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Epigenetics and Cancer II

Image: Nicolas Bouvier and Genevieve Almouzni

Nadine Chapman-Rothe (PhD)

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Overview

1. Definition of Epigenetics

2. Mechanisms of epigenetic regulation

- Histones (modifications, variants and binding proteins)
- RNAi (role in heterochromatin and gene regulation)

3. Epigenetics and Cancer

4. Challenges of 'Epigenetic Therapeutics'

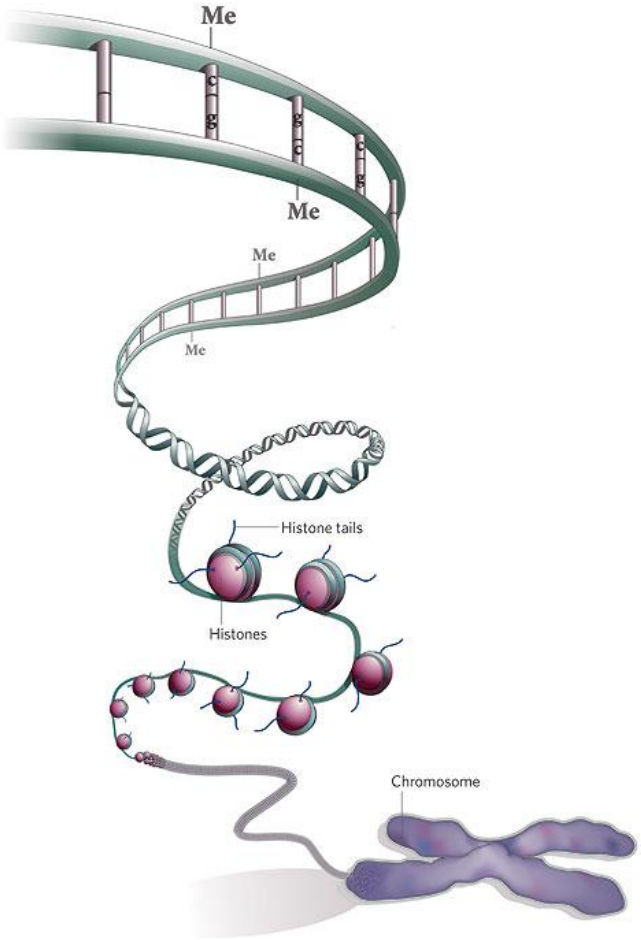

Image: Nature 441, 143-145 (11 May 2006)

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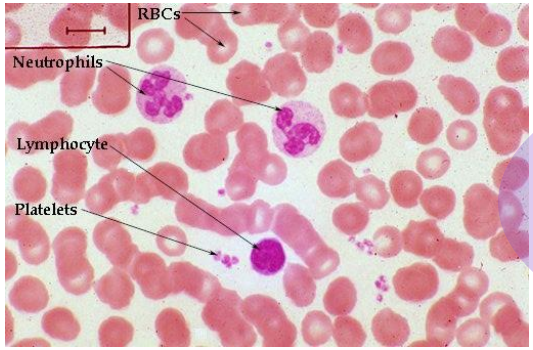
Definition of Epigenetics

- A change in phenotype that is heritable but does NOT involve DNA mutation



http://greenfield.fortunecity.com/rattler/46/blood.htm

Same genotype – different epigenome

http://www.pbs.org/wgbh/nova

BMJ 2009; 339:b5262

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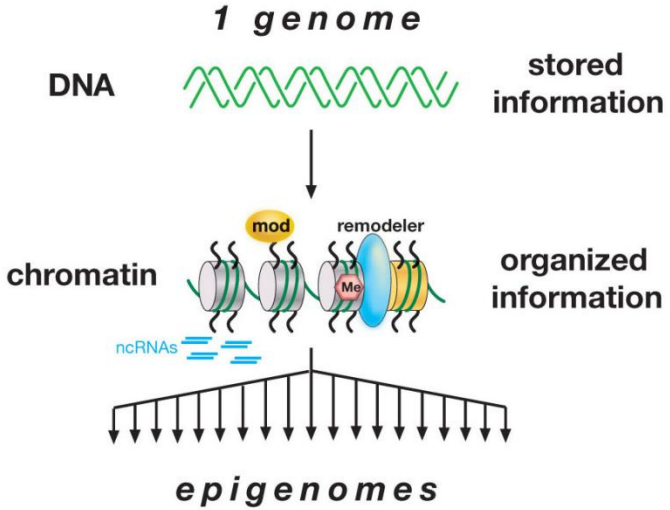
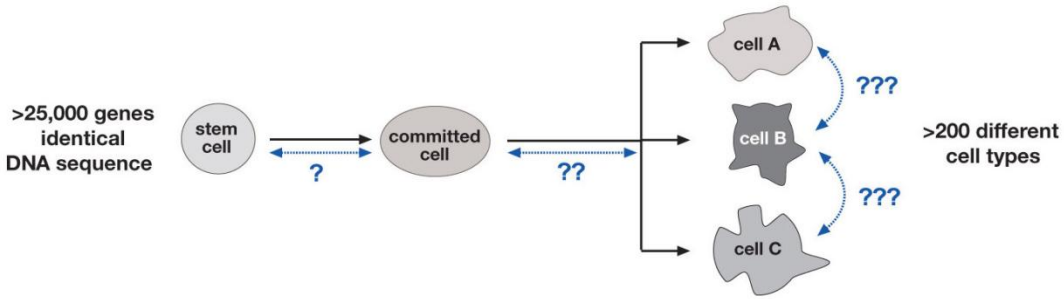
One genome – several epigenomes

Epigenetic Mechanisms control the genome

- **Histone modifications / variants**
- DNA methylation
- Non coding RNAs





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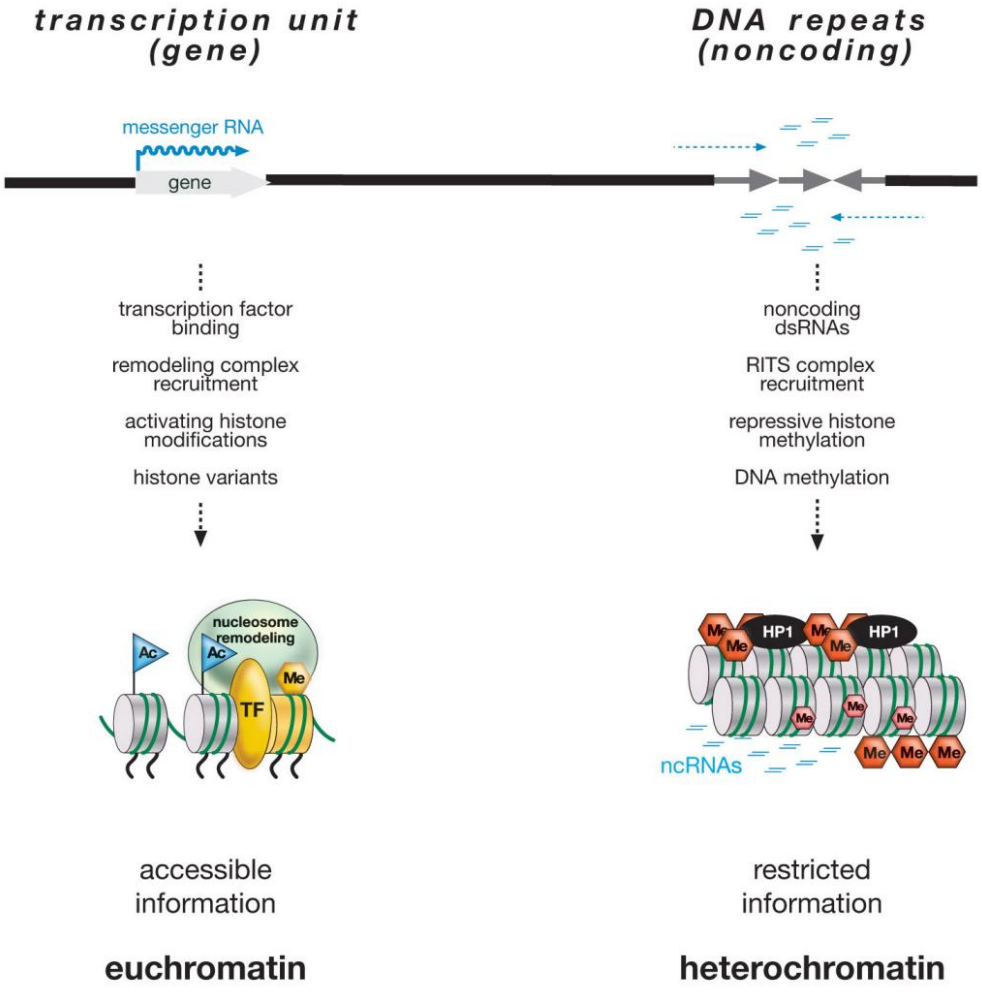
Euchromatin and Heterochromatin



