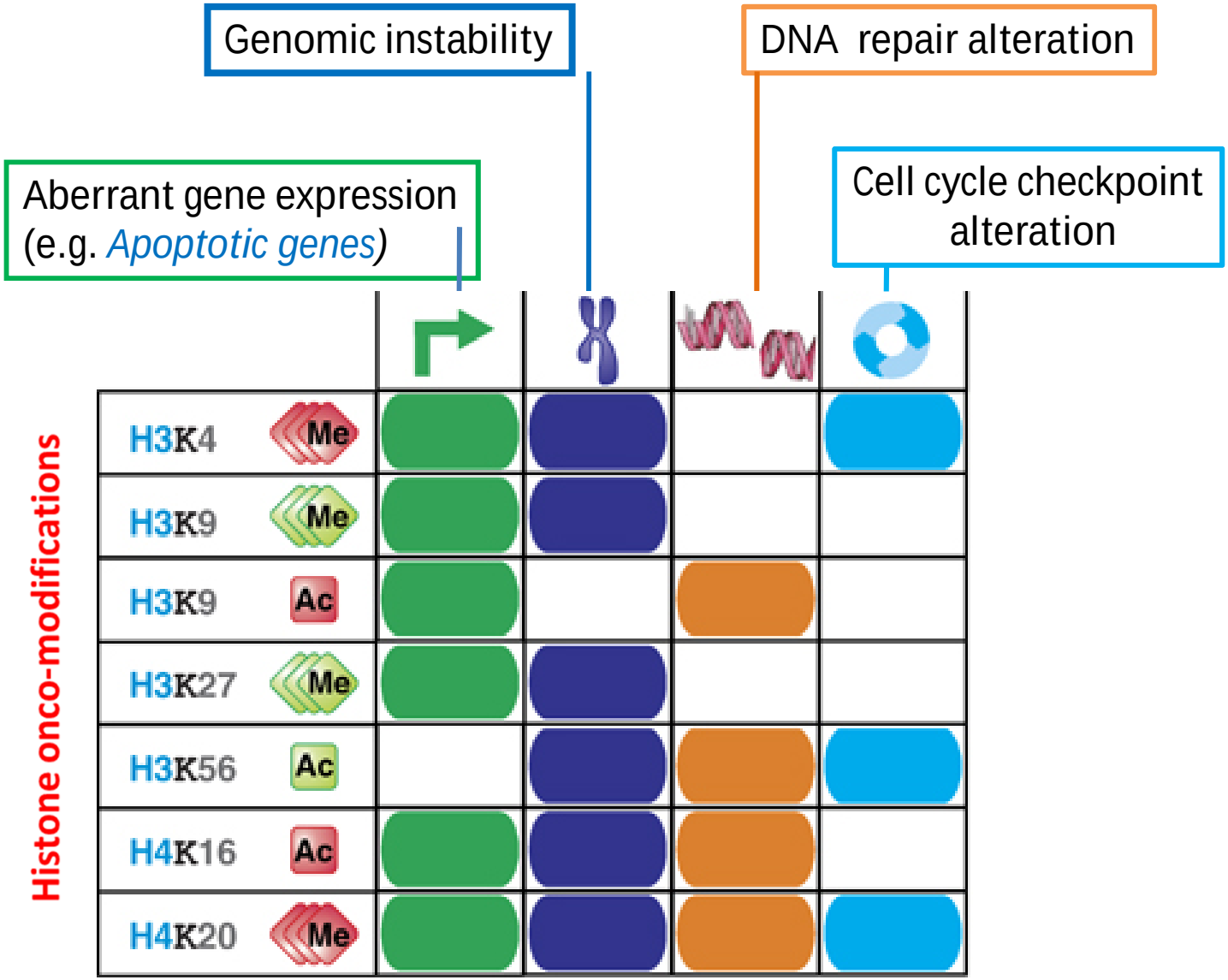
Additional support information

Genes deregulated by post-translational histone modifications in cancer



Histone Onco-modification	Enzymes	*Genes affected*	Gene function	References
H3K4me3	SMYD3	***N-MYC***	Proliferation	(Ren *et al.* 2010)
		CRKL	Proliferation, invasion, apoptosis	
		WNT10B	Proliferation	
		RIZ	Proliferation	
		hTERT	Limitless replication potential	
		Cyclin G1	Cell cycle	
		CDK2	Cell cycle	

J Füllgrabe, N Hajji and B Joseph, CDD . 2010



Functional consequences of histone onco-modifications. Specific histone modifications, which have been shown to occur in cancer cells, are displayed and their implication in cancer associated processes, such as aberrant gene expression (in green), genomic instability (in purple), DNA repair (in orange) and cell cycle checkpoint alterations (in blue). ac, acetylated; H, histone; K, lysine; me, methylated.

