Oncology 520
Epigenetics and Cancer

February 16, 2011

Outline

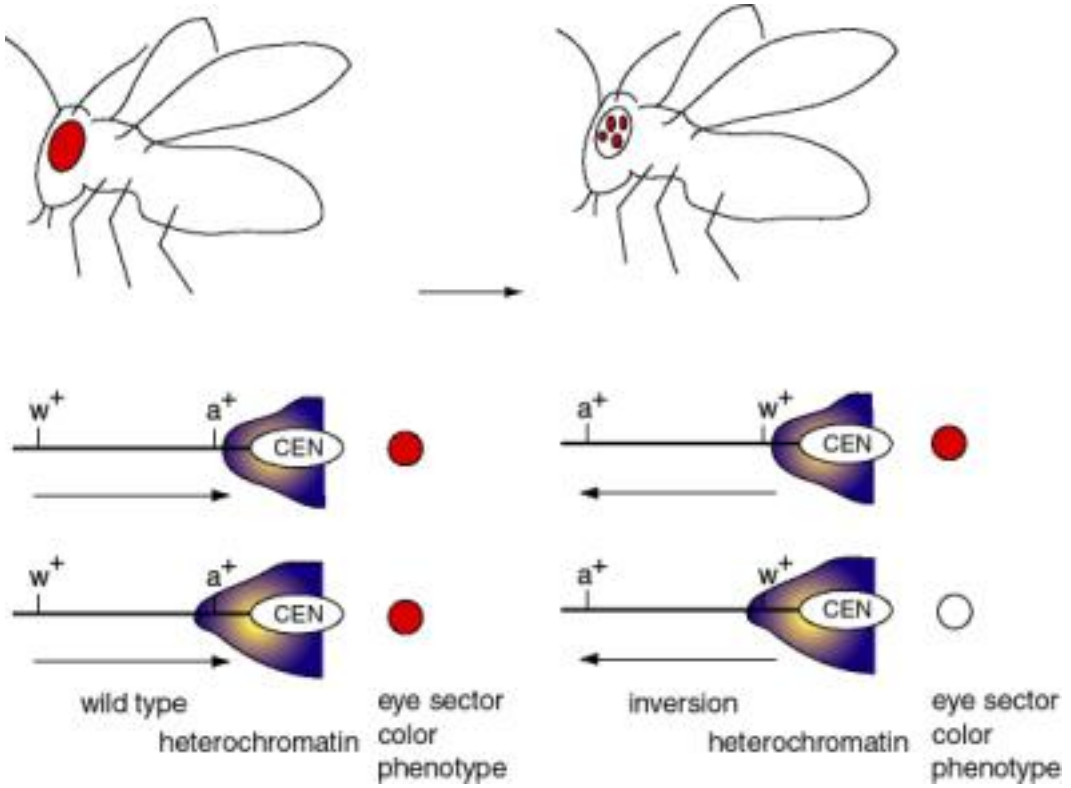
- What is epigenetics?
- What are examples of epigenetic processes?
- What is the epigenome and how is it decoded?
- Are epigenetic mechanisms important in human cancers?
- Does epigenetics provide a link between the genome and the environment?
- What are examples of epigenetic changes that contribute to human cancers?

Definition

- *Epigenetics* (as in "
epigenetic landscape") was coined by C. H. Waddington in 1942 as a portmanteau of the words *genetics* and *epigenesis*.[5] *Epigenesis* is an old[6] word that has more recently been used (see *preformationism* for historical background) to describe the differentiation of cells from their initial totipotent state in embryonic development. When Waddington coined the term the physical nature of genes and their role in heredity was not known; he used it as a conceptual model of how genes might interact with their surroundings to produce a phenotype.
- Robin Holliday defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms."[7] Thus *epigenetic* can be used to describe anything other than DNA sequence that influences the development of an organism.
- The modern usage of the word in scientific discourse is more narrow, referring to heritable traits (over rounds of cell division and sometimes transgenerationally) that do not involve changes to the underlying DNA sequence.[8] The Greek prefix *epi-* in *epigenetics* implies features that are "on top of" or "in addition to" genetics; thus *epigenetic* traits exist on top of or in addition to the traditional molecular basis for inheritance.

Position-effect variegation

http://taputea.lbl.gov/images/flies/fliesgallery/pages/variegation.sat.html



http://www.personal.psu.edu/faculty/r/c/rch8/workmg/TxnlRegChromatinCh20.htm

The Regulation of Chromatin Structure

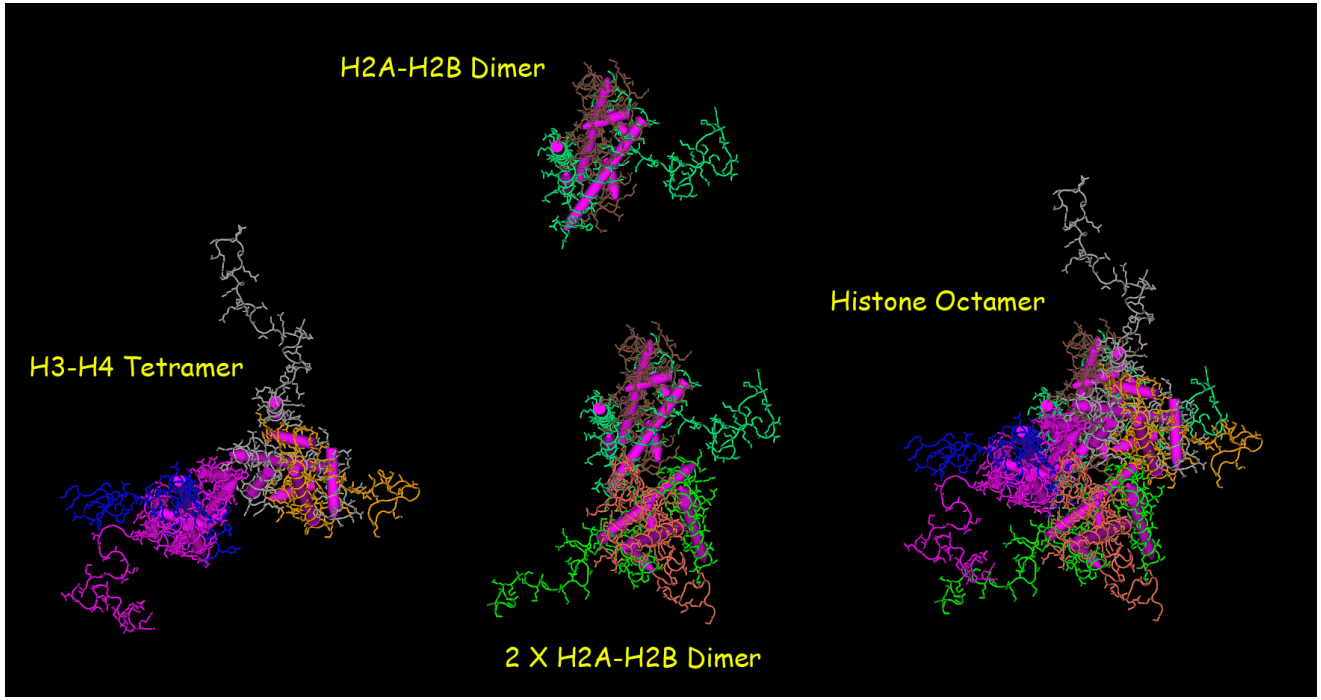
- **Constitutive heterochromatin -**maintains its condensed state throughout the cell cycle and in all cells and cell types. Associated with trimethylation of lysine 20 of histone H4, trimethylation of lysine 9 of histone H3, deacetylation of all four histone types of the nucleosome, and HP1 binding. An example of constitutive heterochromatin is the heterochromatin surrounding the centromere (pericentric heterochromatin).

- **Facultative Heterochromatin** -regions of the genome that maintain a high condensed state during interphase in some cells but where the inclusion of this region of the genome in heterochromatin can vary from cell type to cell type or under different physiological conditions. Associated with the trimethylation of lysine 27 of histone H3. An example of facultative heterochromatin is the inactive X chromosome in mammalian females.

- **Euchromatin**-regions of the genome that are condensed in metaphase but decondensed (below the scale necessary to be visible by conventional light microscopy) during interphase. Associated with increased histone acetylation and the trimethylation of lysine 4 of histone H3

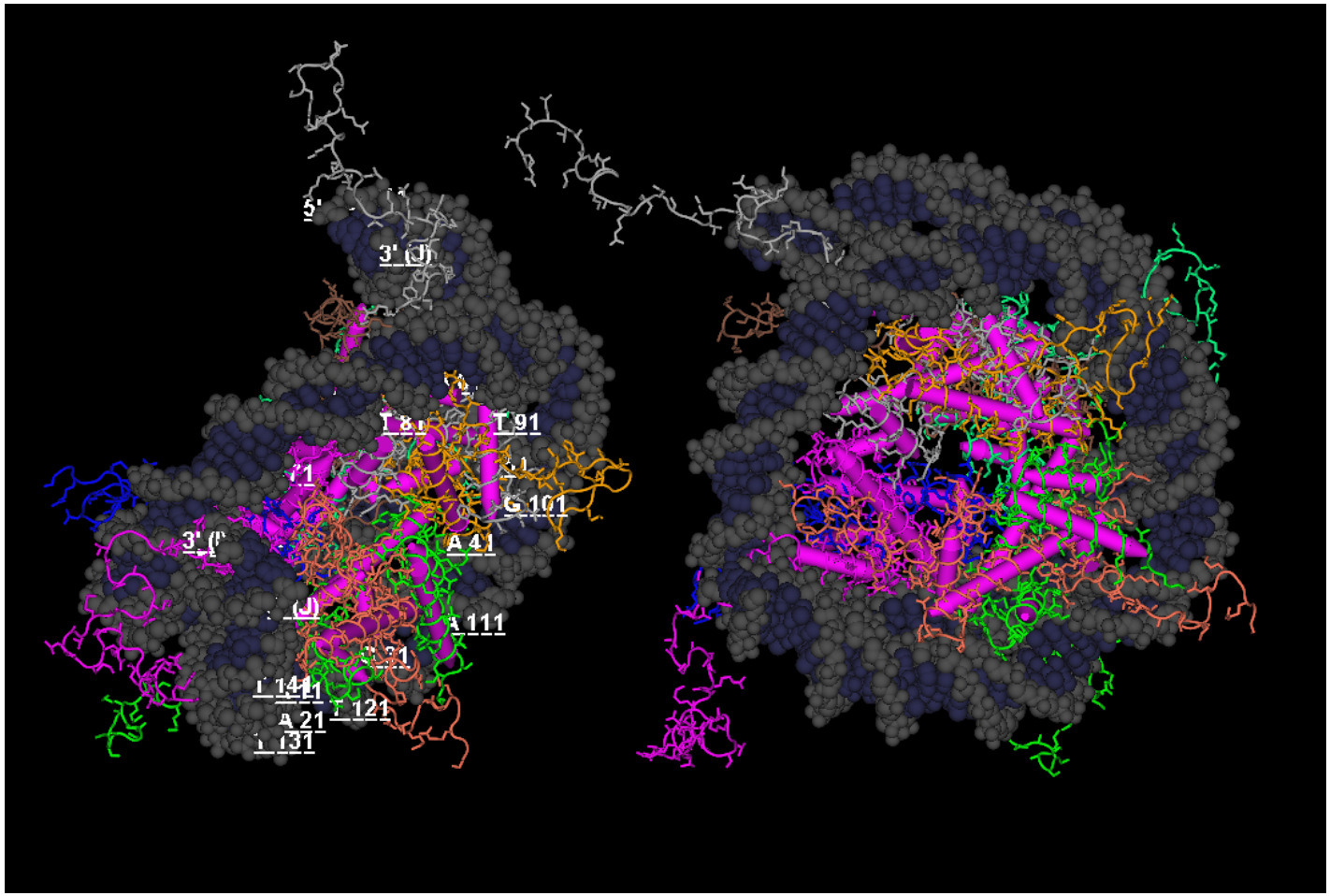
- The state of packaging confers transcriptional competence (can be transcribed if the regulatory proteins are available) or stable repression

The Histone Proteins



There are four histones (H2A, H2B, H3, and H4) present in two copies each to form the nucleosome.