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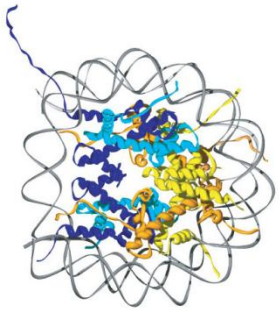
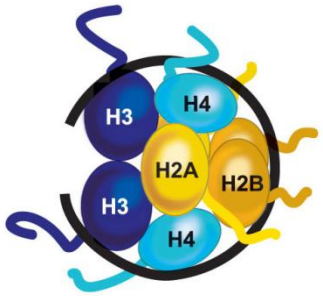
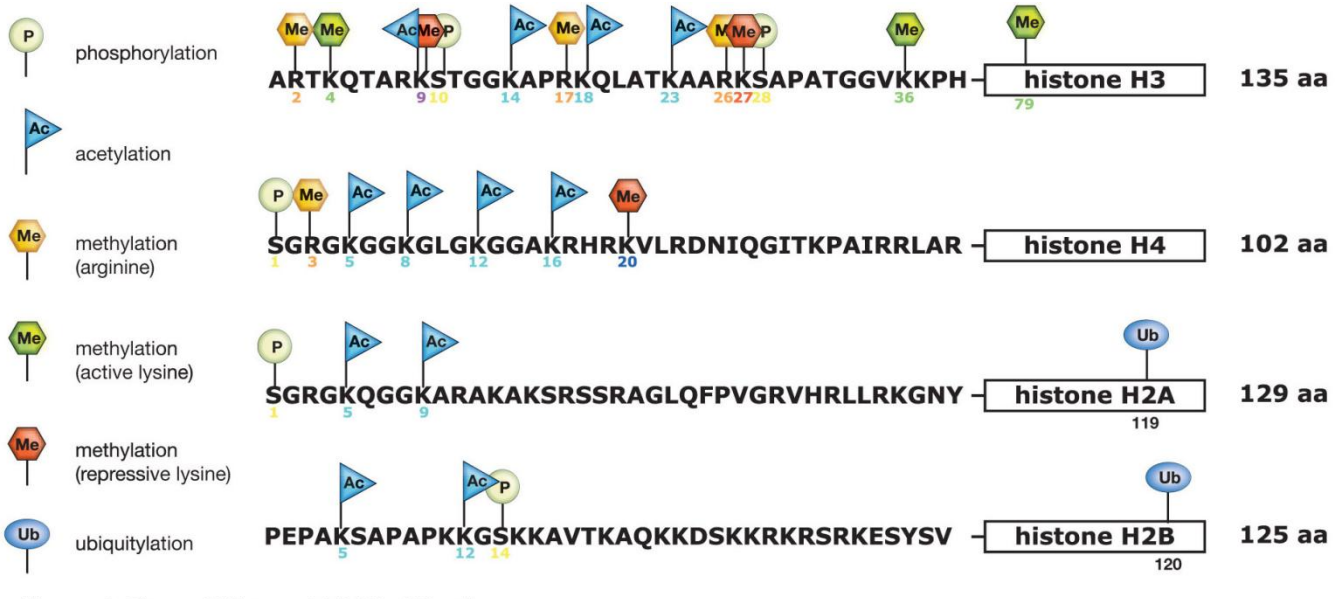
Histones and posttranslational modifications







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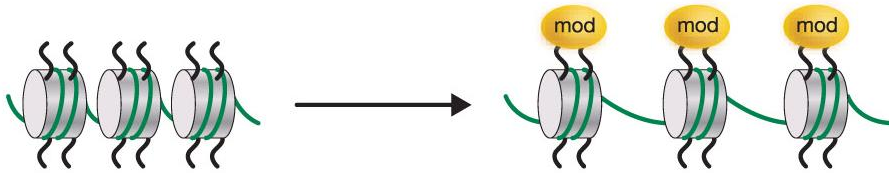
Histone modifications affect chromatin template

- Prevention of crucial contacts necessary for higher order structure

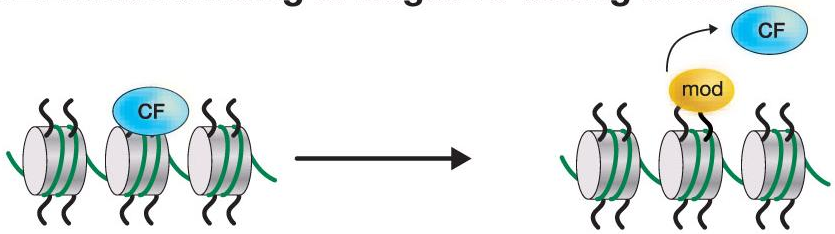
- Disruption of effector protein binding

- Altered binding surface attracts certain effector proteins and complexes

Model 1: Chromatin structural change



Model 2: Inhibit binding of negative-acting factor



Model 3: Recruit positive-acting factor

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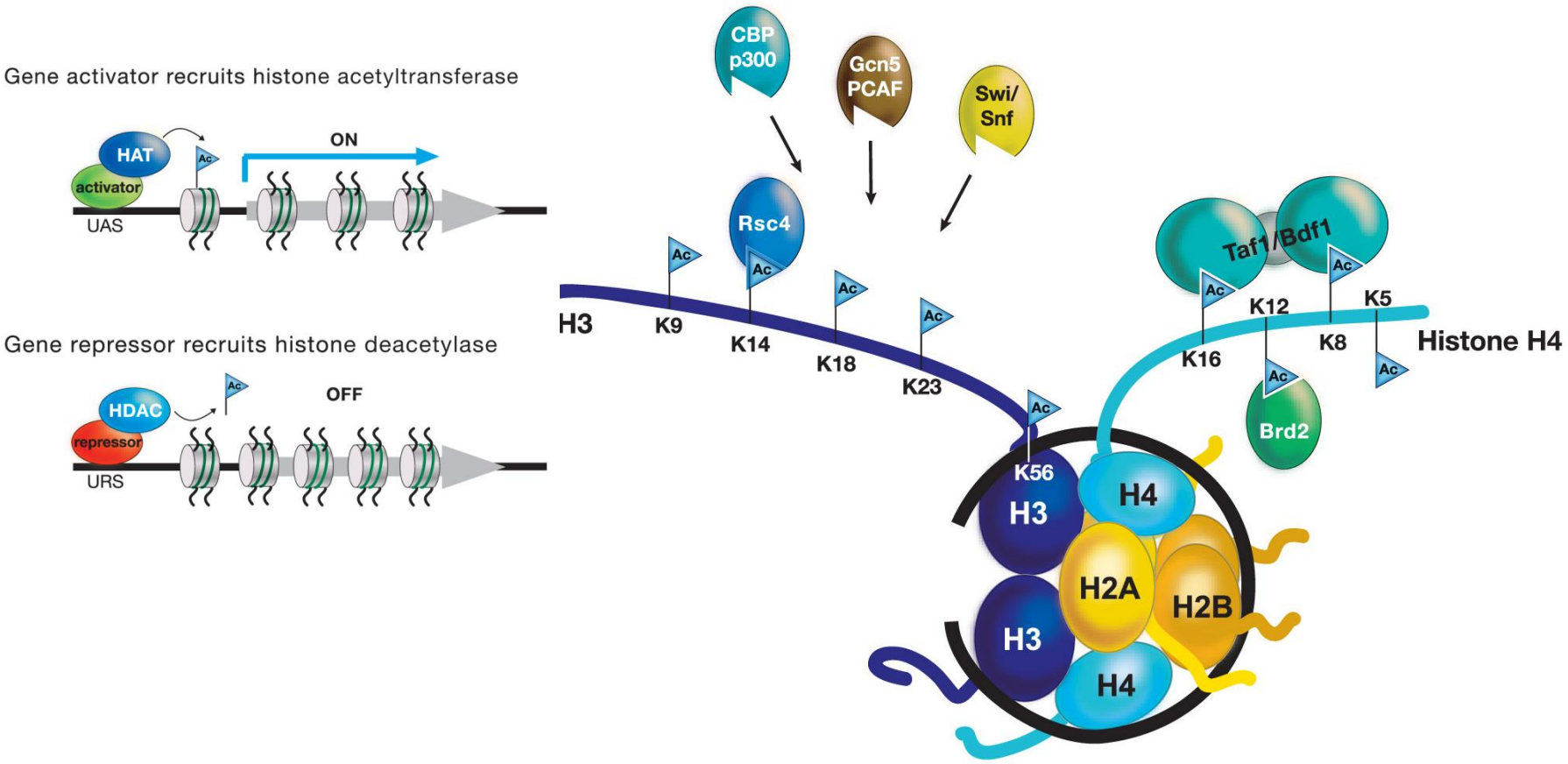
Histone acetylation, transferases and binding proteins