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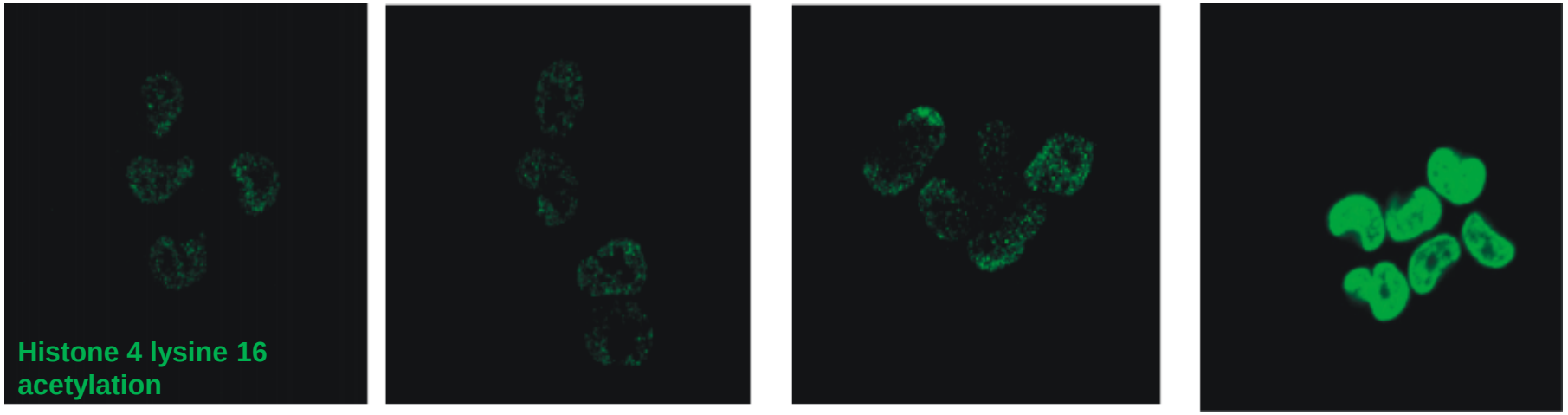
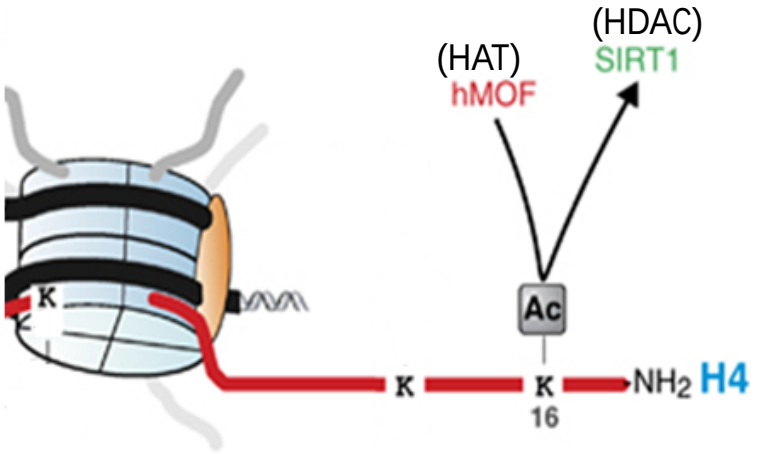
Histone onco-modifications

NSCLC		Neuroblastoma	Neural stem cell
H157	H1299	SK-N-BE(2)	NSC





Decreased H4 K16 acetylation in cancer Lead to aberrant apoptotic gene expression and increase resistance to drug treatment

Hajji N et al., 2010 . Oncogene

The precise balance of the acetylated and deacetylated states of histones is an important feature of gene regulation.

HDAC overexpression

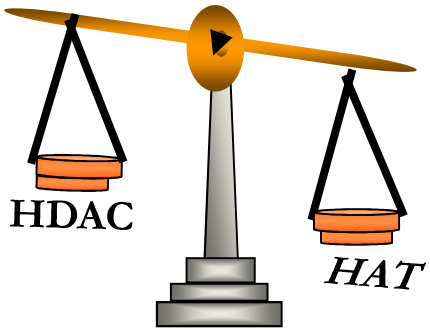
HDAC1 (prostate cancer)

HDAC2 (colon cancer)

SIRT1 Human colon cancer,
Breast cancer,
Prostate cancer,
Squamous cell carcinoma,
Human non-small-cell lung cancer cell
(Associated with oncogenic transcription factors)

HAT downregulation

hMOF (GNAT-MYST family) is frequently downregulated in primary breast carcinomas and medulloblastoma and *constitutes a biomarker for clinical outcome in medulloblastoma* .