The Nucleosome-The fundamental organizational unit of the genome



Nucleosomes are folded into higher-order structures in cells

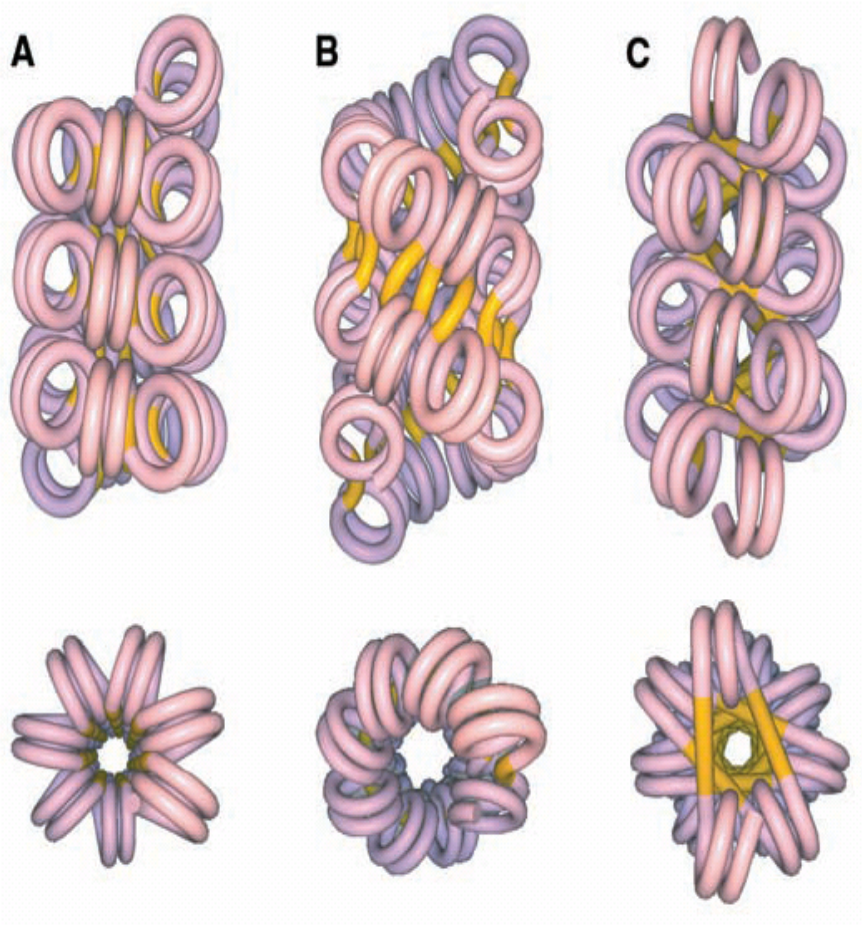


Fig. 1. Models for the DNA path in the chromatin fiber. Higher order structure models: (**A**) one-start solenoidal (*6*), (**B**) two-start supercoiled (*7*), and (**C**) two-start twisted (*12*). Upper views have the fiber axis running vertically; lower views are down the fiber axis. DNA associated with the nucleosome core is red/blue, and linker DNA running between cores is yellow. These models are idealized, with nucleosome cores in each start contacting each other. The open three-dimensional zigzag seen in conditions not fully compacting may be a precursor (*21*).

SCIENCE VOL 306 26 NOVEMBER 2004

Note the relationship between linear sequence and spatial proximity of that sequence

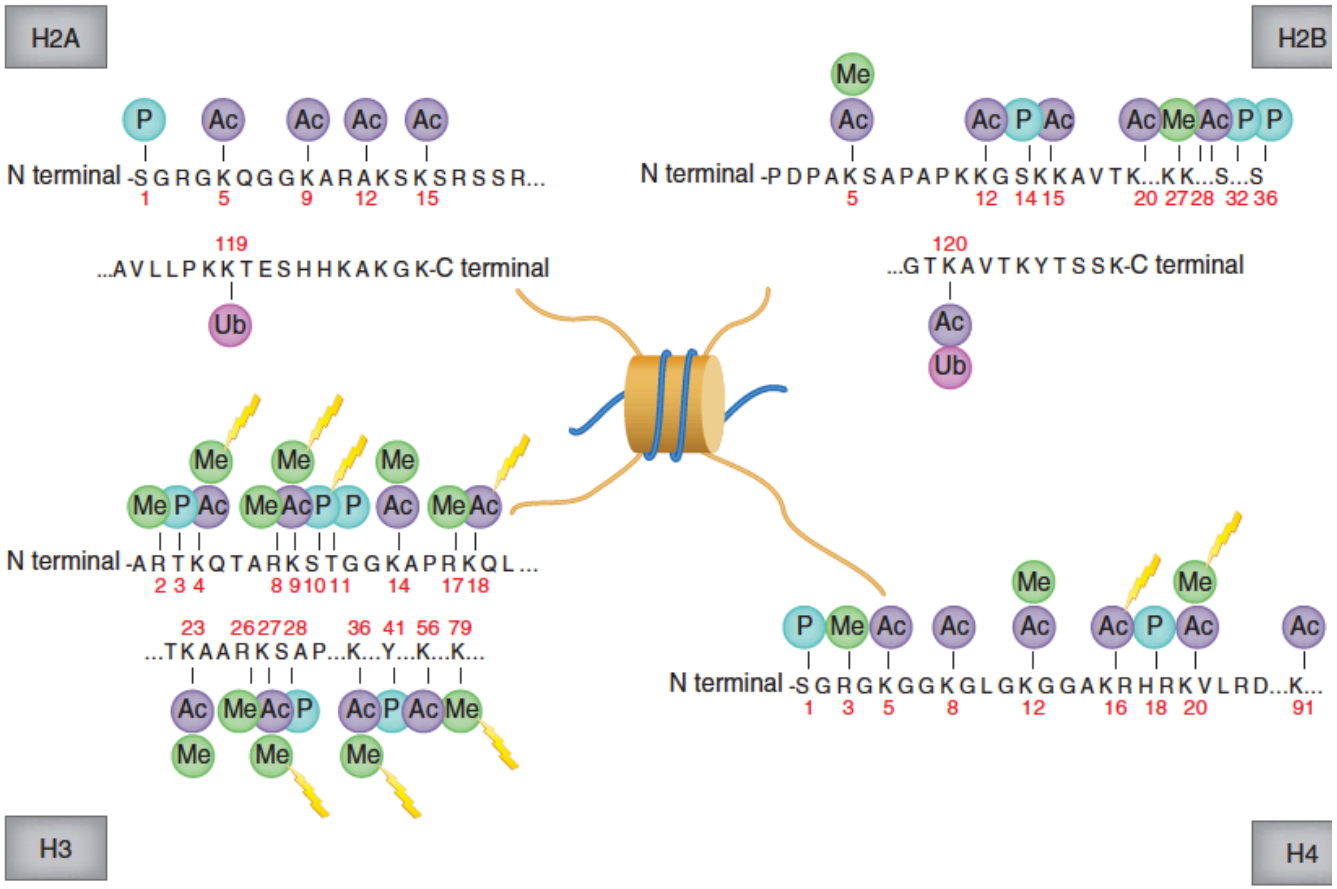
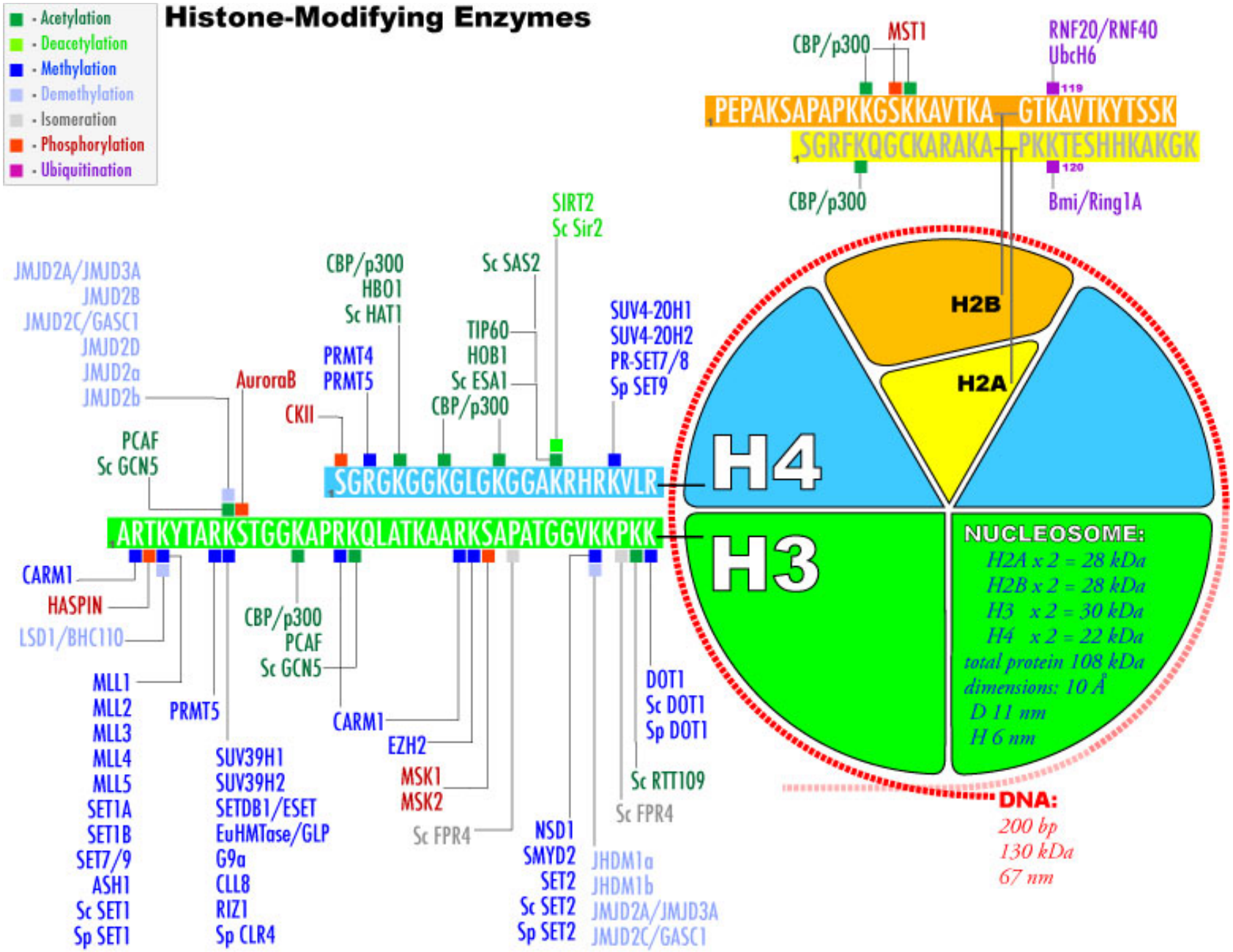


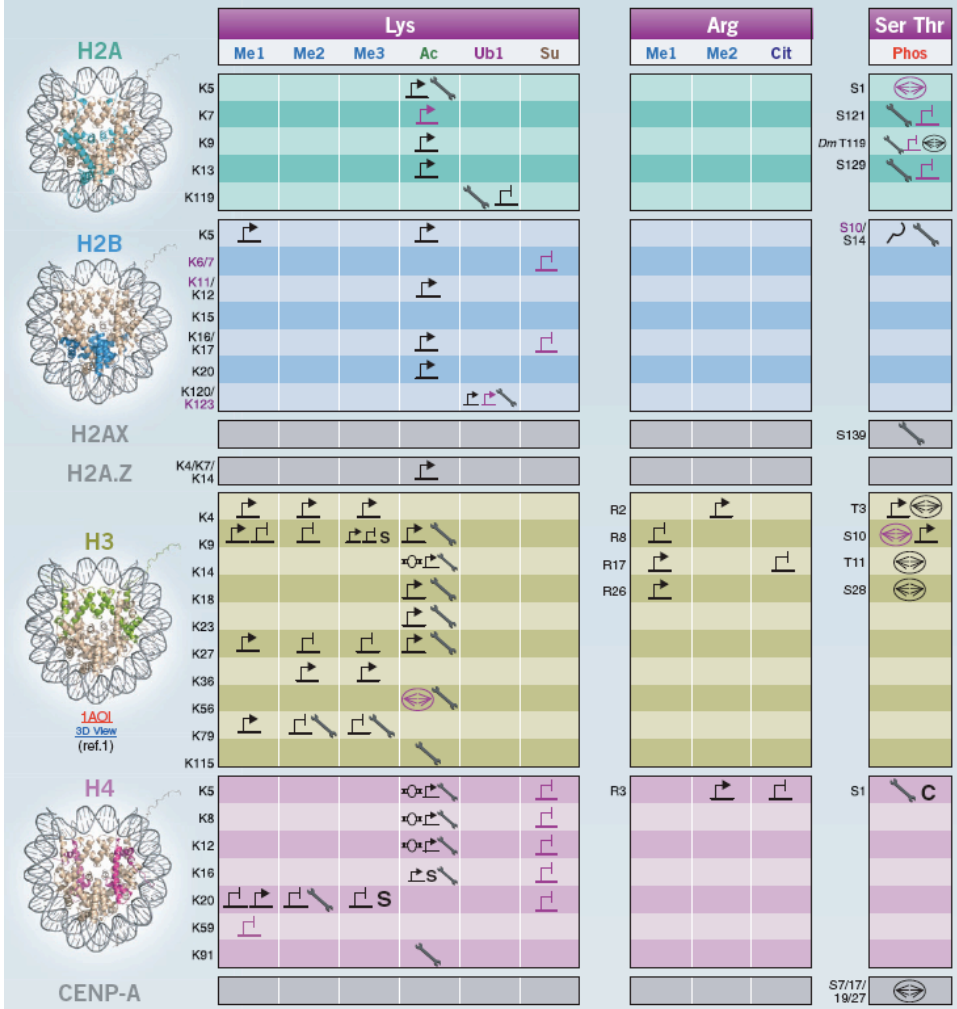
Figure 2 Histone modification patterns in normal and cancer cells. Mainly along their protruding N-terminal tails, but also within their C-terminal regions, histones can undergo diverse post-translational modifications. In the right combination and translated by the appropriate effectors, these modifications contribute to establishing the global and local condensed or decondensed chromatin states that eventually determine gene expression. This figure depicts the main modifications of the four core histones in normal cells (type and position in the amino acid sequence). Furthermore, and because disruption of their normal patterns is related to cancer, histone modifications typically associated with the disease have also been highlighted. Ac, acetylation; Me, methylation; P, phosphorylation; Ub, ubiquitination.

Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330


Histone-Modifying Enzymes
- Acetylation
- Deacetylation
- Methylation
- Demethylation
- Isomeration
- Phosphorylation
- Ubiquitination
CBP/p300
MST1
RNF20/RNF40
UbcH6
PEPAKSAPAPKKGSKKAVTKA
GTKAVTKYTSSK
SGRFKQGCKARAKA
PKKTESHHKAKGK
CBP/p300
Bmi/Ring1A
SIRT2
Sc Sir2
JMJD2A/JMJD3A
JMJD2B
JMJD2C/GASC1
JMJD2D
JMJD2a
JMJD2b
CBP/p300
HBO1
Sc HAT1
Sc SAS2
TIP60
HOB1
Sc ESA1
CBP/p300
SUV4-20H1
SUV4-20H2
PR-SET7/8
Sp SET9
AuroraB
PRMT4
PRMT5
CKII
PCAF
Sc GCN5
SGRGKGGKGLGKGGAKRHRKVLR
ARTKYTARKSTGGKAPRKQLATKAARKSAPATGGVKKPKK
H2B
H2A
H4
H3
NUCLEOSOME:
H2A x 2 = 28 kDa
H2B x 2 = 28 kDa
H3 x 2 = 30 kDa
H4 x 2 = 22 kDa
total protein 108 kDa
dimensions: 10 Å
D 11 nm
H 6 nm
DNA:
200 bp
130 kDa
67 nm
CARM1
HASPIN
LSD1/BHC110
MLL1
MLL2
MLL3
MLL4
MLL5
SET1A
SET1B
SET7/9
ASH1
Sc SET1
Sp SET1
PRMT5
SUV39H1
SUV39H2
SETDB1/ESET
EuHMTase/GLP
G9a
CLL8
RIZ1
Sp CLR4
CBP/p300
PCAF
Sc GCN5
CARM1
EZH2
MSK1
MSK2
Sc FPR4
NSD1
SMYD2
SET2
Sc SET2
Sp SET2
Sc FPR4
JHDM1a
JHDM1b
JMJD2A/JMJD3A
JMJD2C/GASC1
DOT1
Sc DOT1
Sp DOT1
Sc RTT109

Wikipedia

FUNCTIONS ASSOCIATED WITH COVALENT HISTONE MODIFICATIONS



Symbols indicate processes associated or correlated with modification (effects will be context dependent)

C Chromatin condensation S Domain-limited functional chromatin changes DNA repair Cell division Replication/histone deposition

Active transcription Repressed transcription/ silent regions Apoptosis Symbols in purple indicate data from yeast

Other modifications include ADP ribosylation, such as that at H2BE2, proline isomerization, such as that at H3P38 and biotinylation.

'3D ref' columns list structure citations (in some cases including homologs from other species). Further reading can be found in refs. 106–139 and references cited therein.

Additional histone variants have been identified and are modified. These include macroH2A and histone H3.3 (ref. 106). Residues not shown are also modified[106], and the Reviews in this issue cover in detail the functions associated with modifications and cross-talk between modifications[107–112].

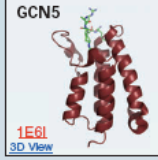
Structure figures used PyMOL (http://pymol.sourceforge.net/), domain assignments used PubMed and/or SMART (http://smart.embl-heidelberg.de/).

Me, methylation; Ub1, monoubiquitination; Ac, acetylation; Cit, citrullination; Phos, phosphorylation; Su, sumoylation.

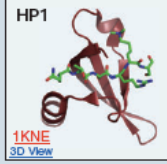
nature structural & molecular biology

HISTONE RECOGNITION DOMAINS

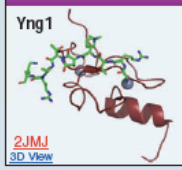
Bromodomain

REPRESENTATIVE STRUCTURE	PROTEIN	TARGET	3D REF	PROTEIN	TARGET	3D REF
GCN5  1E6I 3D View	*Sc* Gcn5	H4K16ac	5,6	CBP/p300	?	140
	PCAF	H4K16ac	10	*Sc* Bdf1/BRD8/dBrd8	H4ac	
	TAF1	H4ac	18	Polybromo/BAF180	H3ac	
	hBRG1	H3K14ac	69,70	*Sc* Rsc1,2,4	H3K14ac (Rsc4)	71 (Rsc4)
	Sc Snf2	H3ac/H4ac		*Dm* NURF301/BPTF	?	81 (BPTF)
	Sc Sth1	?		hACF1/dACF	?	

Chromodomain

HP1  1KNE 3D View	HP1/Swi6	H3K9me2/3	72,73	dMI-2/CHD3/CHD4/CHD5	?	
	PC1/PC2/Polycomb/LHP1	H3K27me3, H3K9me3	74,75	CHD6/CHD7/CHD8/CHD9	?	
	CHD1	H3K4me1/3	76	hBAF155	?	
	Sc Chd1	?	101,102	dMrg15/hMRG15, *Sc* Eaf3[a]	H3K36me, H3K4me	77
	dTip60/hTIP60, MOF, Esa1	?	17 (*Dm* MOF)	CDY1	H3K9me2/3	
	Sp Clr4, SUV39H1	H3K9me	33 (Clr4)			

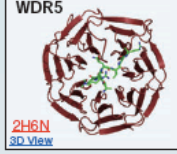
PHD

Yng1  2JMJ 3D View	BHC80	H3K4me0	78	hACF1/dACF	Core histones	
	Yng1	H3K4me2/3	79	Ash1	?	
	ING2	H3K4me2/3	80	JMJD2A/2B/2C	?	
	BPTF/*Dm* NURF301	H3K4me2/3	81	JHDM1a/b	?	
	NSD1	?		JARID1C	H3K9me3	
	MLL	?		dMI-2/CHD3/CHD4	?	

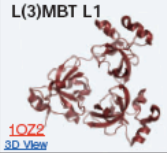
Tudor

53BP1  2IG0 3D View	JMJD2A	H3K4me3/H4K20me3	55	ESET/SETDB1	H3K9	
	53BP1	H4K20me1/2	82	JMJD2B/2C	?	
	Sp Crb2	H4K20me2	82	PHF20	H4K20me2	

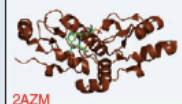
WD40

	PROTEIN	TARGET	3D REF
WDR5  2H6N 3D View	WDR5	H3R2*/H3K4me2*	83–85, 141
	RbAp46/48	?	
	p55	?	
	(*WDR5 can bind unmodified H3 tail)		

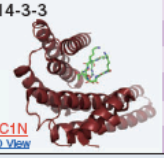
MBT

	PROTEIN	TARGET	3D REF
L(3)MBT L1  1OZ2 3D View	L(3)MBTL1	H1bK26me1/2, H4K20me1/2	89
	SCML2	?	90
	SFMBT	H3K9me1/2, H4K20me1/2	
	PHF20L1	H3K4me1, H4K20me1	

BRCT

	PROTEIN	TARGET	3D REF
MDC1  2AZM 3D View	MDC1	H2AXPh	86,87
	Sp Crb2	H2APh	
	53BP1	?	
	MCPH1	H2AXPh	

14-3-3

14-3-3  2C1N 3D View	14-3-3	H3S10Ph/H3S28Ph	88

See ref. 108 for further information and examples.

Question mark indicates that the exact histone binding specificity is unknown.

[a] Chromo barrel-like motif. List is mostly limited to proteins on these pages.

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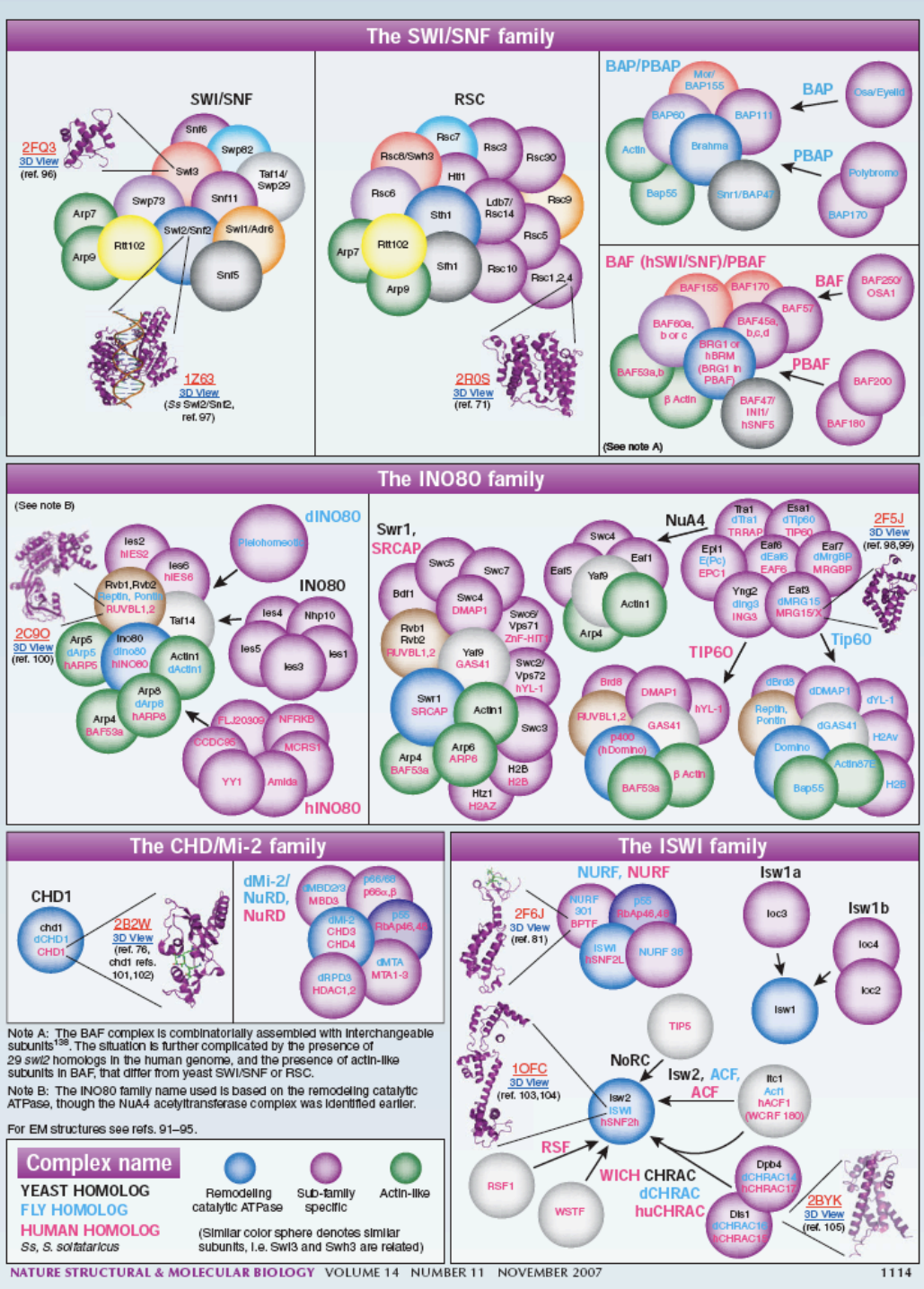
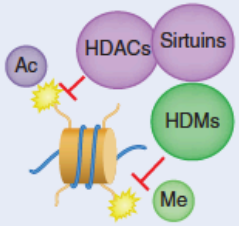
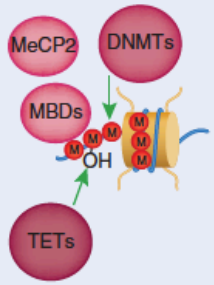

The SWI/SNF family
SWI/SNF
2FQ3
3D View
(ref. 96)
Snf6
Swp82
Swi3
Taf14/
Swp29
Swp73
Snf11
Arp7
Swi2/Snf2
Swi1/Adr6
Arp9
Rtt102
Snf5
1Z63
3D View
(Ss Swi2/Snf2,
ref. 97)
RSC
Rsc7
Rsc3
Rsc8/Swh3
Rsc30
Htl1
Rsc6
Rsc9
Sth1
Ldb7/
Rsc14
Rsc5
Arp7
Rtt102
Sfh1
Rsc10
Arp9
Rsc1,2,4
2R0S
3D View
(ref. 71)
BAP/PBAP
Mor/
BAP155
BAP
Osa/Eyelid
BAP60
BAP111
Actin
Brahma
PBAP
Polybromo
Bap55
Snr1/BAP47
BAP170
BAF (hSWI/SNF)/PBAF
BAF155
BAF170
BAF
BAF250/
OSA1
BAF57
BAF60a,
b or c
BAF45a,
b,c,d
BRG1 or
hBRM
(BRG1 in
PBAF)
PBAF
BAF200
BAF53a,b
β Actin
BAF47/
INI1/
hSNF5
BAF180
(See note A)
The INO80 family
(See note B)
dINO80
Pleiohomeotic
Ies2
hIES2
Ies6
hIES6
Rvb1,Rvb2
Reptin, Pontin
RUVBL1,2
INO80
Taf14
Ies4
Nhp10
Ies5
Ies1
Ies3
2C9O
3D View
(ref. 100)
Arp5
dArp5
hARP5
Ino80
dIno80
hINO80
Actin1
dActin1
Arp8
dArp8
hARP8
Arp4
BAF53a
FLJ20309
NFRKB
CCDC96
MCRS1
YY1
Amida
hINO80
Swr1,
SRCAP
Swc5
Swc7
Bdf1
Swc4
DMAP1
Swc6/
Vps71
ZnF-HIT1
Rvb1
Rvb2
RUVBL1,2
Yaf9
GAS41
Swc2/
Vps72
hYL-1
Swr1
SRCAP
Actin1
Swc3
Arp4
BAF53a
Arp6
ARP6
H2B
H2B
Htz1
H2AZ
NuA4
Swc4
Eaf1
Eaf5
Yaf9
Actin1
Arp4
Tra1
dTra1
TRRAP
Esa1
dTip60
TIP60
Epl1
E(Pc)
EPC1
Eaf6
dEaf6
EAF6
Eaf7
dMrg8P
MRG8P
Yng2
dIng3
ING3
Eaf3
dMRG15
MRG15/X
2F5J
3D View
(ref. 98,99)
Tip60
TIP60
Brd8
DMAP1
hYL-1
RUVBL1,2
GAS41
p400
(hDomino)
BAF53a
β Actin
dBrd8
dDMAP1
dYL-1
Reptin,
Pontin
dGAS41
H2Av
Domino
Actin87E
Bap55
H2B
The CHD/Mi-2 family
CHD1
chd1
dCHD1
CHD1
2B2W
3D View
(ref. 76,
chd1 refs.
101,102)
dMi-2/
NuRD,
NuRD
dMBD2/3
MBD3
p66/68
p66α,β
dMi-2
CHD3
CHD4
p55
RbAp46,48
dMTA
MTA1-3
dRPD3
HDAC1,2
The ISWI family
NURF, NURF
2F6J
3D View
(ref. 81)
NURF
301
BPTF
p55
RbAp46,48
ISWI
hSNF2L
NURF 38
Isw1a
Ioc3
Isw1b
Ioc4
Ioc2
Isw1
TIP5
NoRC
1OFC
3D View
(ref. 103,104)
Isw2
ISWI
hSNF2h
Isw2, ACF,
ACF
Itc1
Acf1
hACF1
(WCRF 180)
RSF
RSF1
WSTF
WICH CHRAC
dCHRAC
huCHRAC
Dpb4
dCHRAC14
hCHRAC17
Dls1
dCHRAC17
hCHRAC17
2BYK
3D View
(ref. 105)
Note A: The BAF complex is combinatorially assembled with interchangeable subunits[198]. The situation is further complicated by the presence of 29 swi2 homologs in the human genome, and the presence of actin-like subunits in BAF, that differ from yeast SWI/SNF or RSC.
Note B: The INO80 family name used is based on the remodeling catalytic ATPase, though the NuA4 acetyltransferase complex was identified earlier.
For EM structures see refs. 91–95.
Complex name
YEAST HOMOLOG
FLY HOMOLOG
HUMAN HOMOLOG
Ss, S. solfataricus
Remodeling catalytic ATPase
Sub-family specific
Actin-like
(Similar color sphere denotes similar subunits, i.e. Swi3 and Swh3 are related)