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Histone acetylation

- Correlates with transcription
- Acetylated regions tend to be transcribed or poised for transcription
- Chromatin has an ‘open’ structure
- HAT-proteins acetylate histones on all 4 core histones
- Different HAT posses distinct specificities to certain substrates
- HAT have non-histone targets as substrate as well
- Histone deacetylases (HDACs) remove acetyl group

Action of acetyl group / HDACs

- Neutralisation of positively charged lysine
- Weakening the binding of basic histones to negatively charged DNA
- Acetyl-group is a target of the ‘Bromodomain’ – recruiting remodelling complexes

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- HDACs: class1 and 2 have related mechanism of deacetylation (no co-factor)
- HDACs: SIR2-family: deacetylation is NAD+-dependent
- Most HDACs act in large multi-subunit repressor-complexes

Classes of HDACs I

HDAC class	Name	Function
HDACs class I	HDAC1 and HDAC2	Complex dependent (found in Sin3, NuRD and Co-REST, can act directly on DNA-binding proteins (YY1, Rb binding protein 1 and Sp1, phosphorylation required for function
	HDAC3	SMRT and N-CoR are necessary for activity, is able to form oligomers with other HDACs like HDAC4, 5, but mostly it interacts with itself, may have role in cell cycle process
	HDAC8	Similar to HDAC3, probably very low abundance of expression
HDACs class II	HDAC6	Exhibits two catalytical domains in tandem, has signal for ubiquitination, probably particularly prone to degradation, functions as tubulin deacetylase but it is also found in the nucleus together with HDAC11
	HDAC10	Exists in two splice variants, interacts with HDAC1, 2, 3, 4, 5, 7
	HDAC4	Exhibit binding domains for CTBP, MEF2; show interaction with SMRT/N-CoR, BCoR and, the N-termini interact with MEF2 and
	HDAC5	

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Classes of HDACs II

	HDAC7	blocks muscle cell differentiation. Suggested to by a link between DNA-binding recruiters and the HDAC3 containing complex
	HDAC9a, 9b, HDRP	Are splice variants, whereas HDRP lacks the catalytic domain, but is able to recruit HDAC3. All three interact with MEF2, indicating function in muscle differentiation.
HDAC11	HDAC11	More closely related to class I than to class II, not present in any known HDAC complex
HDACs class III	SIRT1	Deacetylates histones (preference for H4K16), PCAF/MyoD, EP300, TAF168, HTATSF1, TP53, XRCC6, NKRF and forkhead proteins; Regulation of insulin and glucose homeostasis; Fat reduction, Neuron survival
SIR2 family[1]	SIRT2	Predominantly cytoplasmic, deacetylates α-tubulin and histones, over expression delays mitosis, SIRT2 colocalises with chromatin during the G2/M transition, preference for H4K16 *in vitro*,
	SIRT3	Localised to mitochondrial matrix, *in vitro* deacetylates, multiple substrates including histones and tubulin, maybe important under conditions of energy limitation
	SIRT4	Localised to mitochondria and lacks detectable deacetylase activity but shows ADP-ribosyltransferase activity
	SIRT5	Localised to mitochondria with weak deacetylase activity and no apparent ADP-ribosyltransferase activity
	SIRT6	Nuclear protein, regulates DNA repair, role in aging
	SIRT7	Localised to nucleolus and promotes rRNA transcription, associated with RNA pol I, so far, no deacetylase activity measured, but activity NAD^+-dependent

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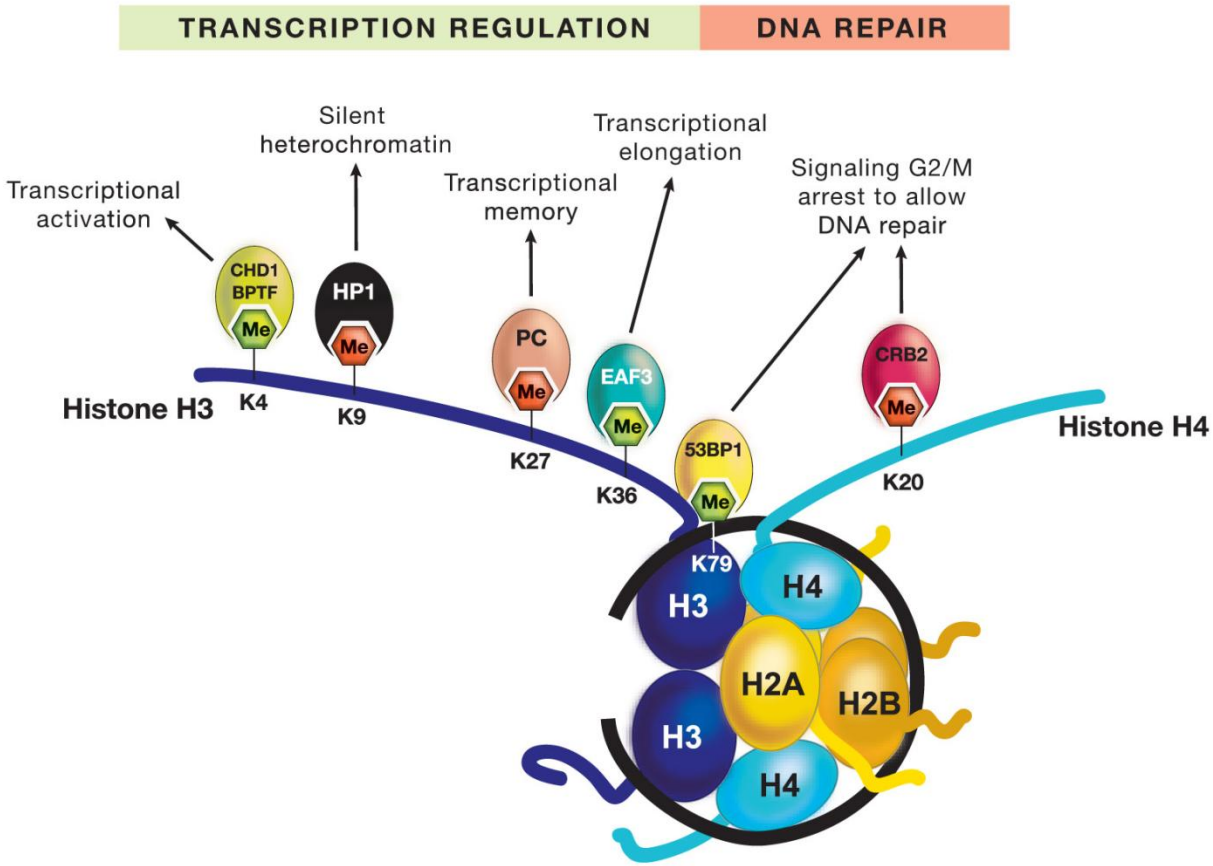
Histone methylation and protein binders



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Histone methylation

- The most complex modification on lysine and arginine
- Can either lead to activation or repression
- Multiple methylated states on each residue (K me1, 2, 3 – R me1, 2)
- Histone lysine methyltransferases (HKMT) share the SET-domain (except Dot1)
- SET-domain contains catalytical domain and binds S-adenosyl-L-methionine
- Binding proteins: Chromo-, tudor- and PHD repeat domain
- Certain methylation states communicate (H3K9me3 prevents H3K4me3)