Gene expression alteration

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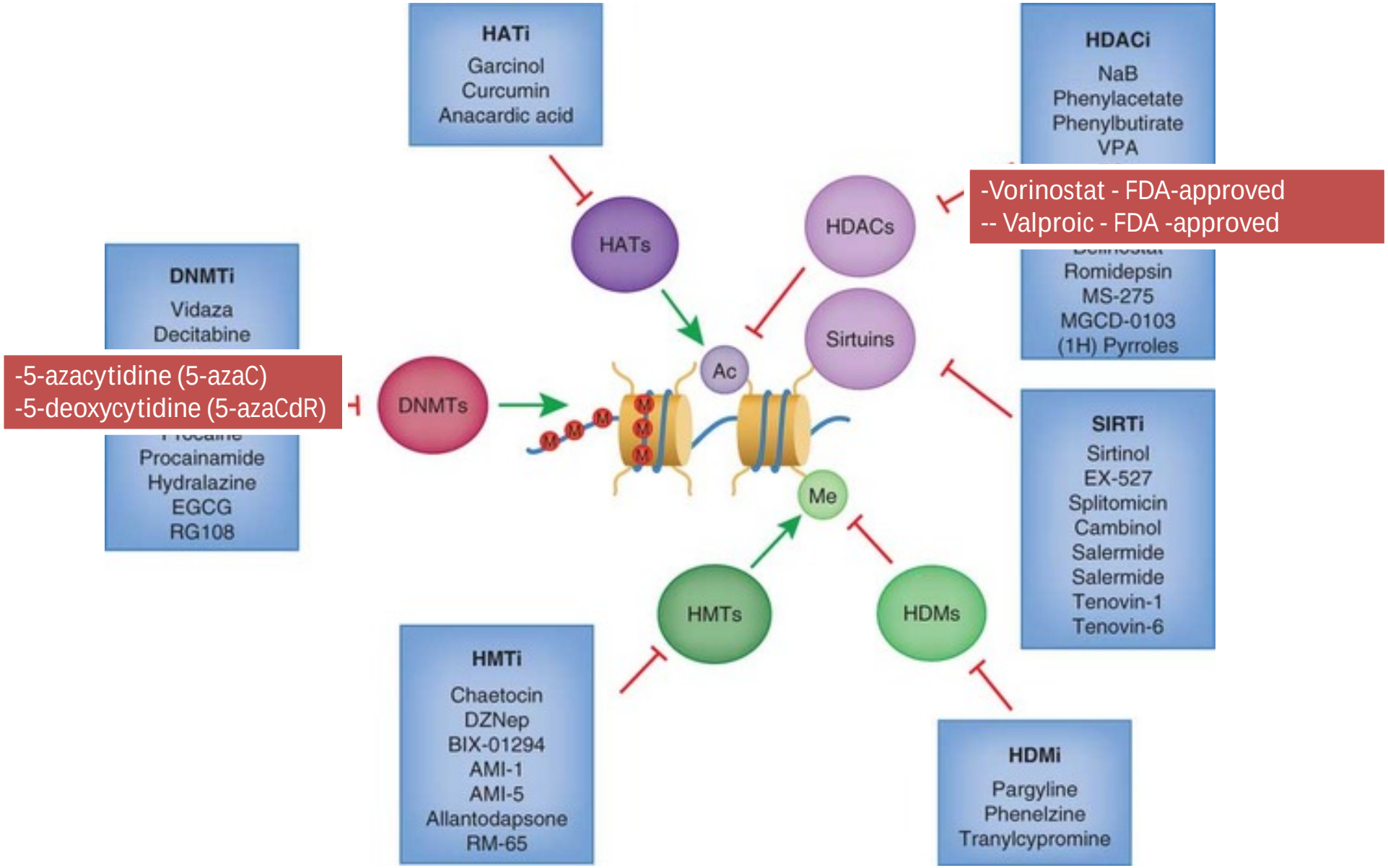
Epigenetic biomarkers in oncology and *EPI-DRUGS*



Food and Drug Administration (*FDA*)

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EPI-DRUGS INDUCED CANCER CELL DEATH

HDAC inhibitors therapy.