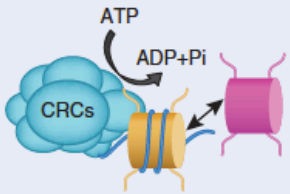
Category	Gene
HDACs	*HDAC1*
	HDAC2
	HDAC6
Sirtuins	*SIRT1*
	SIRT2
	SIRT3
	SIRT7
HDMs	*KDM1A*
	KDM2B
	KDM4C
	KDM5A
	KDM5B
	KDM5C
	KDM6A
	KDM6B



Category	Gene
DNMTs	*DNMT1*
	DNMT3A
	DNMT3B
(2OG)-Fe(II)–dependent oxygenases	*TET1*
	TET2
Methyl-CpG binding proteins	*MBD1*
	MBD2
	MBD3
	MBD4
	MECP2

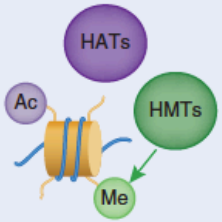


Category	Gene
Histone variants	*H2AFZ*
Chromatin remodeling factors	*ARID1A*
	CHD5
	CHD7
	MTA1
	MTA2
	MTA3
	SMARCA2
	SMARCA4
	SNF5

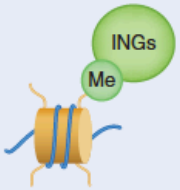


3CACC6

Category	Gene
HATs	*CREBBP*
	EP300
	MYST3
	MYST4
	KAT5
HMTs	*MLL*
	EZH2
	NSD1
	PRDM2
	SMYD3
	WHSC1



Category	Gene
Histone modification readers	*ING1*
	ING2
	ING3
	ING4
	ING5



Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330

Figure 3 Selection of epigenetic genes disrupted in human tumors. Mutation, deletion and/or altered expression of genes encoding components of the various epigenetic machineries are typically observed in human tumors. The figure shows a selection of genes encoding enzymes that add, remove and recognize histone modifications, as well as members of the DNA methylation machinery, whose deregulation is connected to cancer. CRCs, chromatin remodeling complexes; Ac, acetylation; Me, methylation.

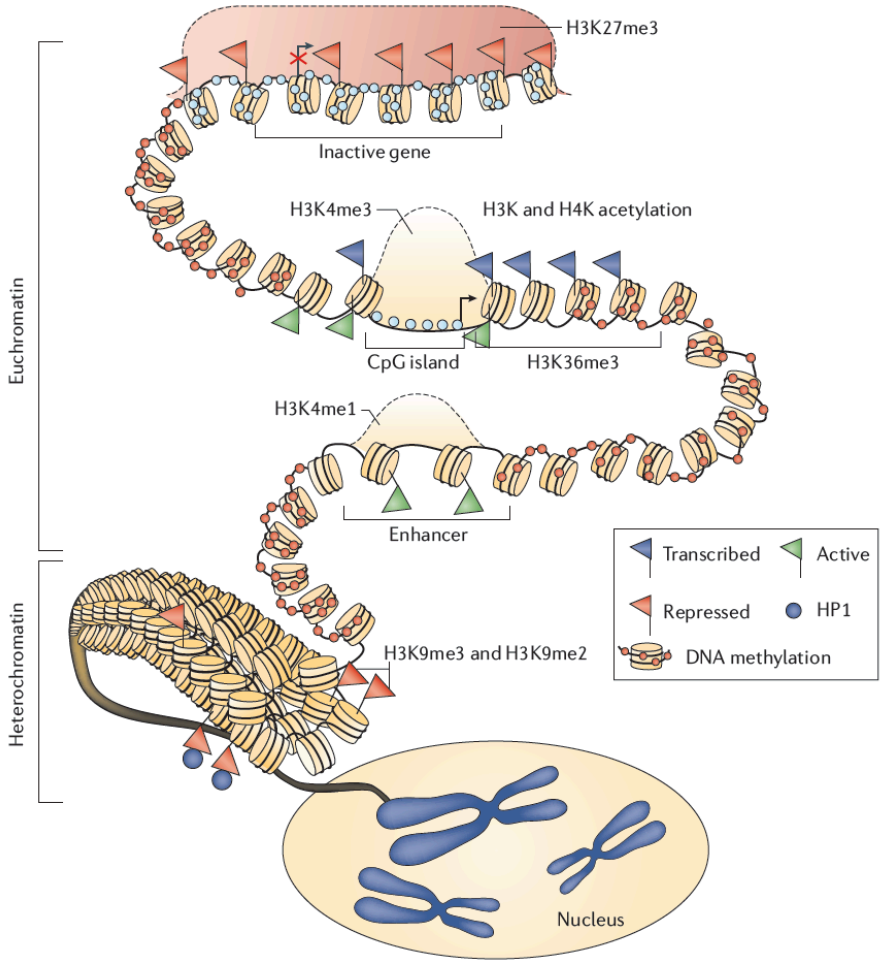
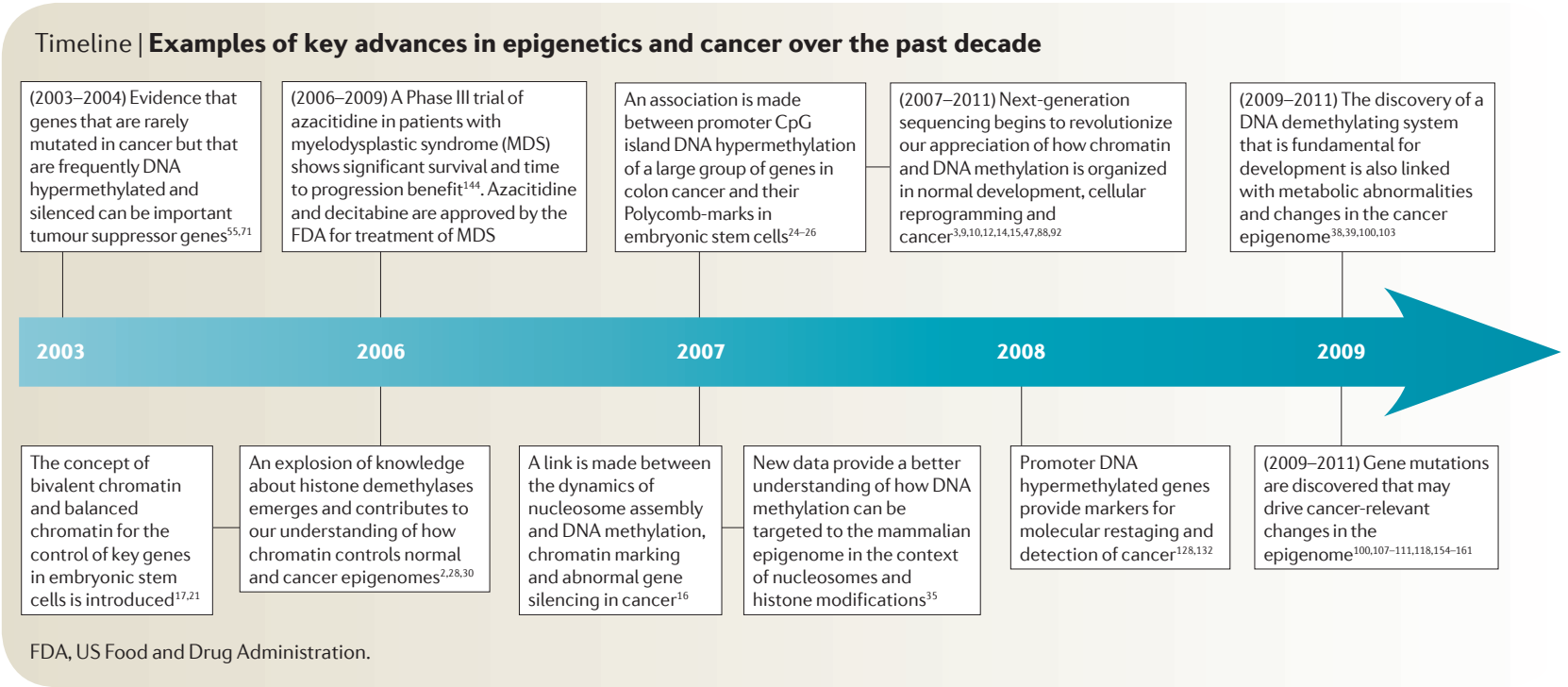


Figure 1 | **Model of the overall structure of the epigenome in normal human cells.** This diagram shows the balanced state of chromatin, nucleosome positioning and DNA methylation, which maintains the normal packaging state of DNA. A silenced gene (indicated by a red X over the transcription start site designated by the arrow) at the top of the figure has its promoter CpG island occupied by a Polycomb group (PcG) complex (indicated by a red shaded area) that mediates chromatin changes that include the repressive histone modification trimethylation of lysine 27 on histone 3 (H3K27me3). There is no CpG DNA methylation within the gene promoter CpG island (shown by pale blue circles) and nucleosomes are positioned over the transcription start site. Sites upstream from the promoter are heavily DNA methylated (shown by red circles). The gene promoter illustrated below the silenced gene has been signalled to adopt a fully active transcription state and retains the active H3K4me3 marks at the promoter. It also has acetylation of key H3 and H4 lysines, the presence of the variant histone, H2A.Z (not shown) and H3K36me3 in the gene body to facilitate transcriptional elongation. The transcription start region (indicated by an arrow) is not occupied by nucleosomes. Just below, a distal enhancer is shown for this gene with an active nucleosome configuration, and the signature histone modification for enhancers, H3K4me1, is present. Finally, towards the bottom of the figure, the packaging of the majority of the cellular DNA into a transcriptionally repressed configuration is depicted, with compacted nucleosomes, the presence of H3K9me2 and H2K9me3, which are signature repressive marks for constitutive heterochromatin, the presence of heterochromatin protein 1 (HP1; also known as CBX5) and extensive DNA methylation. The folding of the heterochromatin into chromosomal locations in the nucleus is shown. Image is adapted, with permission, from REF. 166