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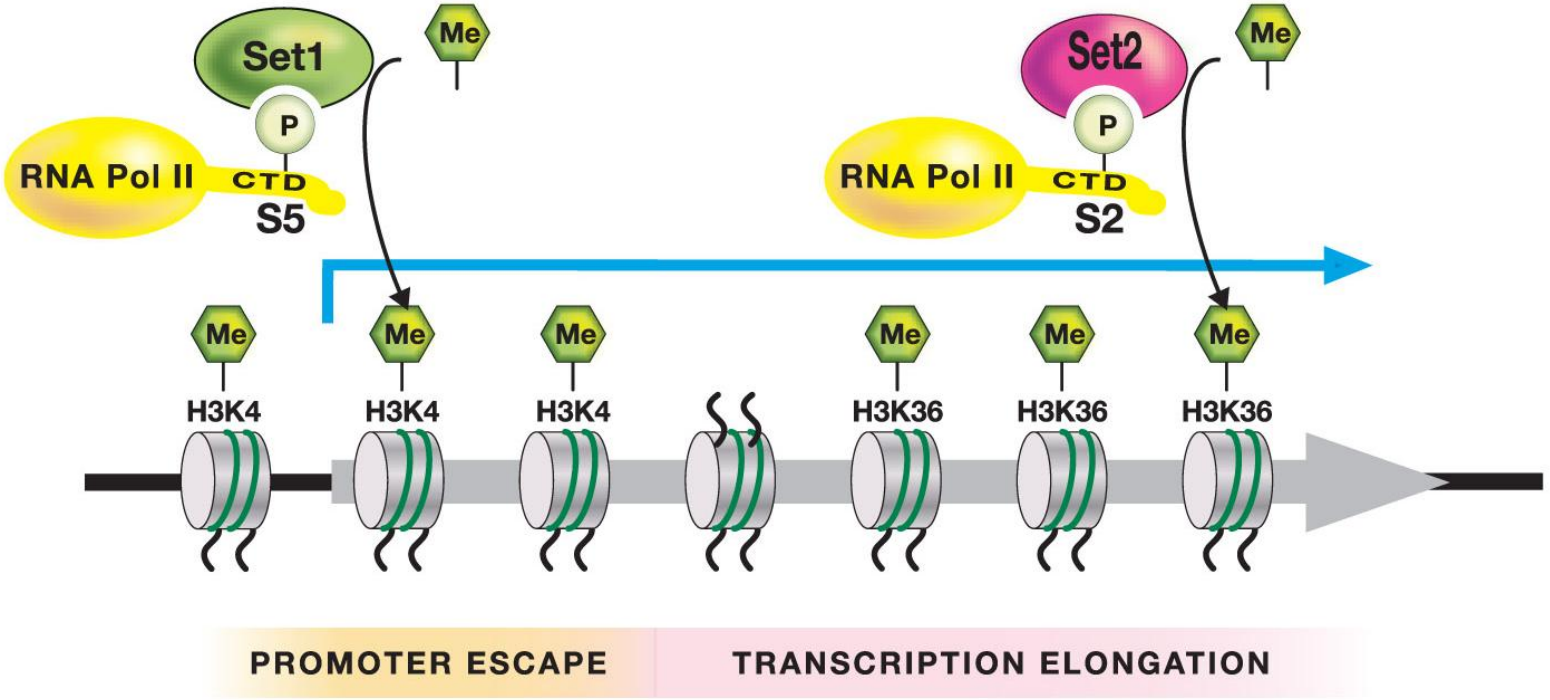
Histone K 4 / K36 methylation in transcription



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Specific Histone methylation and its function

- H3K4me3 recruitment of activating chromatin remodelling factors
- H3K9me3 essential part of heterochromatin formation (spreading of HP1) and necessary for DNA methylation
- H3K9me3 at promoters locks gene into repressive state via restricted HP1 recruitment
- H3K27me3 at 3 distinct locations: euchromatic genes (polycomb repressive complex binding), pericentromeric regions and inactive X (Xi)
- H3K79me, H4K20me role in DNA repair checkpoint

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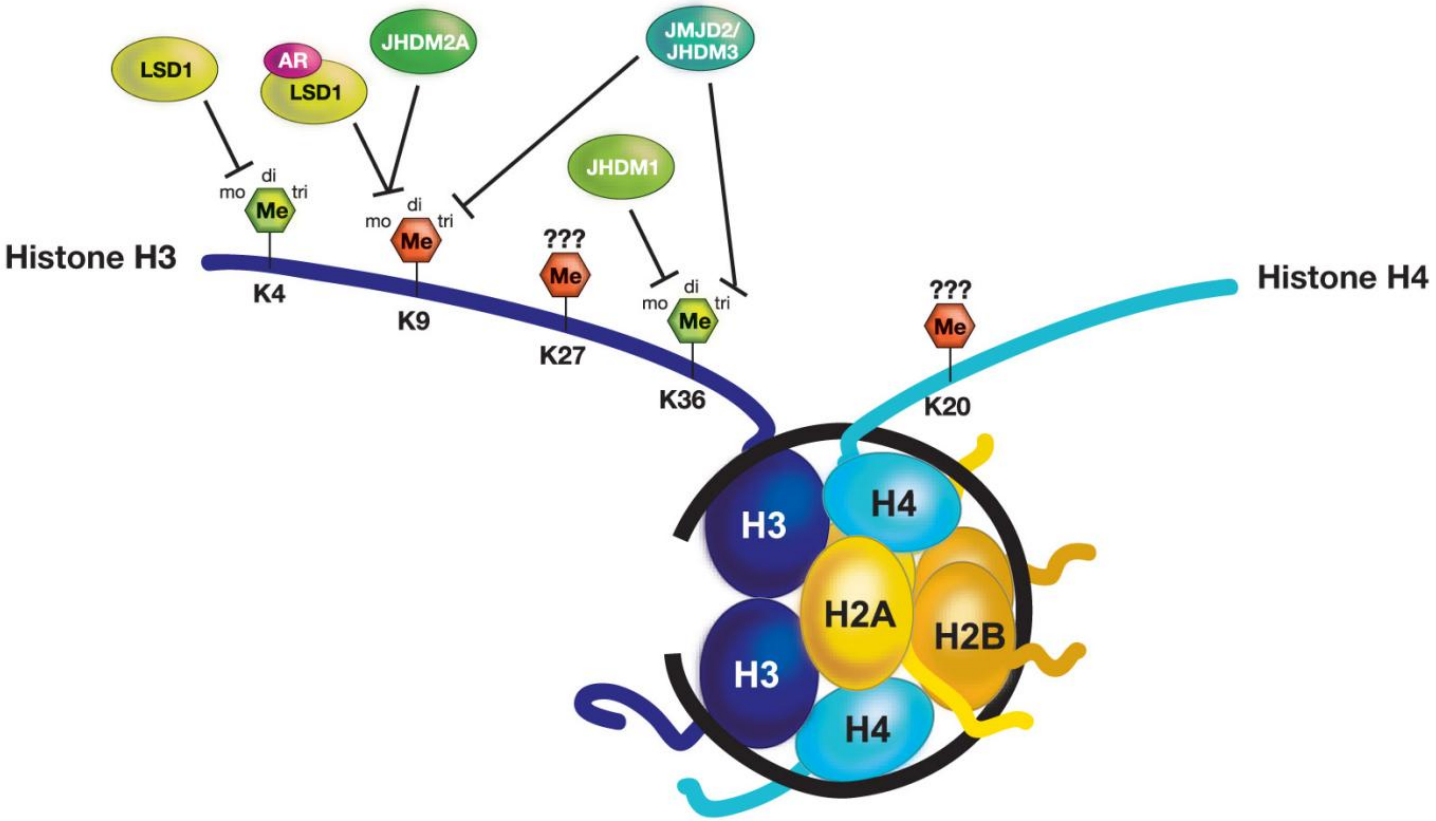
Histone lysin demethylases and their sites of action



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Histone lysine and their transferases/demethylases

Substrate	Histone lysine methyltransferase	Associated function of HKMT	Histone lysine demethylases
H3K4	SET1, MLL, SET9, ASH1L, SMYD3, PRDM9, SETMAR	Active or open chromatin	LSD1/BHC110, JARID1B, JARID family, JHDM1a/FBXL11
H3K9	SUV39H1, SUV39H2, EHMT1, EHMT2, SETDB1, PRDM2, ASH1L	Repressed or condensed (heterochromatic) chromatin	LSD1/BHC110, JMJD2A/3A, JMJD2B, JMJD2C, JMJD2D/2a/2b
H3K27	EZH2, EZH1, EHMT2	Developmentally regulated genes, target of Polycomb	JMJD3, UTX,
H3K36	NSD1, SETD2/HYPB, SETMAR, SMYD2, WHSC1(MMSET)	Actively transcribed chromatin	JHDM1a/FBXL11, JHDM1b/FBXL10, JMJD2A/JHDM3A, JMJD2B, JMJD2C
H4K20	SET8, SUV420H1, SUV420H2, NSD1, ASH1L	Stress and/or DNA damage response	-