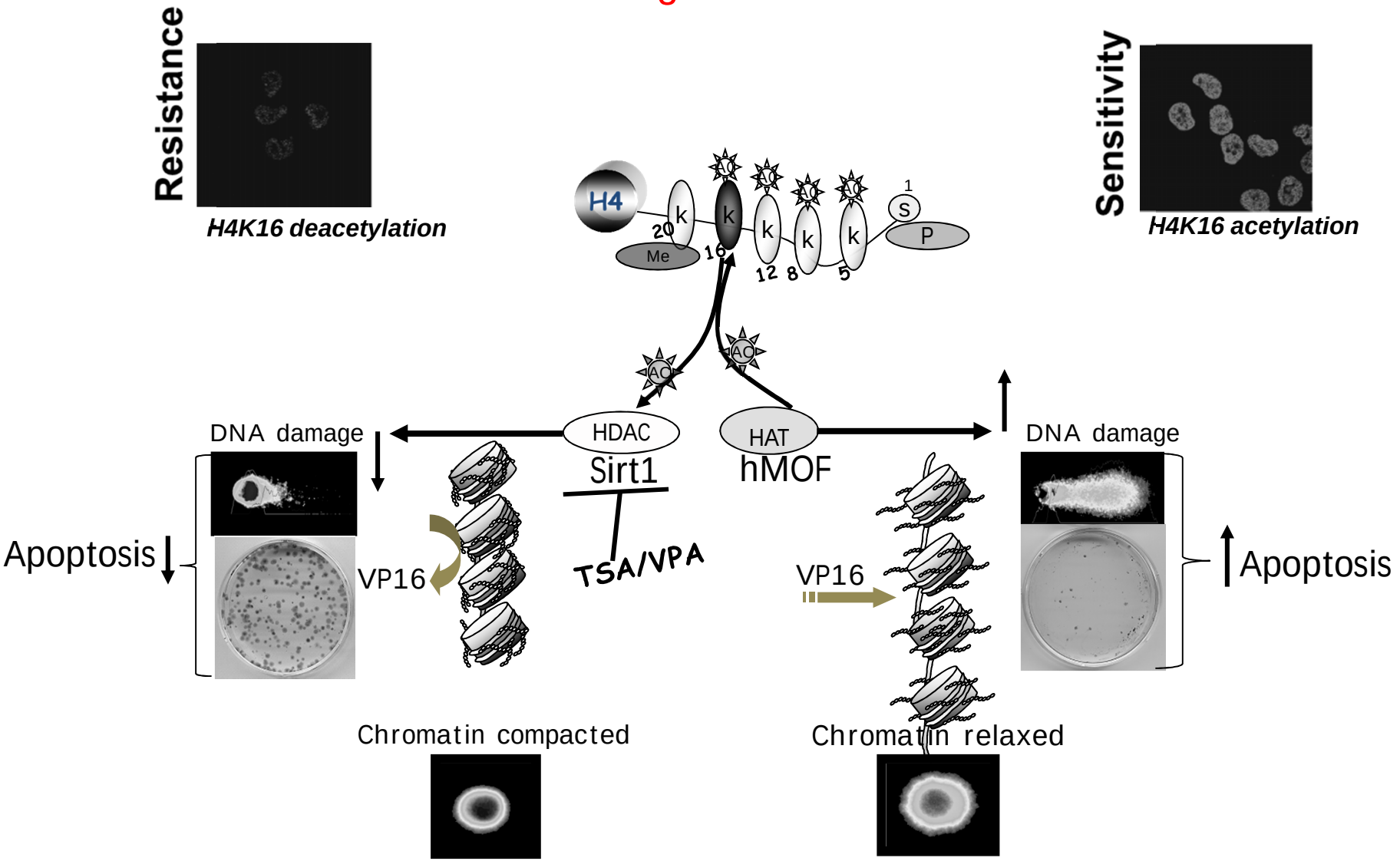
-Induce cancer cell death whereas normal cells are relatively resistant. Modulate the expression of several gene related to apoptosis

-Act through the reactivation of dormant tumor suppressor genes.

-HDAC inhibitors have been shown to enhance radio- and chemosensitivity of tumor cells.

- Increase DNA damage response.
- Increase Transcription factor -DNA accessibility

Opposing effects of hMOF and SIRT1 regulate the responsiveness of cancer cells to DNA damage-induced cell death.