Baylin and Jones (2011). Nature Reviews Cancer 11:726-734

X-chromosome inactivation

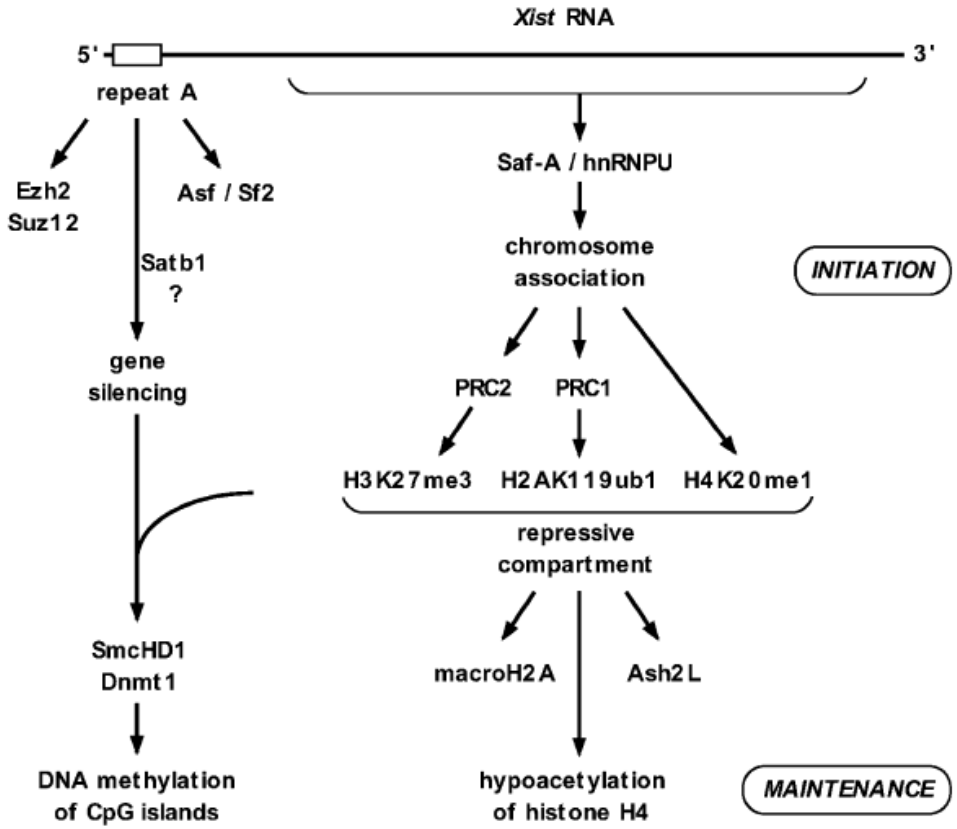
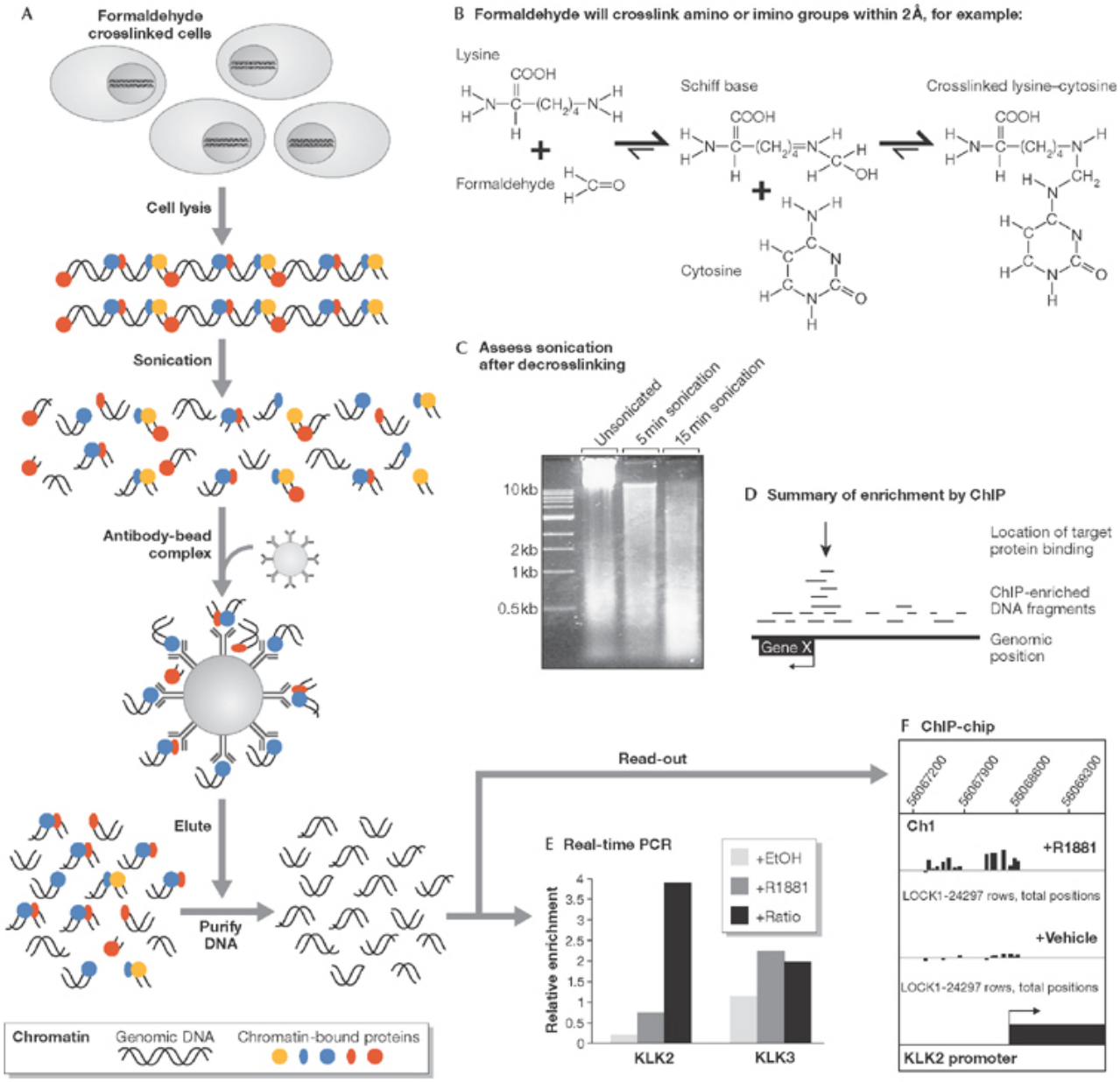


Fig. 2 Sequence–function relation of mouse *Xist* RNA. A schematic overview of *Xist* sequences and their connections with chromosomal changes is shown. The repeat A in a 500 nucleotide (nt) long region at the 5′ of *Xist* has been shown to be required for initiation of gene silencing. Pathways considered important for gene silencing and factors that are associated with repeat A RNA are linked by *arrows*. Initiation of gene silencing requires a special cellular context that in T cell differentiation is correlated with Satb1 expression. Unknown factors with similar functions to Satb1 are expected to contribute to gene silencing in other cell systems (indicated by the *question mark*). Maintenance of gene repression in differentiated somatic cells involves DNA methylation of CpG dinucleotide rich gene promoters and depends on the SmcHD1 protein that has been shown to be enriched on the Xi. The 17,000–19,000 nt long body of *Xist* contains multiple elements that mediate association of *Xist* with the chromosome and trigger the formation of a repressive compartment that is thought to contribute to gene silencing. Saf-A/hnRNPU has been shown to be tightly linked with *Xist* localization and to be required for chromosome attachment. Recruitment of chromatin modifying complexes and chromatin proteins is mediated by direct or indirect mechanisms following *Xist* accumulation on the Xi. During cell differentiation histone H4 hypoacetylation is established that can be maintained independent of *Xist* by unknown pathways

Human Genetics 130:295-305

What does X-chromosome inactivation tell us about gene regulation and DNA sequence?

Chromatin immunopreciptiation for mapping epigenetic changes in the genome



Charles E Massie & Ian G Mills

EMBO reports ***9, 4, 337–343 (2008)***

Genome-wide histone modification map

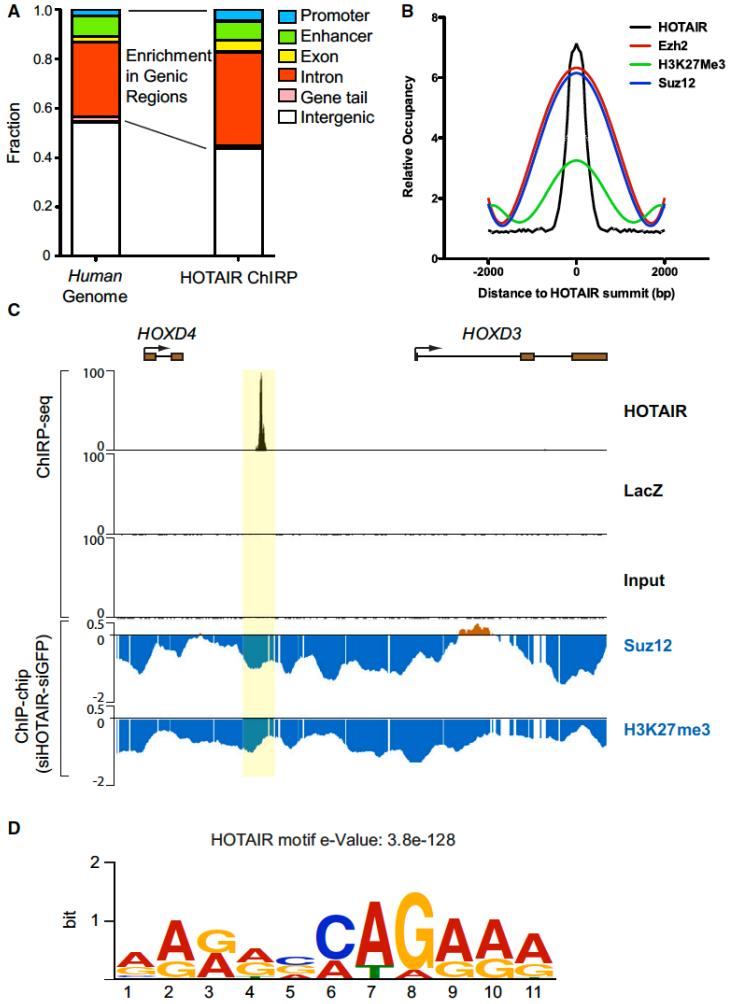


Figure 5. HOTAIR ChIRP-Seq Suggests Mechanisms of HOTAIR Recruitment of PRC2
(A) HOTAIR binding sites are enriched in genic regions, notably promoters and introns.
(B) Metagene analysis of genomic regions aligned by 832 HOTAIR ChIRP peaks show focal HOTAIR peaks in association with broad domains PRC2 occupancy (evidenced by subunits EZH2 and SUZ12) and H3K27Me3.
(C) HOTAIR nucleates broad domains of PRC2 occupancy. A HOTAIR binding site between *HOXD3* and *HOXD4* lies in the center of a broad domain of SUZ12 and H3K27Me3 occupancy that are both lost upon HOTAIR knock down (Tsai et al., 2010, Rinn et al., 2007).
(D) GA-rich homopurine motif enriched in HOTAIR binding sites.

Chu et al. (2011). Molecular Cell 44:1-12

Epigenetics and Diet:

There have long been correlations between diet and cancer. While carcinogens in our food can explain some of this relationship, what is the basis for foods that have been associated with reduced cancer risk?

1) There is a relationship between metabolism and epigenetic state. While this is still an emerging field with much to be learned, some of these relationships are well established. In particular, the importance of nutrients that regulate the methylation state of DNA and proteins is well established.
2) Some foods associated with lowered cancer risk have been found to contain chemicals that inhibit epigenetic processes. For example:
    a) Dietary fiber—generates short chain fatty acids in the colon. Short chain fatty acids are histone deacetylase inhibitors
    b) Sulfranone (broccoli)-inhibits histone deacetylase
    c) Curcumin (curry)-inhibits CBP and P300 (histone acetyltransferases)
    d) Epigallocatechin-3-gallate (green tea)-inhibits DNA methylation