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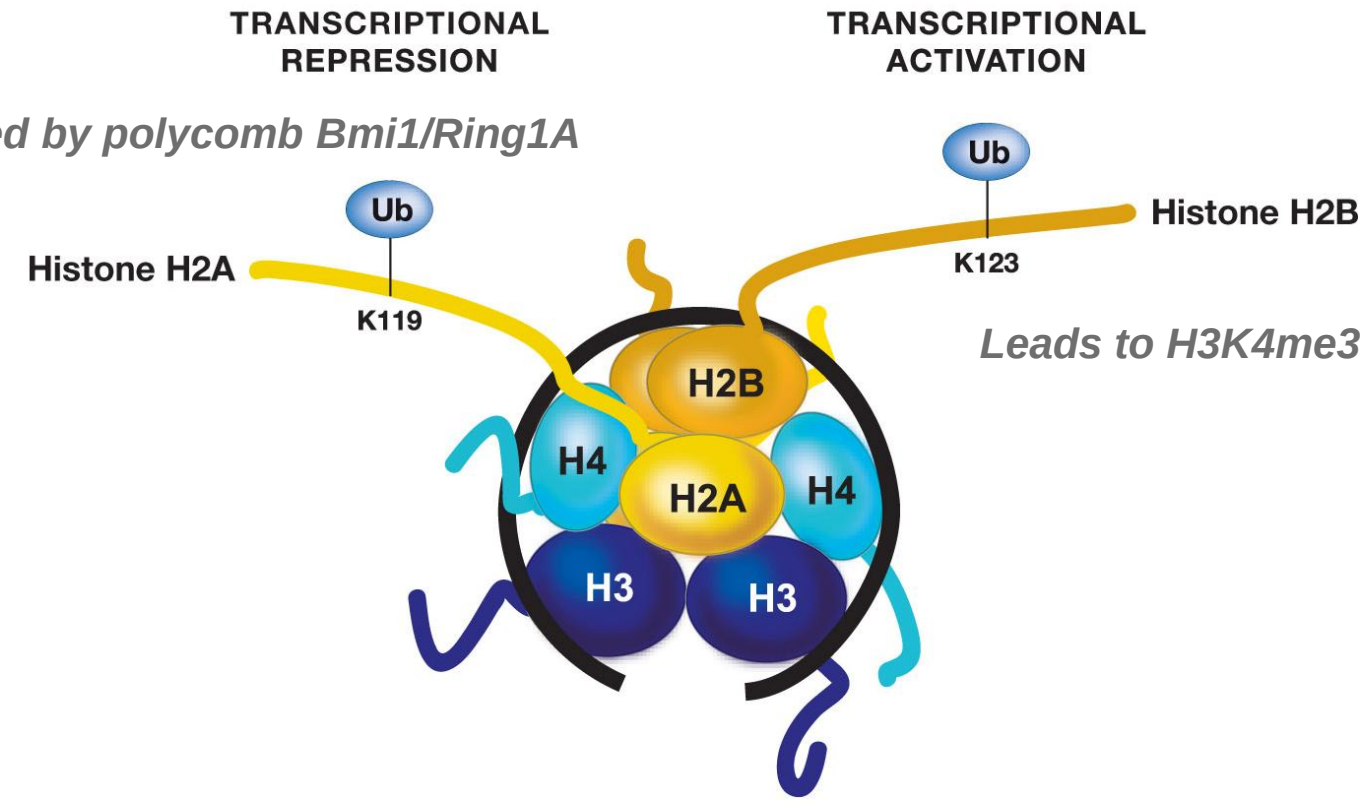
Histone ubiquitylation



Increases size of histone by 2/3

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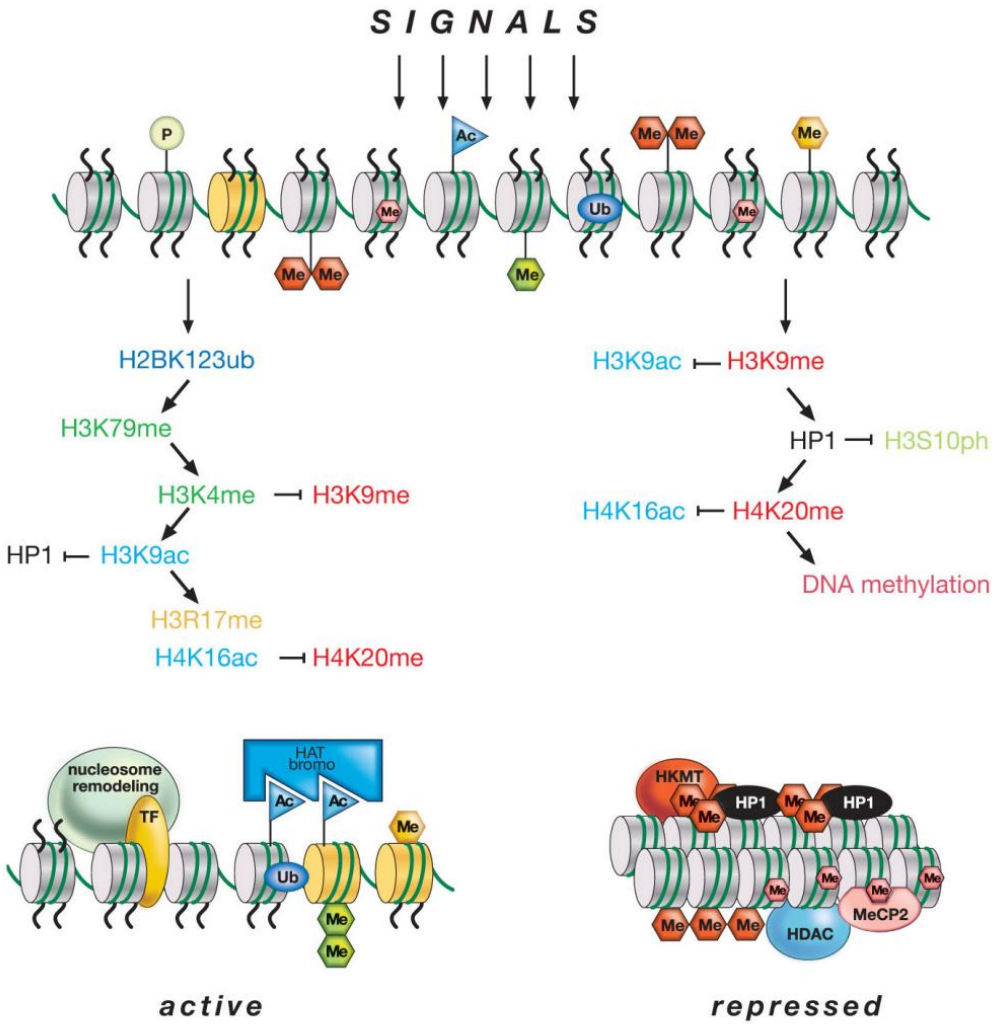
Histone code



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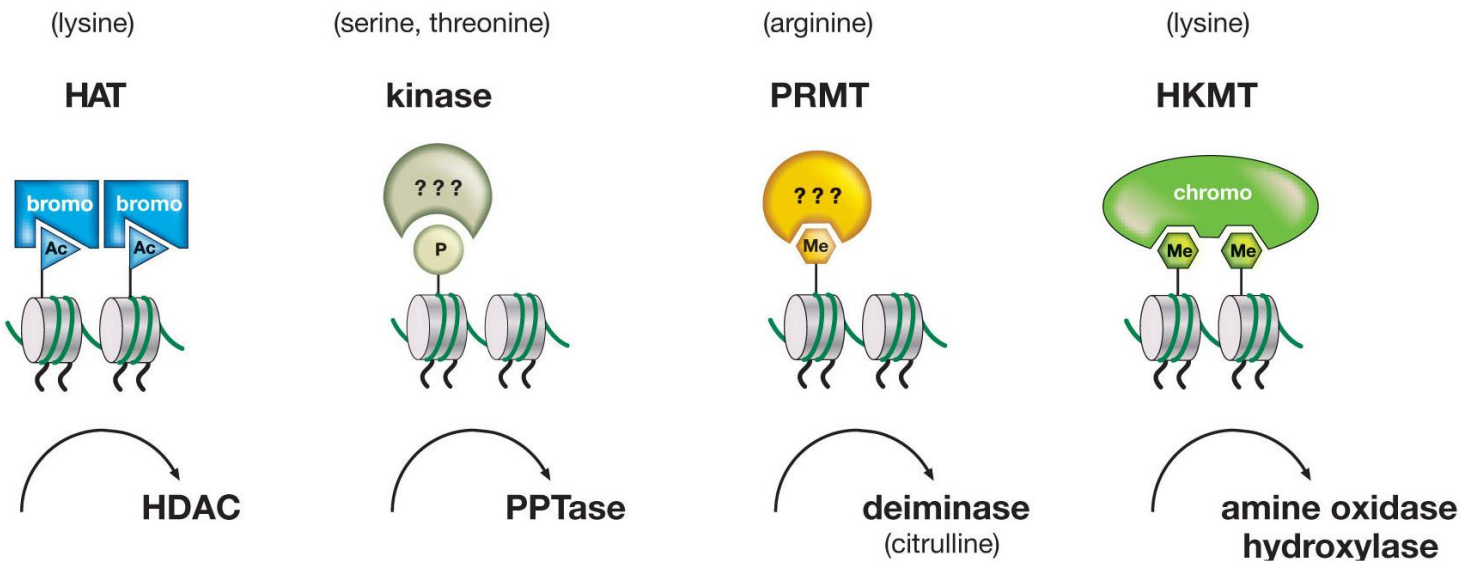
Histone modifying enzymes