Hajji N et al 2010. Oncogene

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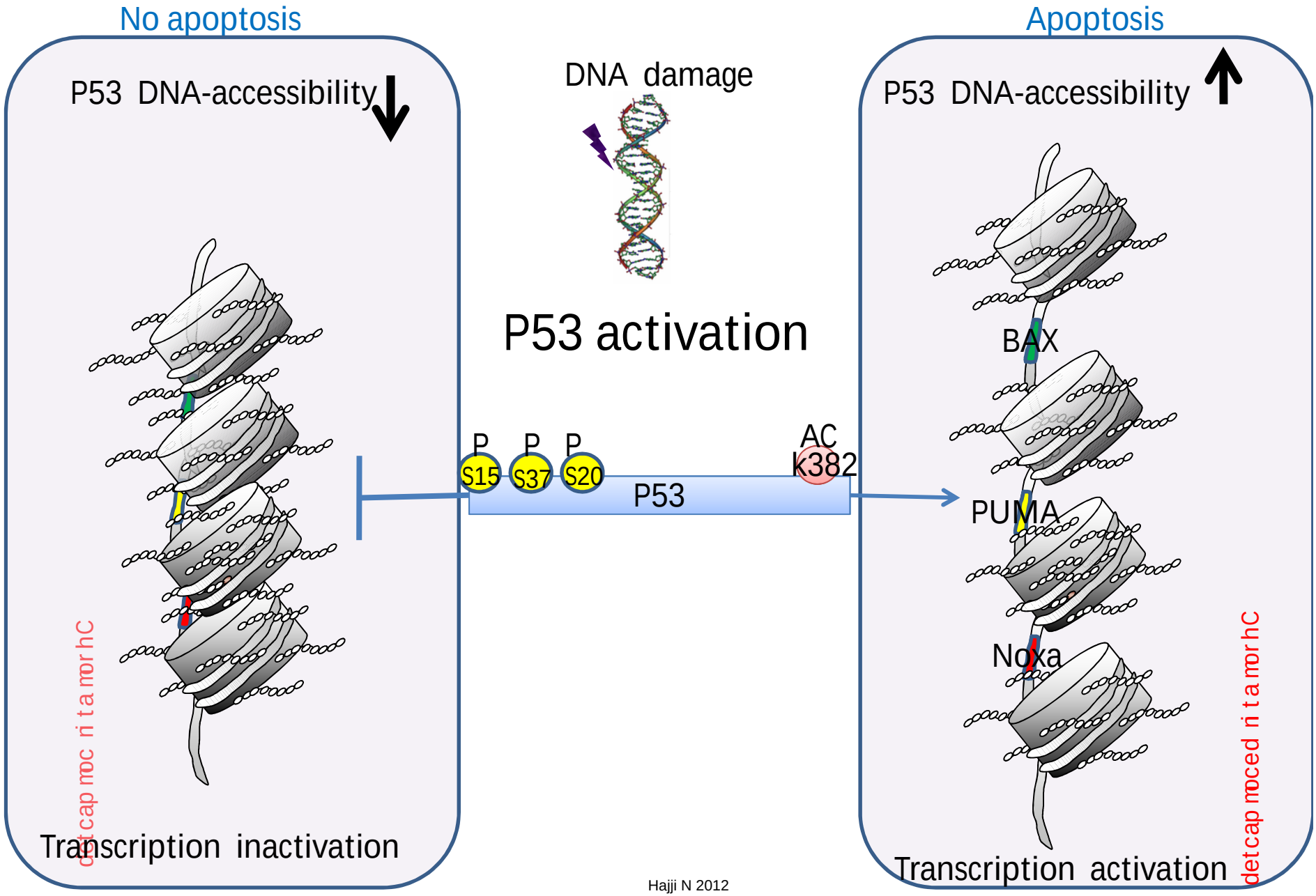
Acetylation of histone H4 lysine 16 regulates transcription factor -DNA accessibility