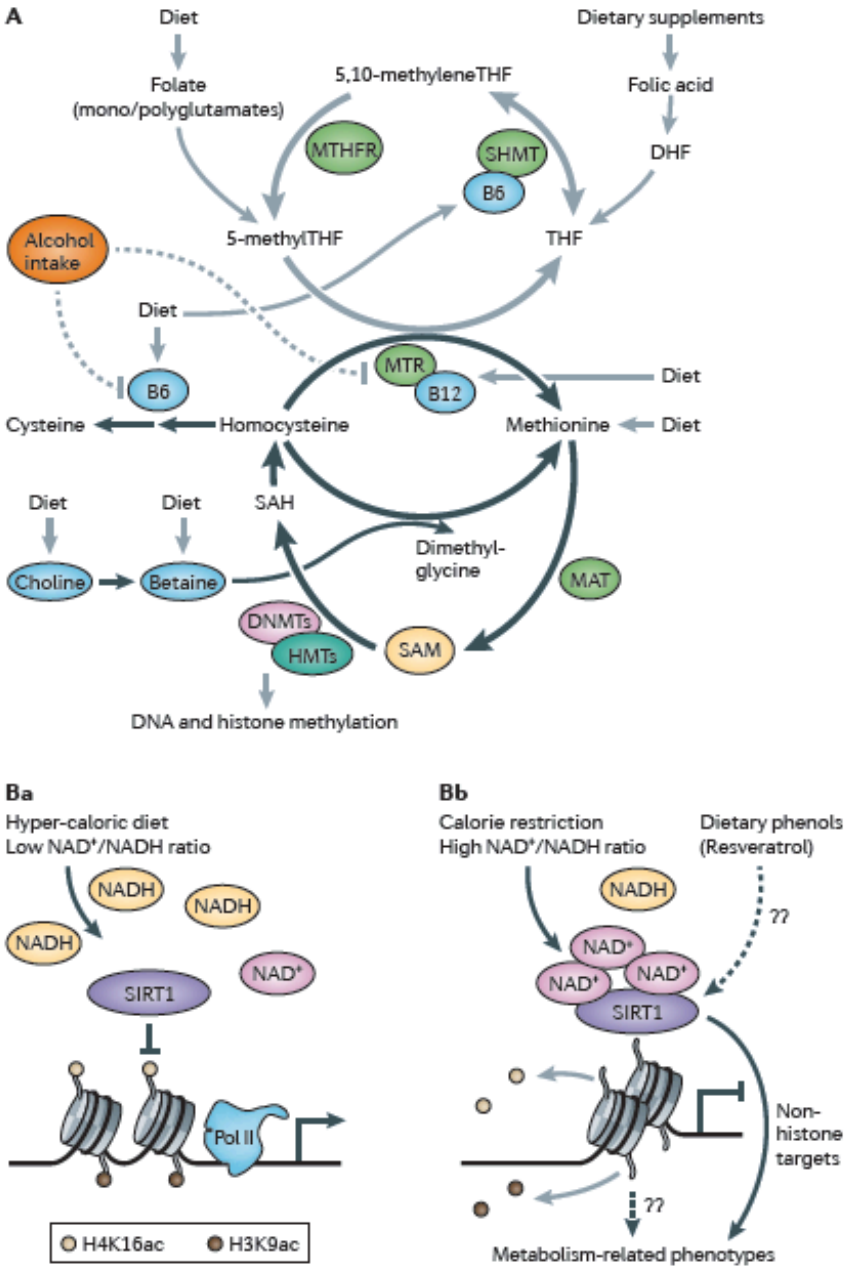


Figure 4 | **Molecular mechanisms that mediate environmental effects. A** | Levels of *S*-adenosylmethionine (SAM) affect global DNA and histone methylation. In cells, SAM is generated by the methionine cycle (also known as the one-carbon cycle; thick black arrows). The cycle incorporates methyl groups from dietary folate in another multistep cyclic pathway, called the folate cycle (thick grey arrows). The folate cycle includes the enzymes serine hydroxymethyltransferase (SHMT), methylenetetrahydrofolate reductase (MTHFR) and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR). Before its incorporation into the folate cycle, folic acid (the synthetic form of natural folate) from dietary supplements must be converted to dihydrofolate (DHF) and then to tetrahydrofolate (THF). MTR uses methyl groups from the folate cycle to convert homocysteine to methionine. Methionine adenosyltransferase (MAT) catalyses the synthesis of SAM from methionine. SAM is then converted to *S*-adenosylhomocysteine (SAH) by DNA- and histone-methyltransferases (DNMTs and HMTs) that use its methyl group to methylate DNA and histones. SAH is hydrolysed to homocysteine to close the cycle. The methionine cycle can also incorporate methyl groups from betaine. Two important cofactors that are involved in SAM biosynthesis are vitamins B6 and B12. Vitamin B6 is involved in the conversion of homocysteine to cysteine, and of THF to 5,10-methyleneTHF. Vitamin B12 is a cofactor of MTR. Alcohol intake can have an effect on SAM production at least at two different levels: the conversion of homocysteine to methionine, and the conversion of homocysteine to cysteine (by altering the levels of vitamin B6). **B** | Sirtuins remove acetyl groups from histones and other proteins in a reaction that consumes NAD^+. Sirtuin 1 (SIRT1) specifically targets H4K16ac and H3K9ac. Hyper-caloric diets give rise to a low NAD^+/NADH ratio (**Ba**) and, consequently, low SIRT1 activity. Calorie restriction gives rise to a high NAD^+/NADH ratio (**Bb**), and can therefore increase the activity of SIRT1. Sirtuins have important roles in the establishment of the adaptive response to calorie restriction[108]. They can be activated in an indirect manner by dietary phenols such as resveratrol[109,110].

Feil and Fraga (2011). Nat. Rev. Genetics 13:97-109,

DNA methylation in human cancers

- Paradoxically, human cancers tend to be hypomethylated relative to normal tissue while, at the same time, they have commonly inactivated key regulators of cellular growth by hypermethylation. It is the latter process that is targeted for therapeutic purposes. In 2004, the first epigenetic drug, a DNA methyltransferase inhibitor,was approved for the treatment of myelodysplastic syndrome.
- In colon cancer, where a multi-stage transformation process can be followed from pre-malignant to malignant transformation, hypomethylation occurs early in the transformation stage, in pre-malignant stages.

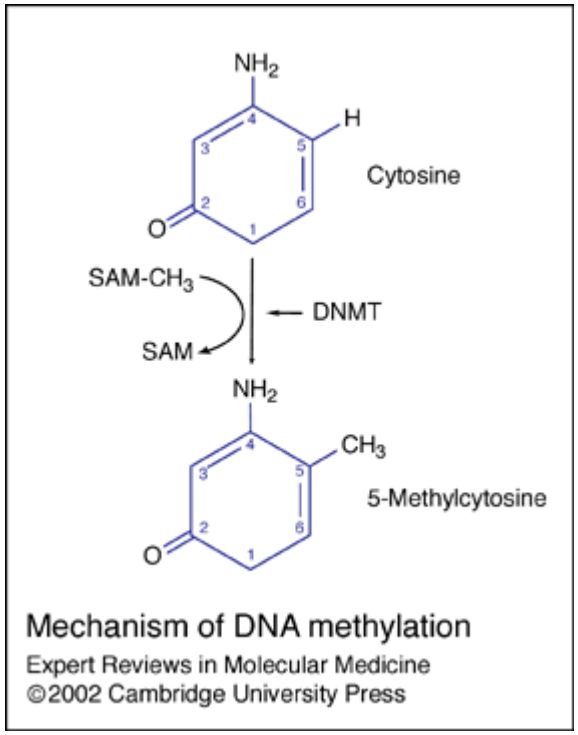
Table 1 | **Main principles of DNA methylation analysis**

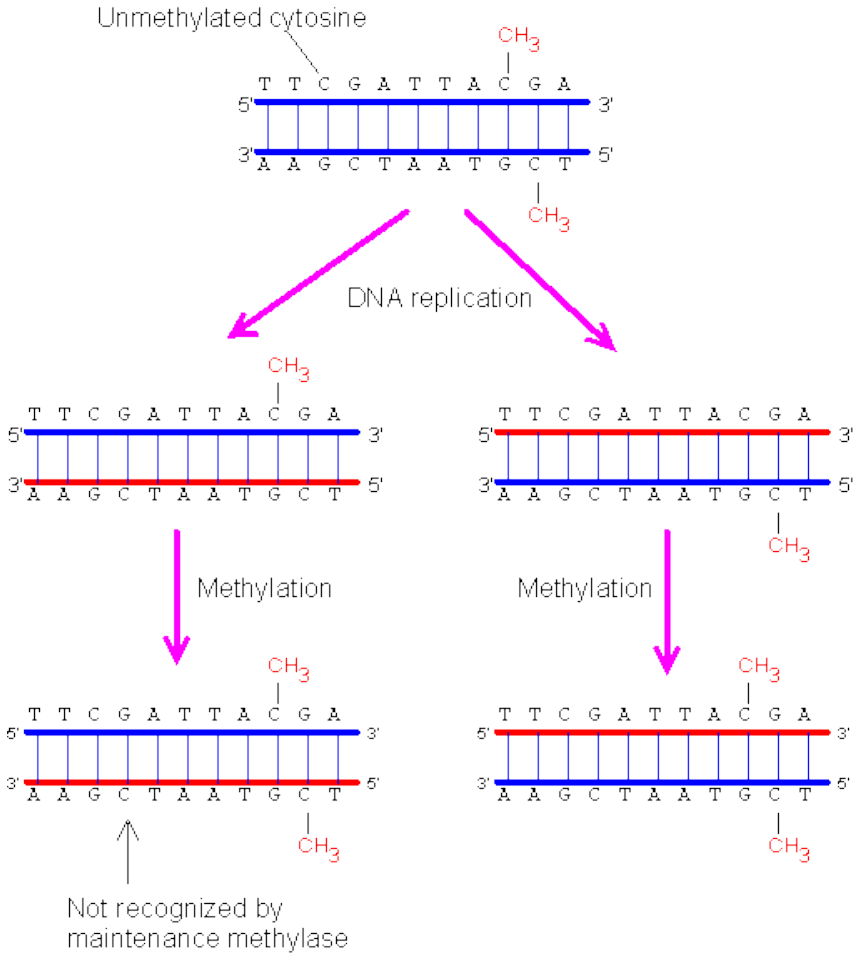
Pretreatment	Analytical step			
	Locus-specific analysis	**Gel-based analysis**	**Array-based analysis**	**NGS-based analysis**
Enzyme digestion	• *Hpa*II-PCR	• Southern blot • RLGS • MS-AP-PCR • AIMS	• DMH • MCAM • HELP • MethylScope • CHARM • MMASS	• Methyl–seq • MCA–seq • HELP–seq • MSCC
Affinity enrichment	• MeDIP-PCR		• MeDIP • mDIP • mCIP • MIRA	• MeDIP–seq • MIRA–seq
Sodium bisulphite	• MethyLight • EpiTYPER • Pyrosequencing	• Sanger BS • MSP • MS-SNuPE • COBRA	• BiMP • GoldenGate • Infinium	• RRBS • BC–seq • BSPP • WGSBS

AIMS, amplification of inter-methylated sites; BC–seq, bisulphite conversion followed by capture and sequencing; BiMP, bisulphite methylation profiling; BS, bisulphite sequencing; BSPP, bisulphite padlock probes; CHARM, comprehensive high-throughput arrays for relative methylation; COBRA, combined bisulphite restriction analysis; DMH, differential methylation hybridization; HELP, *Hpa*II tiny fragment enrichment by ligation-mediated PCR; MCA, methylated CpG island amplification; MCAM, MCA with microarray hybridization; MeDIP, mDIP and mCIP, methylated DNA immunoprecipitation; MIRA, methylated CpG island recovery assay; MMASS, microarray-based methylation assessment of single samples; MS-AP-PCR, methylation-sensitive arbitrarily primed PCR; MSCC, methylation-sensitive cut counting; MSP, methylation-specific PCR; MS-SNuPE, methylation-sensitive single nucleotide primer extension; NGS, next-generation sequencing; RLGS, restriction landmark genome scanning; RRBS, reduced representation bisulphite sequencing; –seq, followed by sequencing; WGSBS, whole-genome shotgun bisulphite sequencing.

Peter Laird, 2010 Nature Reviews Genetics 11: 191-203

DNA methylation





http://www.web-books.com/MoBio/Free/Ch7F2.htm

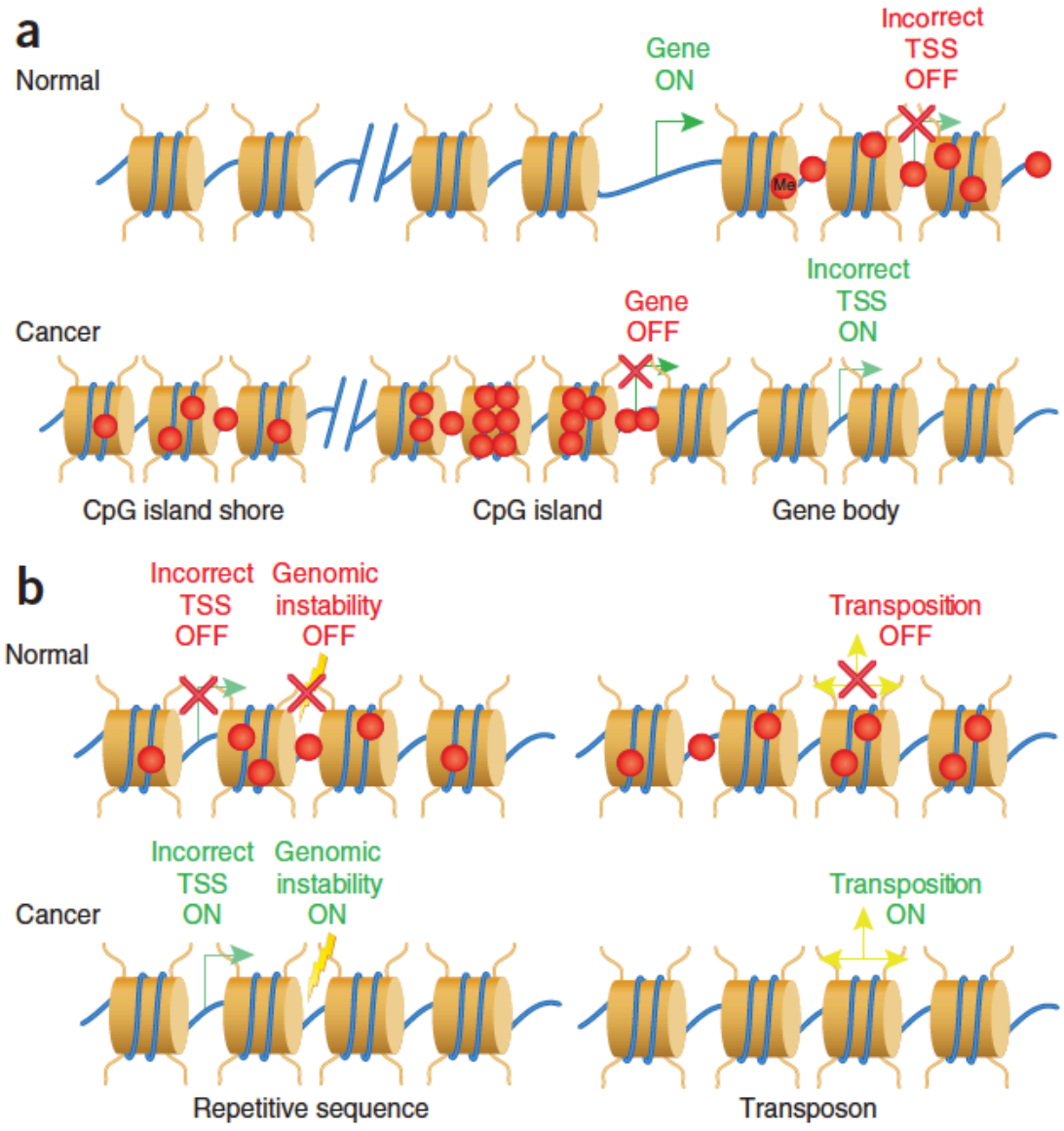


Figure 1 DNA methylation patterns in normal and cancer cells. DNA methylation takes place along the whole genome, and its disruption is a typical hallmark of cancer. (**a**) In normal cells (top), CpG islands and CpG island shores usually remain unmethylated, allowing gene transcription. Additionally, DNA methylation within the gene bodies avoids spurious transcription initiations. In cancer cells (bottom), by contrast, although both CpG islands and CpG island shores may be strongly methylated, gene bodies lack this modification. As a result, transcription of many genes gets blocked, and aberrant transcription may occur from incorrect transcription start sites (TSSs). (**b**) In normal cells (top), methylation of repetitive sequences prevents genomic instability and, again, spurious transcription initiations. Moreover, transposable elements cannot be activated in a methylated environment. In cancer cells (bottom), global hypomethylation triggers genomic instability and aberrant transcription initiations. Concomitant activation of transposons may lead to gene disruption.

Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330

Genes that are bound by polycomb group proteins in stem/progenitor cells tend to silenced by DNA methylation in human cancers

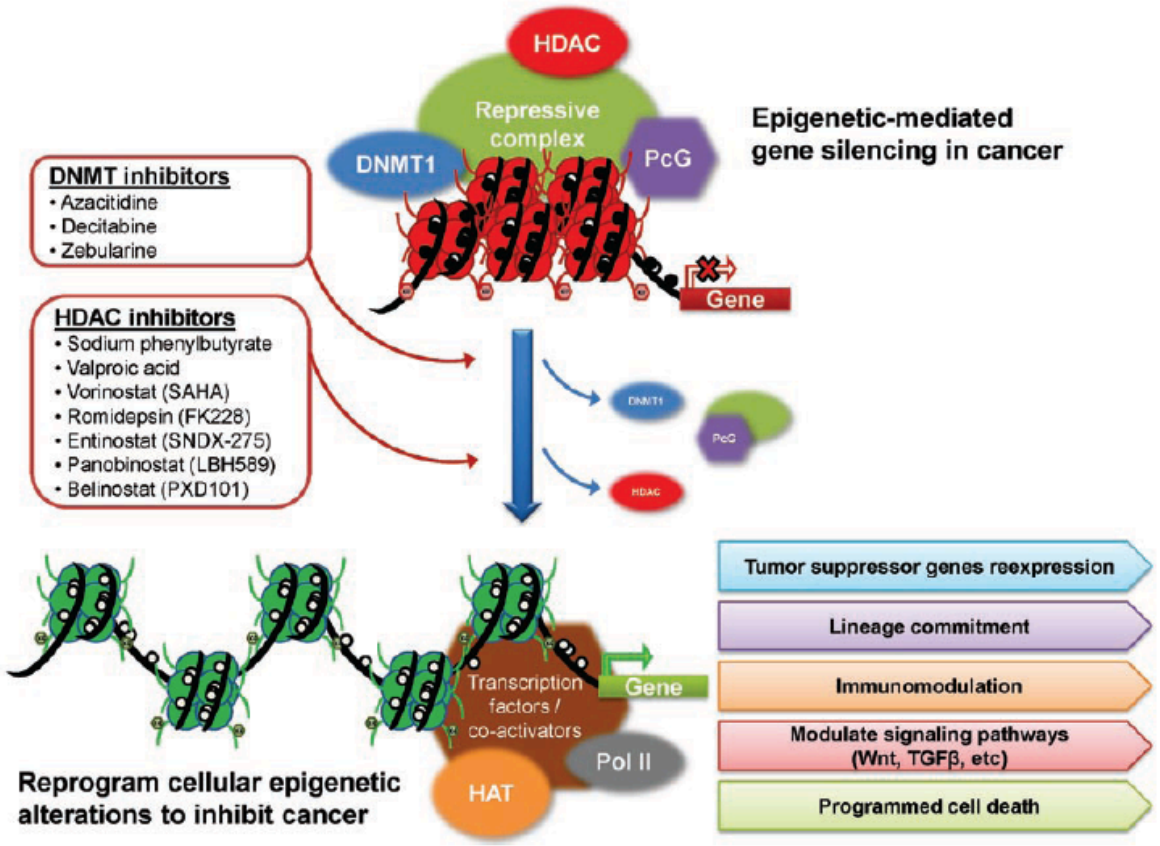


Figure 2 DNA methylation-mediated aberrant gene silencing in cancer involves transcriptional repressive complexes in the gene promoter region and interactions between DNA methylation machinery, chromatin modifiers (such as histone deacetylase, HDAC) and polycomb (PcG) proteins. Pharmacological inhibition of individual components in the repressive complex with DNMT inhibitors and HDAC inhibitors, either alone or in combination, may result in DNA demethylation and complex disintegration leading to reactivation of critical genes and reversal of genome-wide epigenetic alterations in cancer through resetting multiple cellular processes, including lineage commitment, immunomodulation, major cell signaling pathways, programmed cell death, and others. HAT: histone acetylase. Pol II: RNA polymerase II.

Tsai and Baylin (2011) Cell Research 21:502-517

Histone H3 Lysine Methylation in Human Cancer

Di and trimethylation of lysine 9 are associated with transcriptional repression. Trimethylation is associated with pericentric heterochromatin and telomeres and is important in the maintenance of genomic stability

artkqtark stggkaprkq latkaarksa patggvkkph

Trithorax homologues (MLL) Trimethylation of lysine 4 is associated with transcriptional activation and can be mistargeted in MLL fusion proteins.

Trimethylation of lysine 27 is associated transcriptional repression and facultative heterchromatin formation (e.g. .X-inactivation)
EZH2 methyltransferase is part of the polycomb family and often overexpressed in human cancers
Essential for the maintenance of pluripotency

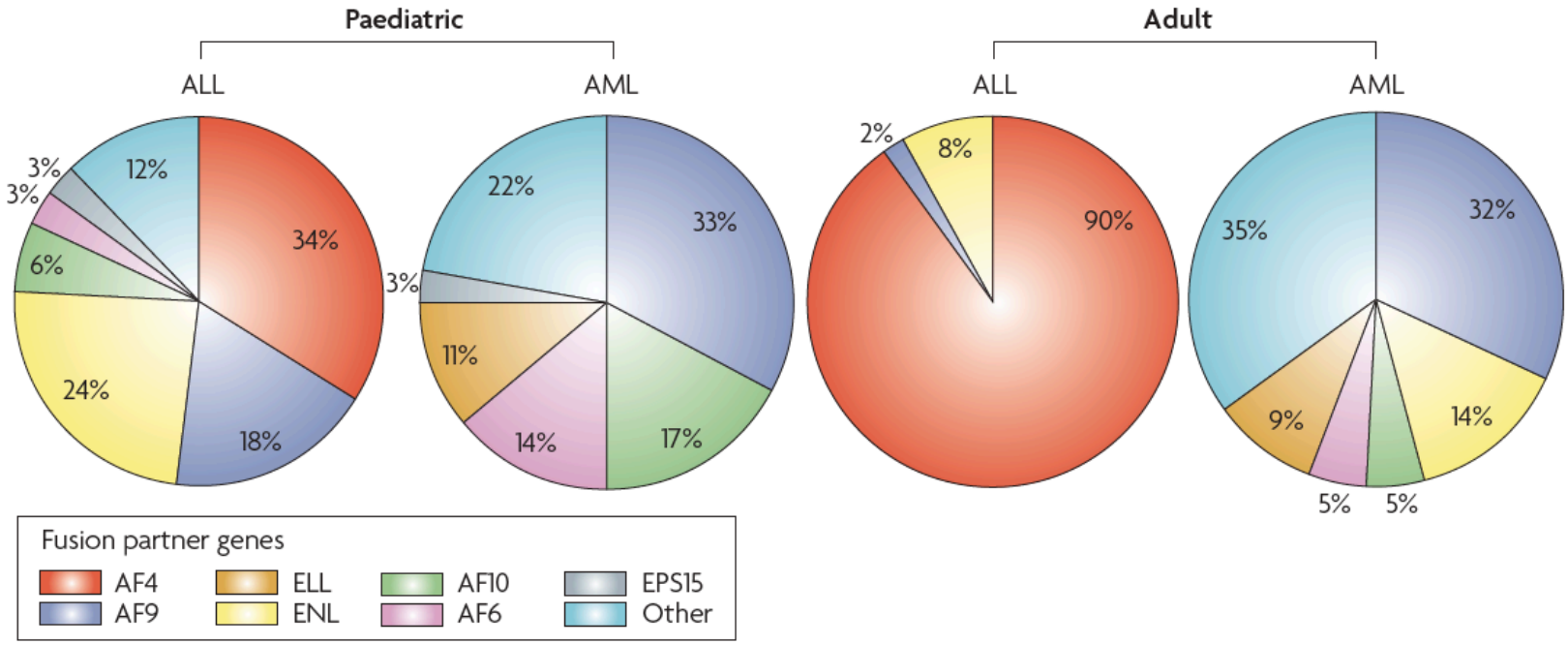


Figure 1 | **Distribution of major MLL fusion partner genes in *de novo* childhood and adult leukaemias.** Mixed lineage leukaemia (*MLL*) rearrangements are found in approximately 5% of acute lymphoblastic leukaemias (ALL), approximately 5–10% of acute myeloid leukaemias (AML) and virtually all cases 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.

MLL translocations are found in 70 percent of all infant leukemias and approximately 10 percent of all human luekaemias.

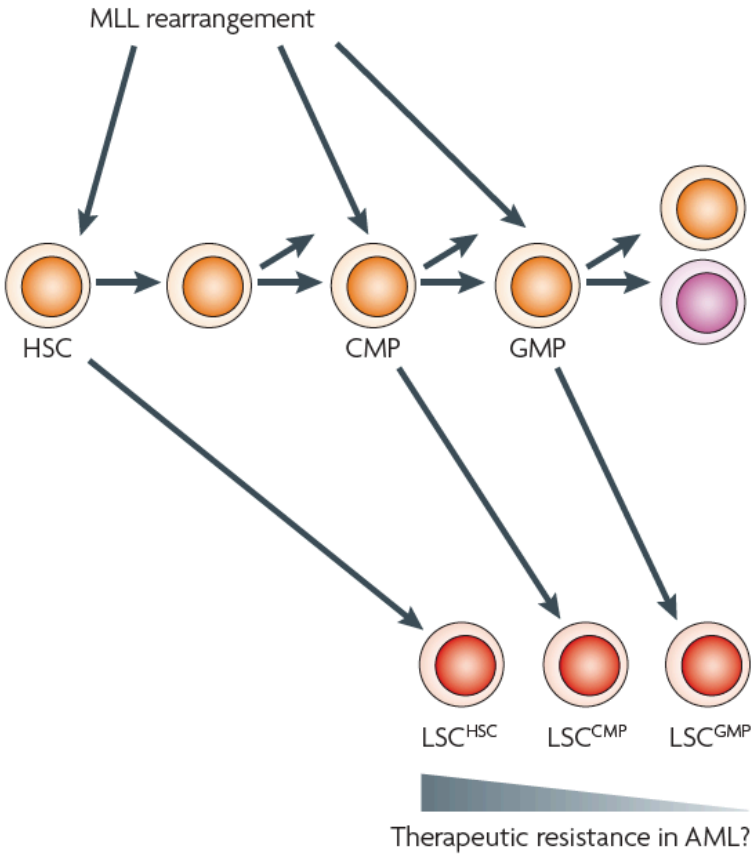


Figure 5 | **The leukaemia cell of origin and the LSC phenotype.** Mouse studies suggest that mixed lineage leukaemia (MLL) fusions can transform haematopoietic stem cells (HSCs), common myeloid progenitors (CMPs), and granulocyte macrophage progenitors (GMPs)[90,99], resulting in immunophenotypically similar acute myeloid leukaemia (AML). As HSCs, CMPs and GMPs possess inherent differences in processes such as apoptosis and drug resistance[103–105], these inherited differences might be maintained in the resultant leukaemia stem cells (LSCs) that originate from HSCs (LSC^{HSC}), CMPs (LSC^{CMP}) and GMPs (LSC^{GMP}).

Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes

Gillian L. Dalgliesh[1], Kyle Furge[2], Chris Greenman[1], Lina Chen[1], Graham Bignell[1], Adam Butler[1], Helen Davies[1], Sarah Edkins[1], Claire Hardy[1], Calli Latimer[1], Jon Teague[1], Jenny Andrews[1], Syd Barthorpe[1], Dave Beare[1], Gemma Buck[1], Peter J. Campbell[1], Simon Forbes[1], Mingming Jia[1], David Jones[1], Henry Knott[1], Chai Yin Kok[1], King Wai Lau[1], Catherine Leroy[1], Meng-Lay Lin[1], David J. McBride[1], Mark Maddison[1], Simon Maguire[1], Kirsten McLay[1], Andrew Menzies[1], Tatiana Mironenko[1], Lee Mulderrig[1], Laura Mudie[1], Sarah O'Meara[1], Erin Pleasance[1], Arjunan Rajasingham[1], Rebecca Shepherd[1], Raffaella Smith[1], Lucy Stebbings[1], Philip Stephens[1], Gurpreet Tang[1], Patrick S. Tarpey[1], Kelly Turrell[1], Karl J. Dykema[2], Sok Kean Khoo[3], David Petillo[3], Bill Wondergem[2], John Anema[4], Richard J. Kahnoski[4], Bin Tean Teh[3,5], Michael R. Stratton[1,6] & P. Andrew Futreal[1]

nature Vol 463|21 January 2010|doi:10.1038/nature08672

Table 2 | Mutation summary of highlighted genes in ccRCC

Gene	Initial screen mutations	Follow-up screen mutations	Further RCC cell line mutations*	Total mutations
HIF1A	1 nonsense	1 splice/del, 1 frameshift		3
JARID1C	1 nonsense, 1 missense	5 nonsense, 2 splice/del, 4 frameshift, 1 missense		14
MLL2	1 nonsense, 2 missense	9 missense, 1 nonsense, 4 silent	ND	17
NBN	1 frameshift	1 frameshift	ND	2
NF2	3 frameshift, 1 splice	1 frameshift	1 nonsense, 1 splice/del	7
PMS1	1 frameshift	2 nonsense (germline)		3
SETD2	4 frameshift, 1 nonsense, 2 missense	4 frameshift, 3 nonsense, 1 missense	1 frameshift	16
UTX	3 frameshift, 1 splice, 2 missense	1 frameshift, 1 splice/del, 3 missense, 1 nonsense (germline)		12
WRN	1 nonsense	1 splice/frameshift, 1 missense	ND	3
ZUBR1	1 frameshift, 1 missense, 1 silent	3 frameshift, 4 missense	ND	10

del, deletion; ND, not done. Detailed mutation annotation can be found in Supplementary Table 8.
* No matching normal sequence available, presumptive somatic mutation.