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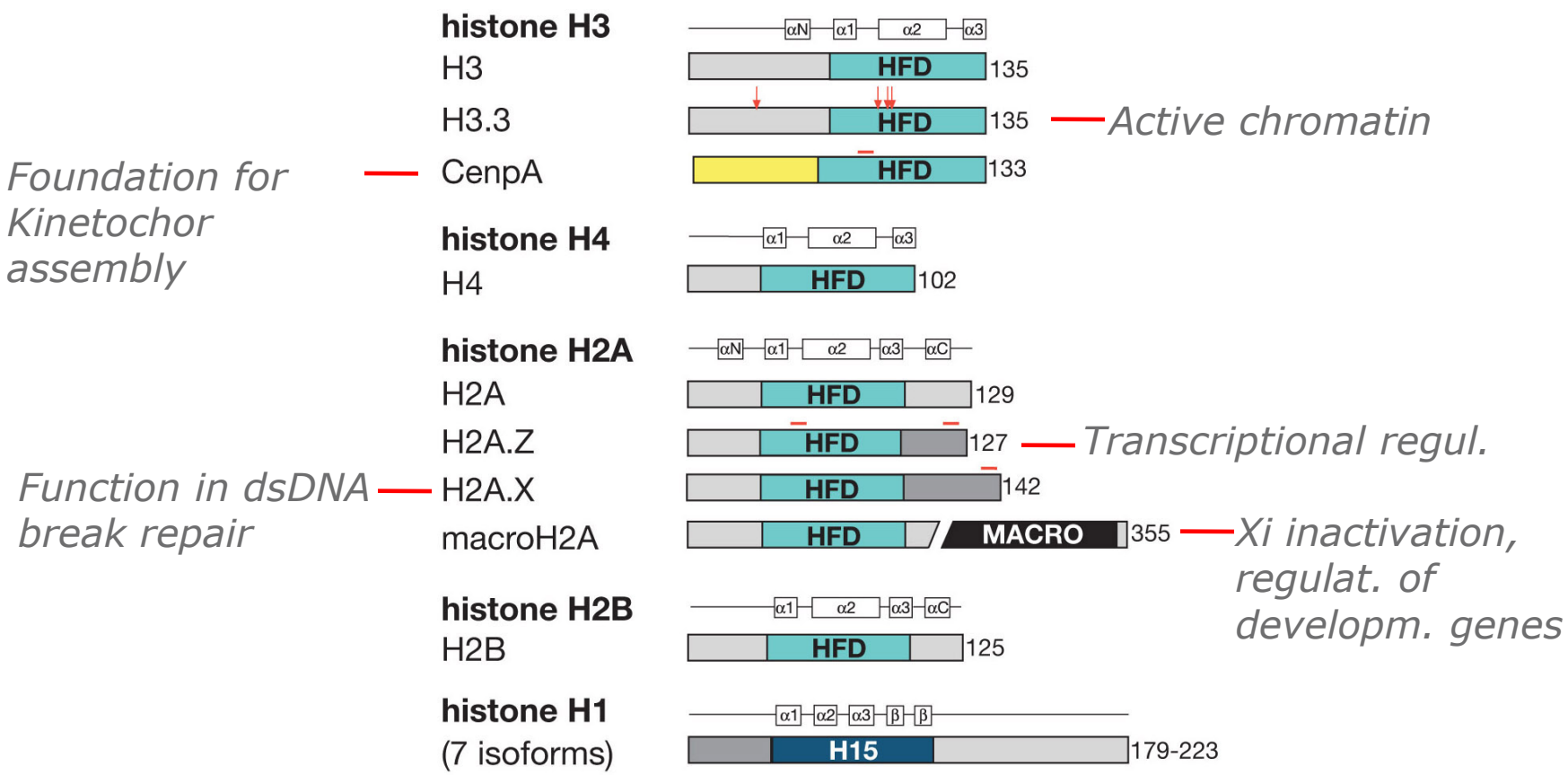
Histone variants and their function



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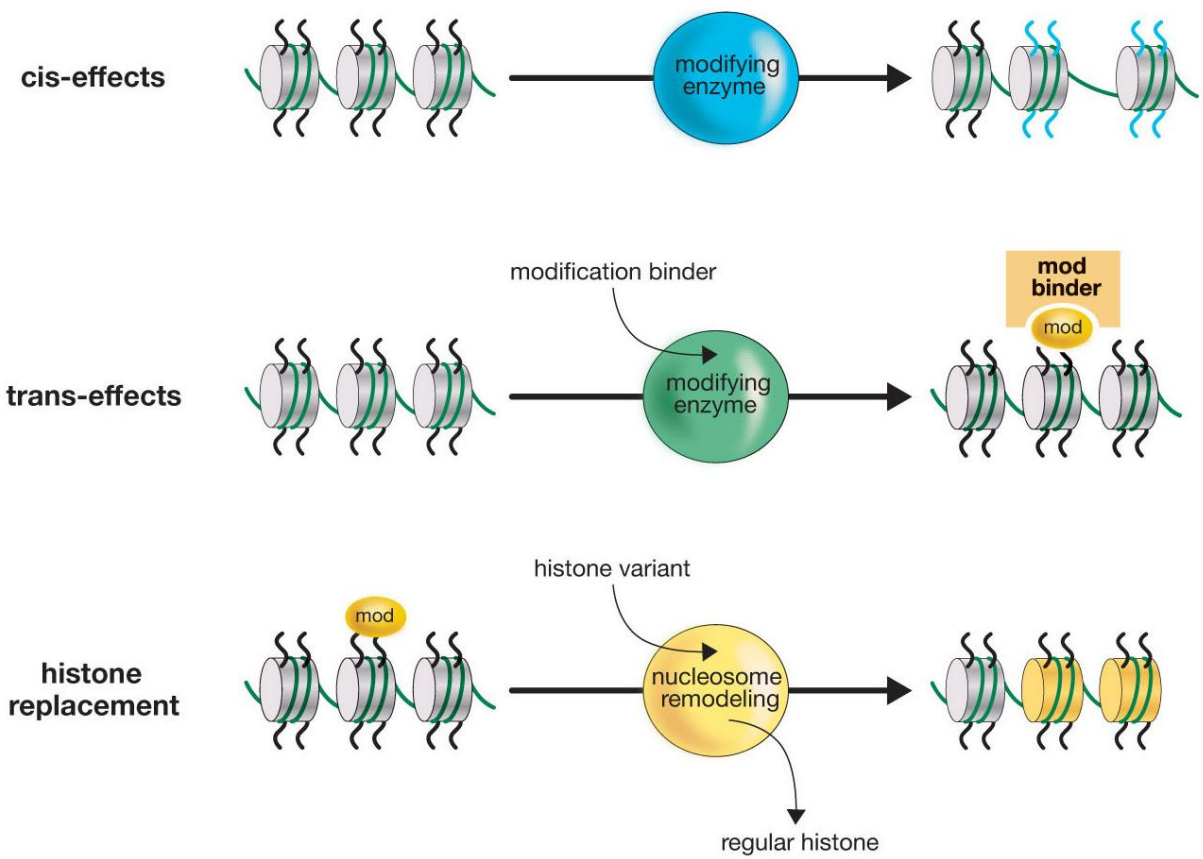
Summary: Transitions in chromatin