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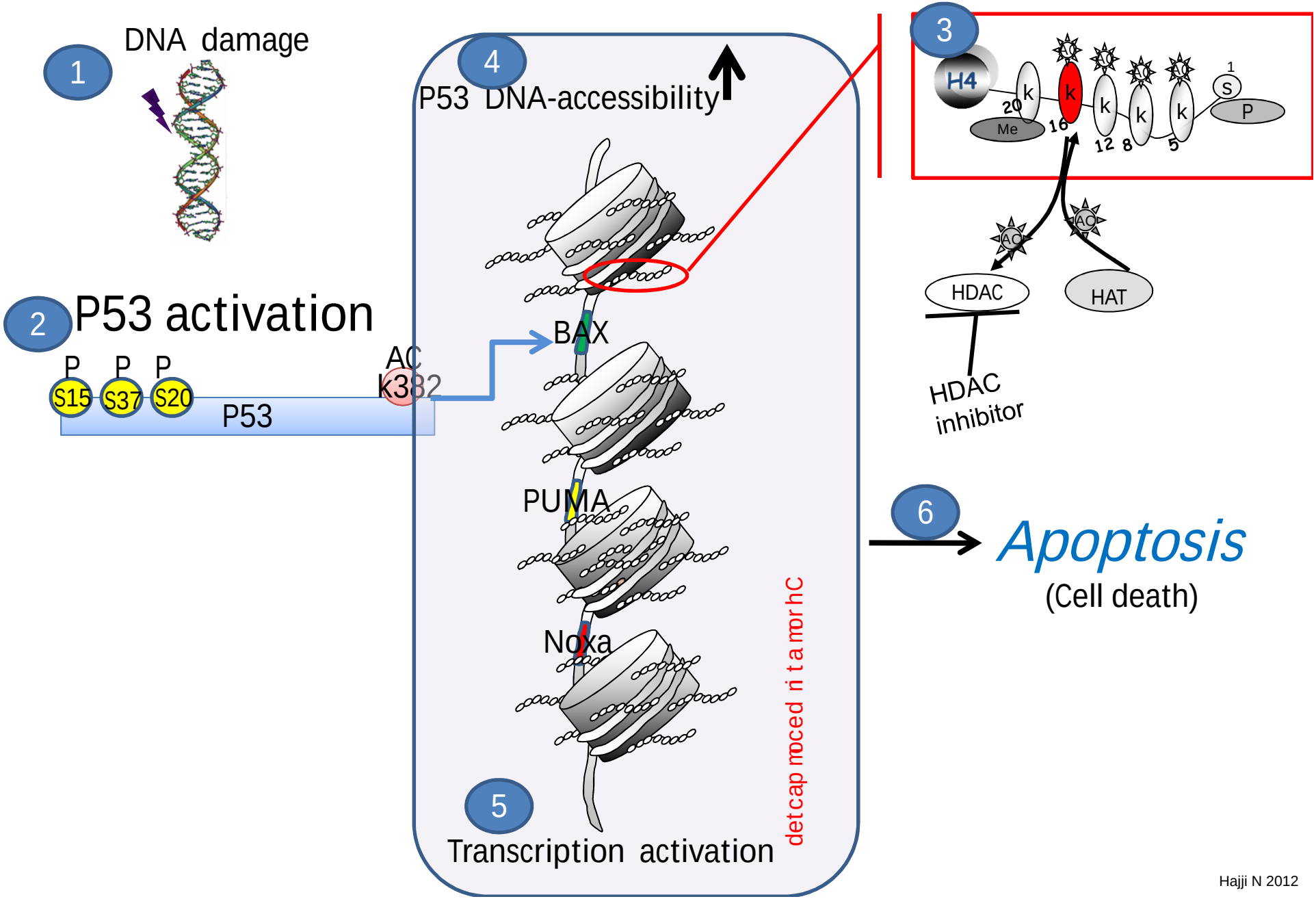
P53 DNA accessibility is important for its transcription activity