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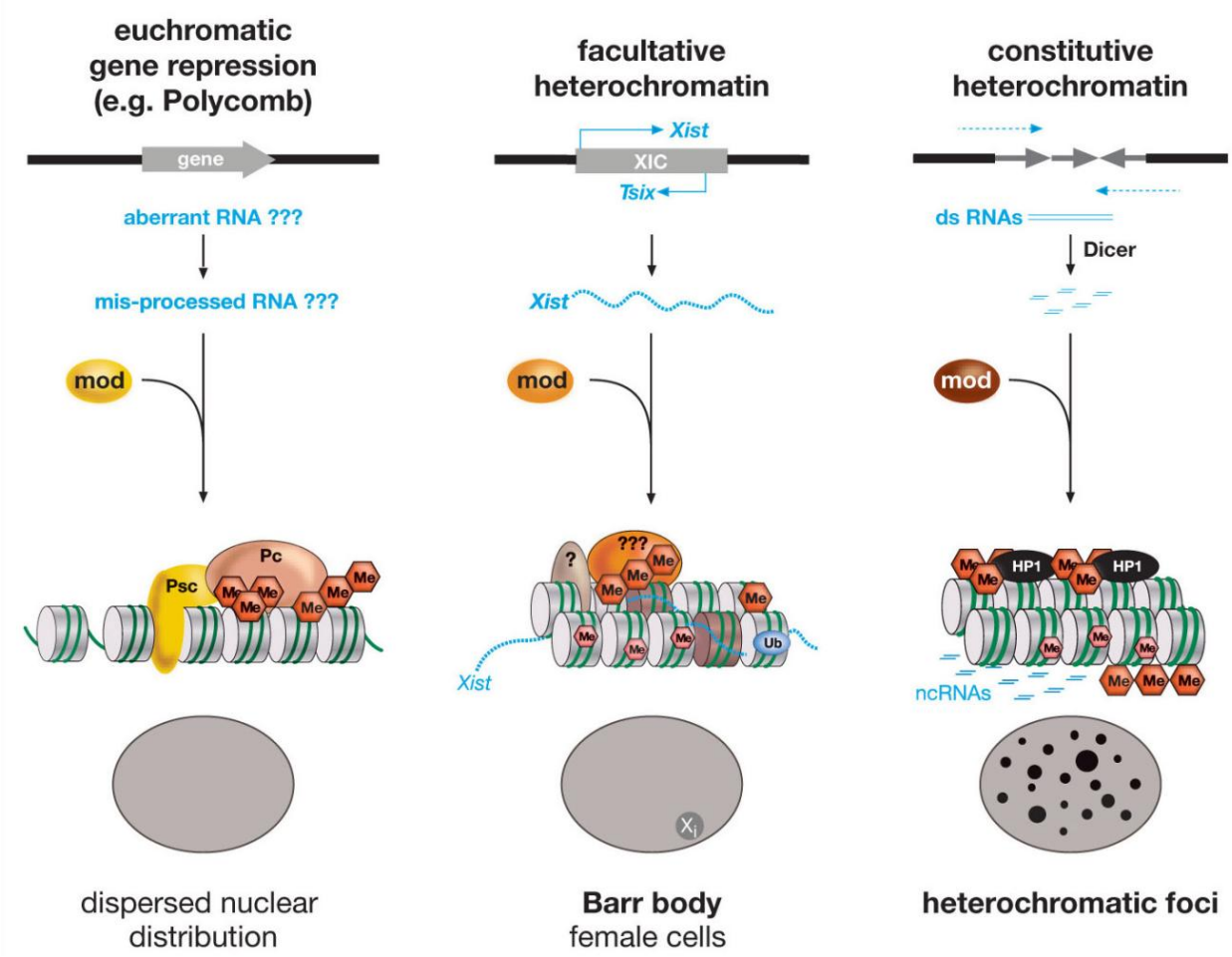
Non coding RNAs and their function



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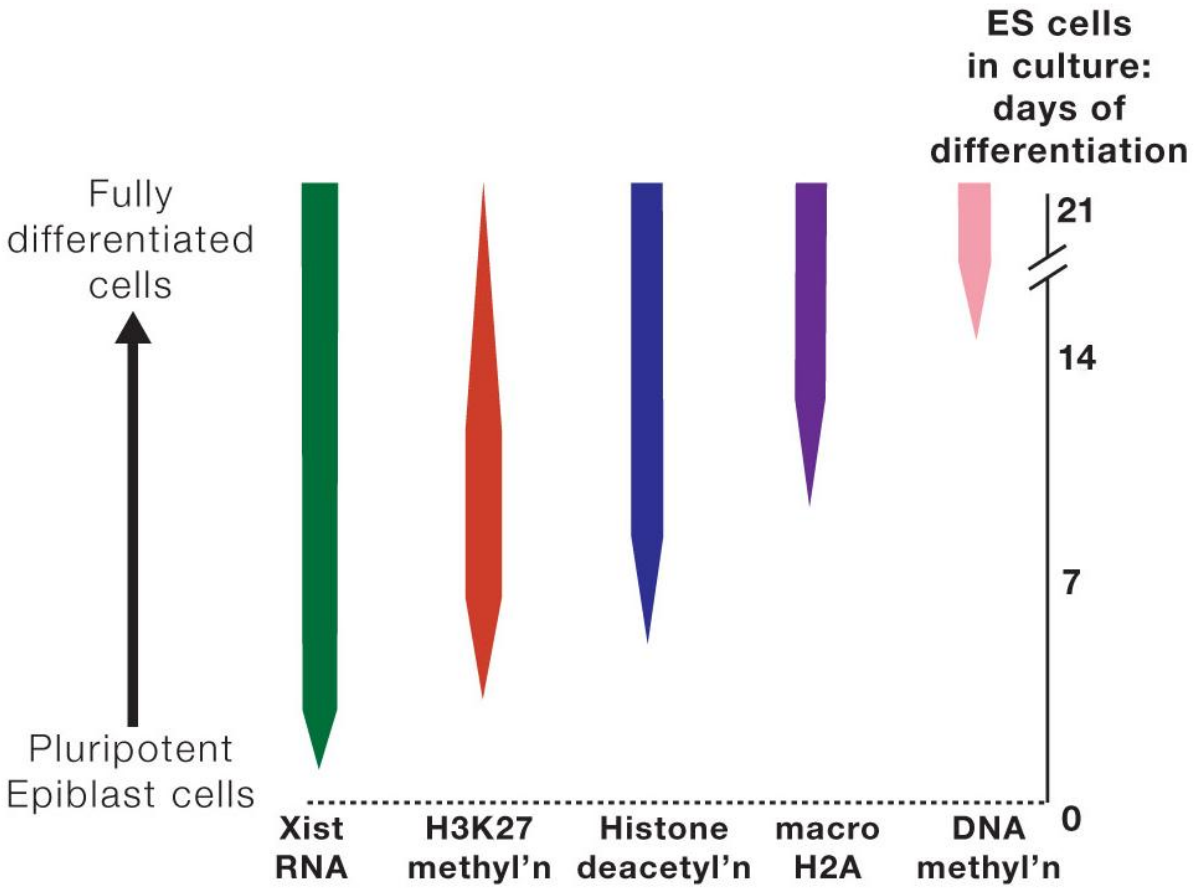
Epigenetic silencing in mammalian X-inactivation



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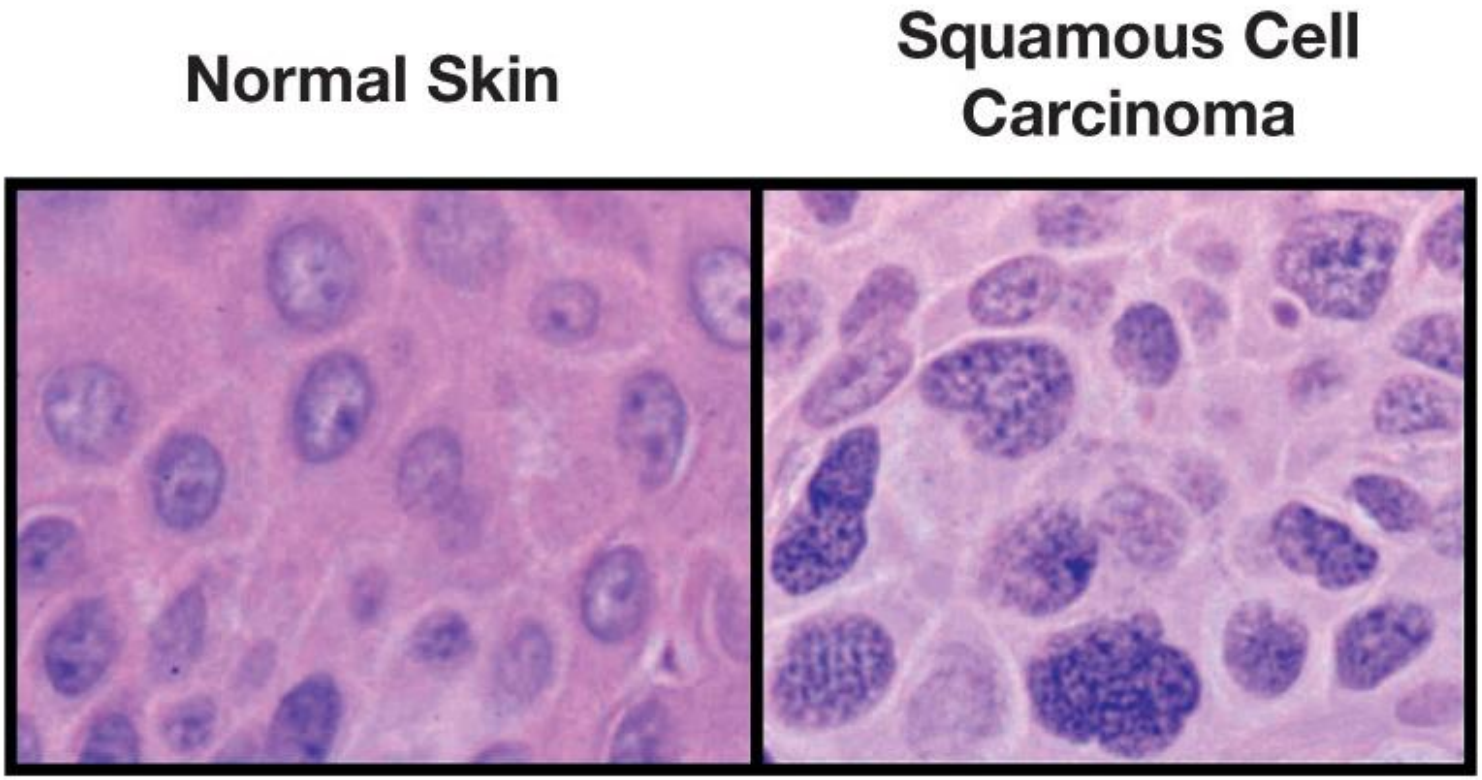
Epigenetics and Cancer