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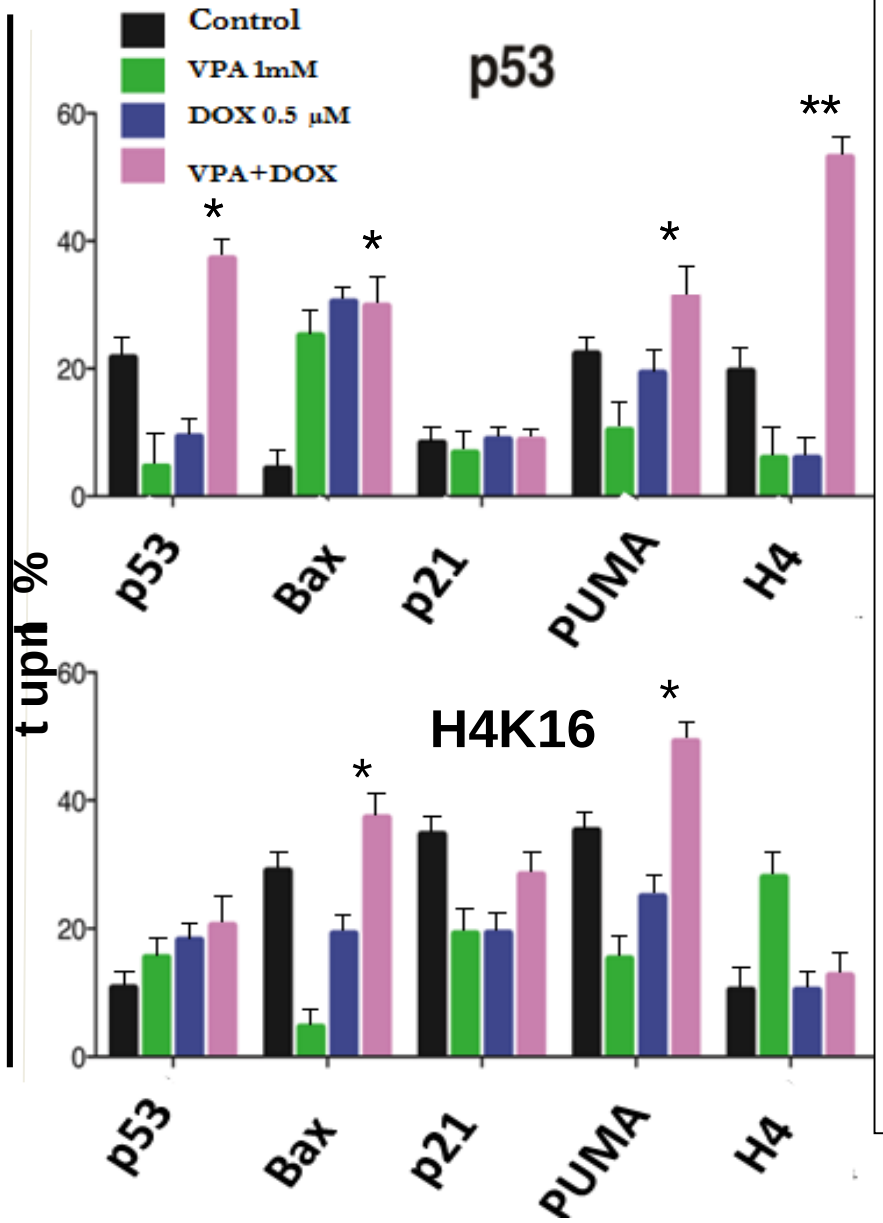
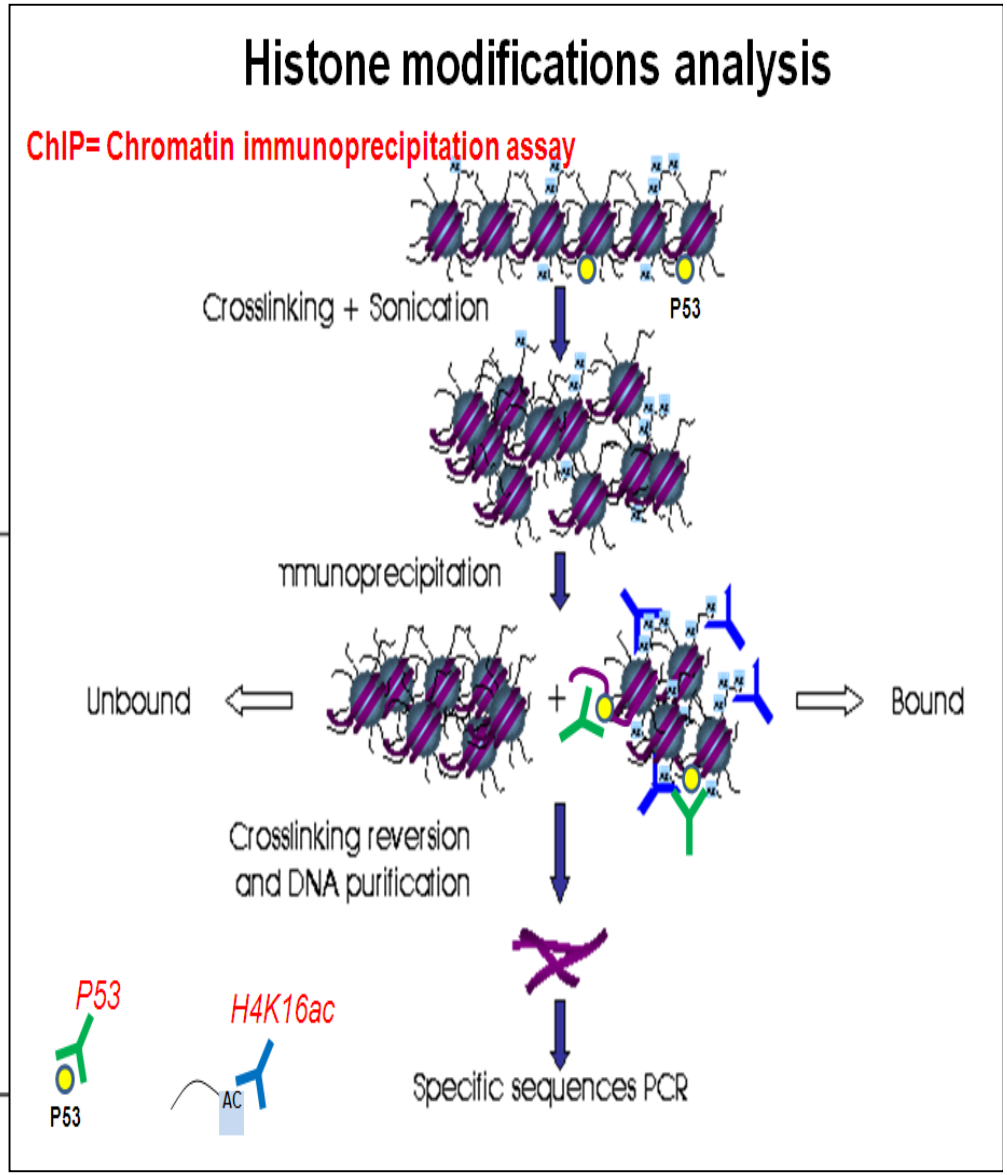
Acetylated H4 k16 acetylation increases p53 binding to apoptotic gene promoters