Epigenetics, Allis, Jenuwein, Reinberg, Caparros, 2009

Epigenetic Determinants of Cancer

- Heritable deregulation of genes
- Usually genes involved controlling cell division and apoptosis
- Interplay of oncogenes (dominant) and tumor suppressor genes (recessive) results in formation of cancer

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- Gene inactivation via:

  gene mutation, gene loss or gene switched off
- Gene activation via:

  point mutation or gene switched on

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Genes methylated and epigen. silenced (tumour)

Cell cycle: *Rb, $p16^{INK4a}$, $p15^{INK4a}$, $p14^{ARF}$*

Signal transduction: *RASSF1, APC*

Apoptosis: *DAPK, Caspase 8*

DNA repair: *MLH1, MGMT, BRCA1*

Senescence: *TERT, TERC*

Invasion/metastasis: *TIMP-3, E-cadherin*

APC = adenomatous polyposis coli
DAPK = death-associated protein kinase
MGMT = O-6-methylguanine-DNA methyltransferase

1. Jones & Baylin. Cell 2007;128:683–92
2. Teodoridis JM, et al. Drug Resistance Updates 2004;7:267–78

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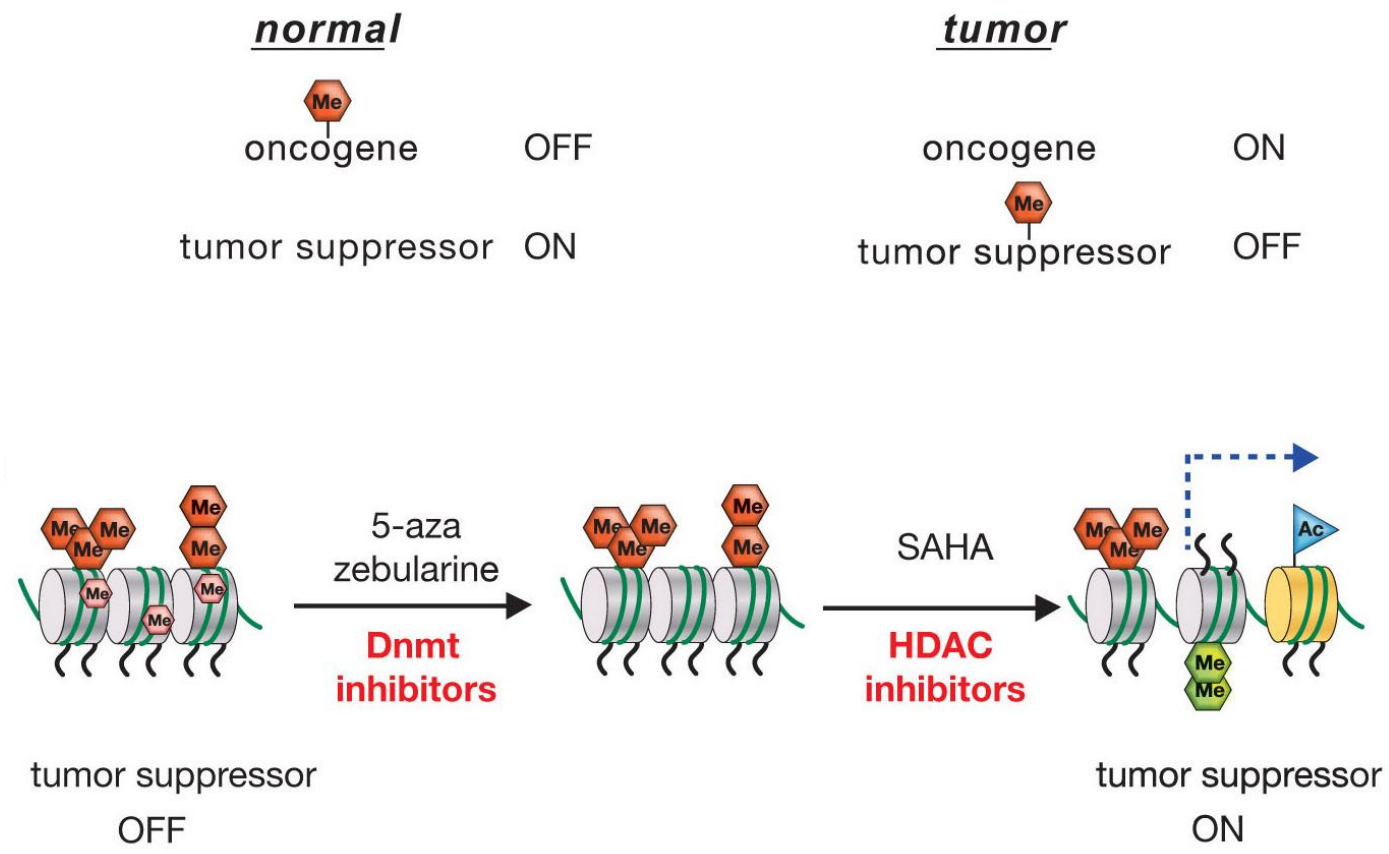
Epigenetic Marks are Reversible



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HDAC inhibitors (HDACi)

Class of HDACi	Compounds	HDAC target	Clinical trials
Hydroxamate	Suberoylanilide hydroxamic acid (SAHA, Vorinostat)	Class I, II	Approved for advanced T-cell lymphoma
	101, LAQ824, LBH589	Class I, II	Phase II
	Trichostatin (),	Class I, II	N/A
	Oxamflatin, Scriptaid, Suberic bishydroxamic acid (SBHA), Azelaic bishydroxamic acid (ABHA), CG-1521	N/A	N/A
	Pyroxamide	Class I, unknown effect on class II	Phase I
	SK-7041, SK-7068	HDACs1 and 2	N/A
	Tubacin	HDAC6	N/A
Alipathic acid	Phenylbutyrate, Valproic acid (VPA)	Class I, II	Phase I, II
	AN-9 (prodrug), Savicol	N/A	N/A
	Baceca	Class I	Phase I, II
Benzamide	MS-275	HDACs 1, 2, 3 and slightly 8	Phase I, II
	MGCD0103	HDAC1, 2, 3, 11	Phase I, II
Cyclic peptide	Depsipeptide (FK228)	Class I	Phase I, II
	Trapoxin A	Class I, II	N/A
	Apicidin	HDAC 1, 3	N/A
	CHAPs	Class I	N/A

(Chapman-Rothe & Brown, Landes Bioscience, 2010)

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Non histone targets of HDACi

Function	Proteins
binding transcriptional factors	P53, c-Myc, 1, -6, E2F1, E2F2, E2F3, GATA-1, GATA-2, GATA-3, GATA-4, Ying Yang 1 (YY1), NF-κB, MEF2, , HIF-1a, 2, -1, -2, -7, SRY, EKLF
Steroid receptors	Androgen receptor, estrogen receptor a, glucocorticoid receptor
Transcription co-regulators	RB, DEK, -3, HMGI(Y)/HMGA1, CtBP2, PGC-1α
Signalling mediators	3, Smad7, β-catenin, -1
repair enzymes	Ku70, WRN, TDG, NEIL2, FEN1
Nuclear import	Rch1, importin-α7
Chaperon protein	90
Structural protein	α-tubulin
Inflammation mediator	HMGB1
Viral proteins	E1A, L-HDAg, S-HDAg, T-antigen, HIV Tat

(Chapman-Rothe & Brown, Landes Bioscience, 2010)

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Epigenetic Therapies

Histone Deacetylase (HDAC) Inhibitors

- Vorinostat: approval in US for treatment of advanced cutaneous T-cell lymphoma

Development of new small compounds

- Targeting new enzyme classes such as HKMT
- Developing more specific compounds

Challenges

- Off-target effects
- Lack of gene specificity
- Uncertain mechanism of action in responsive tumours
- Delivery

Better Epigenetic Therapies?

- More specific, less toxic anti-cancer therapies
- Effort in developing more target specific approaches
- Compound development against new targets such as HKMT
- Rational approaches to combination studies of epigenetic therapies: optimise schedule and maximise efficacy
- Stratifying patient populations: prognostic or predictive epigenetic biomarkers
- Targeting tumour cell subpopulations: drug resistant and stem (sustaining) cell population