Additional support information

ChIP- HCT116 p53 +/+





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HDAC inhibitors

Additional support information

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Chemical class	Selected members	Comments
Short-chain fatty acids	Sodium *n*-butyrate (NaB)	Butyrates such as NaB inhibit proliferation of colon, prostate, endometrial and cervical carcinomas at high millimolar concentrations.
	Phenylacetate	Valproate is quite active against HDACs 1–5, 7 and 9 but less so against HDACs 6 and 10. It is more efficient as an inducer of differentiation in carcinoma cells, transformed hematopoietic progenitor cells and leukemic blasts from individuals with AML.
	Phenylbutyrate	
	Valproate	
Hydroxamic acids	Trichostatin A	Trichostatin A inhibits HDACs 1–7 and 9 at the single-digit nanomolar level and HDAC8 at the single-digit micromolar level. Despite its proven antitumoral activity, it has too many side effects to be used clinically.
	Vorinostat (SAHA)	Vorinostat is FDA-approved for hematological malignancies.
	Panobinostat	Panobinostat is highly active against HDACs 1–4, 7 and 9 but less so against HDAC6 and, especially, HDAC8. It is undergoing clinical trials for the treatment of CML, refractory CTCL and multiple myelomas. It may also be relevant to the treatment of hormone-dependent breast cancers, as it causes strong inhibition of their typically upregulated aromatase gene.
	Belinostat	Belinostat is quite active against HDACs 1–10. It is in clinical trials for the treatment of hematological malignancies and solid tumors.
Cyclic peptides	Romidepsin (formerly FK-228)	A natural, stable prodrug that, once converted to its active form (redFK) by cellular reducingactivity, is capable of inhibiting HDACs 1, 2, 4 and 6. After showing strong preclinical antitumoral activity, it was approved by the FDA and has undergone clinical trials for the treatment of AML, CML and CTCL.
Benzamide derivatives	MS-275 (or entinostat)	MS-275 inhibits HDACs 1–3 and 9 and has also been used in clinical trials in conjunction with other agents.
	MGCD-0103	MGCD-0103 can inhibit HDACs 1 and 2 and, to a lesser extent, HDACs 3 and 11. It is also in clinical trials for the treatment of hematological malignancies and solid tumors.

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Encouraging use of epi- drugs

-Research in epigenetics has led to improved survival of patients with certain forms of lymphoma and leukemias through the use of drugs that alter DNA methylation and histone acetylation.

-Numerous other clinical applications :

.Cancer screening and early detection.
. Molecular stratification of cancer
.Predicting outcomes after standard therapy.

e.g. Vorinostat inhibits proliferation and induces cell death of a variety of transformed cells.

- Lymphoma. Breast adenocarcinoma
- Myeloma. Pancreatic cancer
- Leukaemia. Glioblastoma
- Mesothelioma. Prostate cancer
- Non-small cell lung carcinoma. Ovarian cancer
- Bladder carcinoma Melanoma
- Colon carcinoma. Renal cell carcinoma
- Thyroid cancer. Endometrial cancer

Limitations of the epi-drugs

-Increasing list of enzymes involved in the epigenome together with genes subject to abnormal epigenetic regulation.

-Limitation of our comprehension of the molecular mechanism that contributes to cancer development or progression that involve epigenetic.

-Epigenetic diversity is also a characteristic of cancer, and should be considered, if not cell specific, at least as tumor type specific.

- Lack of Epi-drug specificity (e.g pan-HDAC inhibitors may also cause numerous side effects such as bone marrow depression, diarrhea, weight loss, taste disturbances, electrolyte changes, disordered clotting, fatigue, and cardiac arrhythmias)

Summary

Epigenetic

Covalent modification of histones

Histone post-translational modifications:

Existence of an Histone code?

Histone acetylation machinery

Histone methylation machinery

Epigenetic & CANCER

•Histone post-translational modifications (PTMs) involvement in cellular functions and cancer .

•Genes deregulated by post-translational histone modifications in cancer (e.g apoptotic genes).

•(e.g. Histone onco-modifications Histone modifications analysis.

Epigenetic as biomarkers in oncology and *EPI-DRUGS INDUCED CANCER CELL DEATH*

Encouraging aspect of epi-drugs and limitations

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