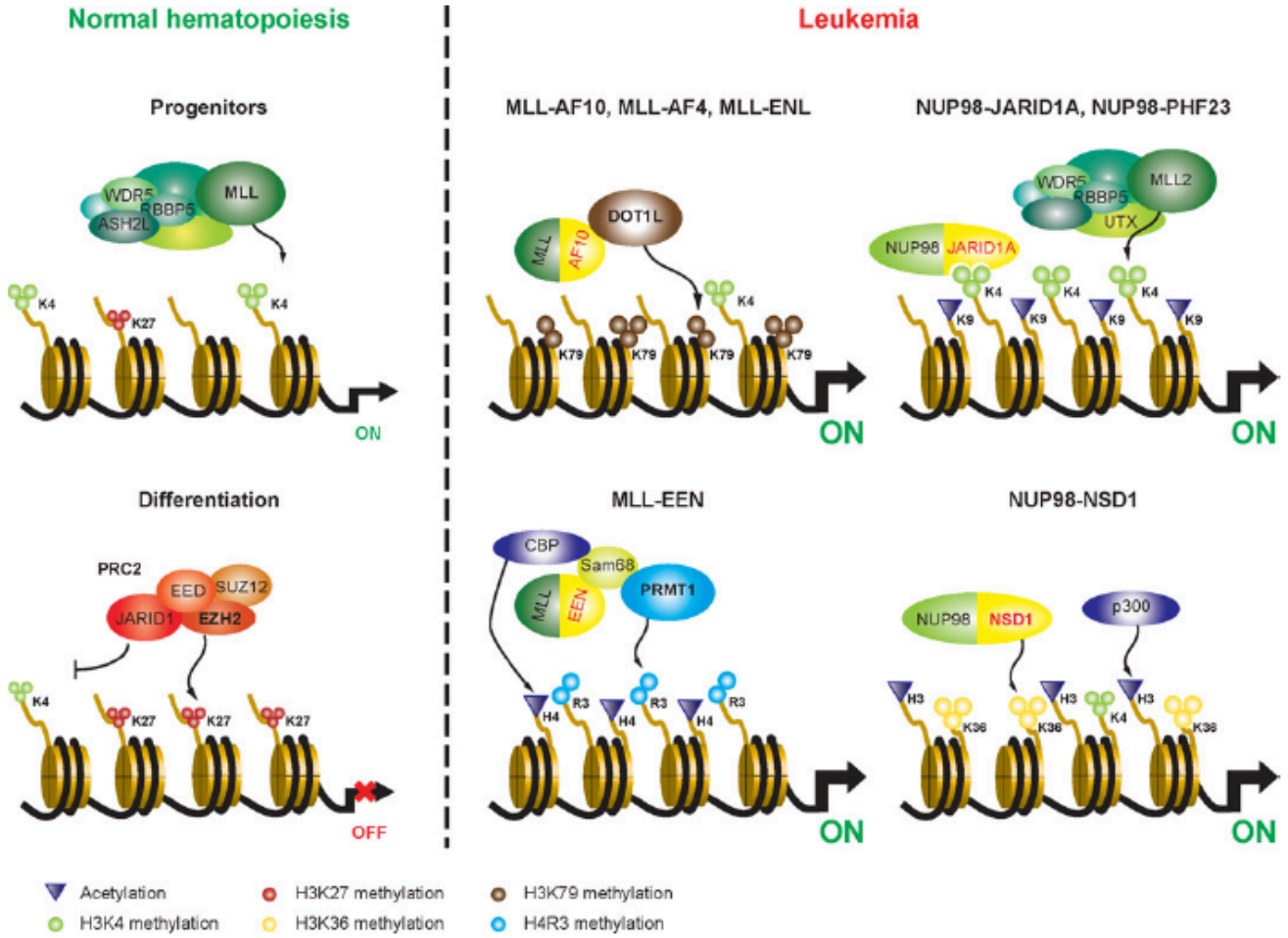
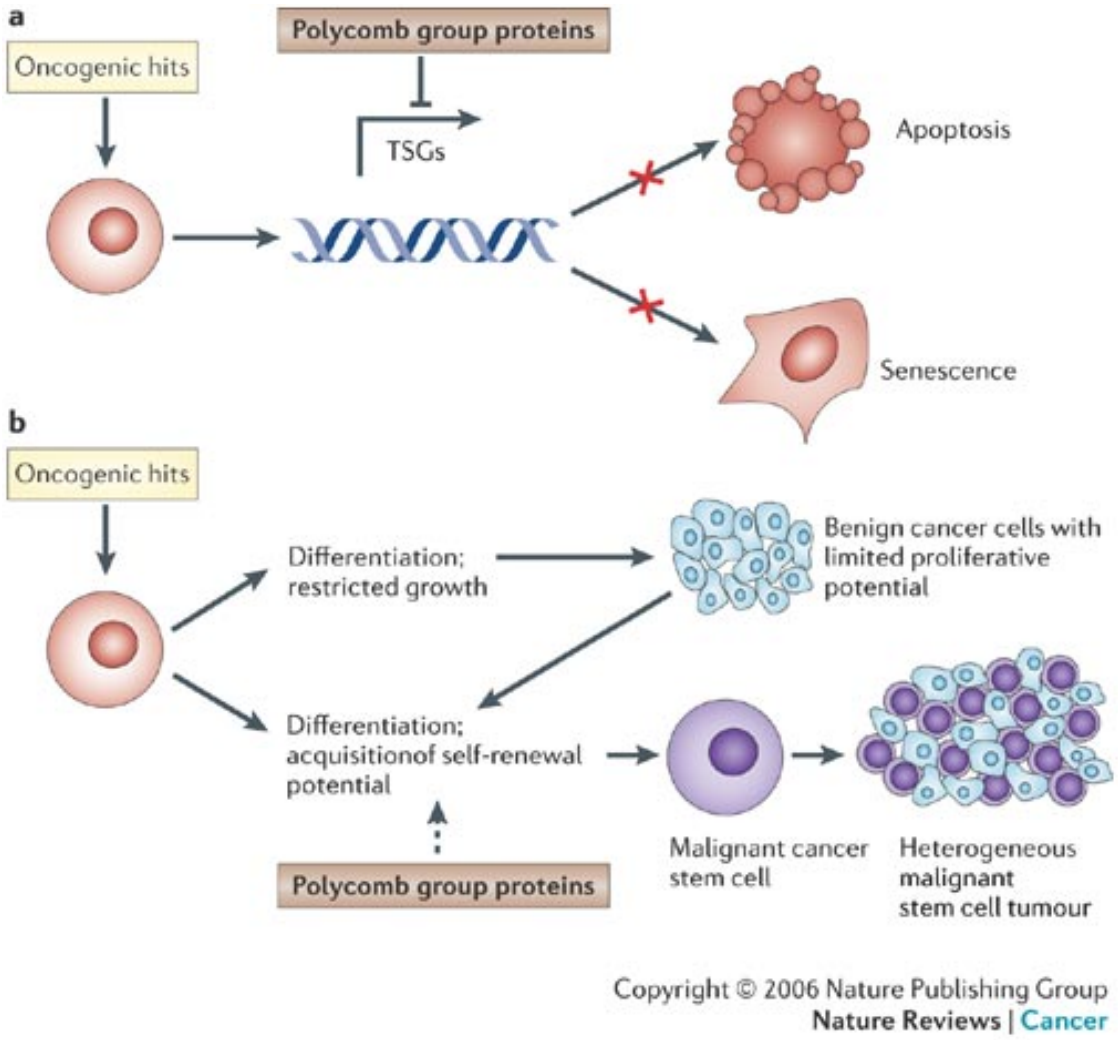


Fig. 3. Aberrant chromatin modifications at leukemia-inducing genes. In normal hematopoietic stem and progenitor cells, genes involved in proliferation and self-renewal, like *HoxA5*, *HoxA9* and *Meis1*, are marked by H3K4me4 associated with moderate levels of gene transcription. During normal differentiation, these genes are downregulated correlating with loss of H3K4me3 and gain of Polycomb-mediated repressive H3K27me3. In various MLL or NUP98 fusion-mediated leukemias, the *HoxA9* gene is highly overexpressed linked with the aberrant acquisition of active chromatin modifications, like H3K4, H3K36, H3K79 and H4R3 hypermethylation and H3/H4 hyperacetylation.



Sparmann and Lohuizen *Nature Reviews Cancer* **6**, 846–856 (November 2006) | doi:10.1038/nrc1991

nature REVIEWS CANCER

Polycomb Group Proteins and Lysine 27 trimethylation

- Associated with gene silencing and the establishment of facultative heterochromatin
- The EZH2 protein has histone methyltransferase activity that methylates lysine 27 of histone H3. Polycomb group proteins are transcriptional repressors that contain a chromo domain that specifically recognizes lysine 27 trimethylation
- The EZH2 protein is commonly overexpressed and associated with poor prognosis in solid tumors in humans.

Table 1. Polycomb group (PcG) proteins and associated human cancers

Table 1. Polycomb group (PcG) proteins and associated human cancers

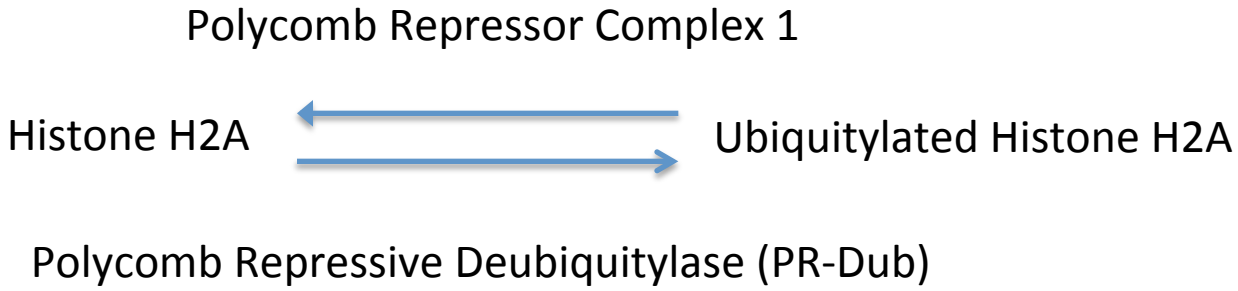
Drosophila homolog	Human homolog	Protein domain/function	Expression	Cancer type	Reference
PRC2 initiation complex					
Enhancer of zeste, E(z)	EZH2	SET/Histone methyl transferase	Gene amplification and/or overexpression	B-cell non-Hodgkin lymphoma	[216]
				Bladder	[156, 217–219]
				Breast	[156, 186, 220–222]
				Colon	[156, 186, 220–223]
				Glioblastoma	[156]
				Hodgkin lymphoma	[224]
				Larynx	[156]
				Liver	[225]
				Lung	[156]
				Mantle cell lymphoma	[226]
				Melanoma	[156, 221]
				Prostate	[86, 211, 214, 221, 227–229]
				Sarcoma	[156]
				Stomach	[156, 230]
				Testis	[156]
				Uterus	[221]
Suppressor of zeste, Su(z)	SUZ12	Zinc-finger domain	Overexpression	Breast	[231]
				Colon	[153, 231]
				Liver	[231]
PRC1 maintenance complex					
RING	RING1/RNF1/RING1A/ RNF2/RING1B	RING-finger domain/ ubiquitin ligase	Overexpression	Prostate	[227]
				Bladder	[212]
				Breast	[212]
				Cervix	[212]
				Colon	[212]
				Kidney	[212]
				Larynx	[212]
				Liver	[212]
				Lymphoma	[212]
				Non-small cell lung cancer	[212]
				Ovary	[212]
				Pancreas	[212]
				Parathyroid	[212]
				Prostate	[212]
				Thymus	[212]
				Thyroid	[212]
				Uterus	[212]
Posterior sex combs, Psc	BMI1	RING-finger domain/ ubiquitylation	Gene amplification and/or overexpression	B-cell non-Hodgkin lymphoma	[216]
				Bronchial squamous cell cancer	[232]
				Cervix	[212]
				Colon	[212, 233]
				Ependymoma	[212]
				Head and neck squamous cell cancer	[234]
				Hodgkin	[235]
				Leukemia	[236]
				Liver	[212]
				Mantle cell lymphoma	[212, 213]
				Medulloblastoma	[116]
				Meningeoma	[212]
				Nasopharyngeal carcinoma	[237]
				Neuroblastoma	[238]
				Non-small cell lung cancer	[239, 240]
				Oral cancer	[241]
				Pituitary	[212]
				Prostate	[211, 227]
Pleiohomeotic, Pho	YY1	Zinc finger/sequence-specific DNA binding	Overexpression	Prostate	[152]
				Retinoblastoma	[242]
Polycomblike, Pcl	PCL3	PHD	Overexpression	Cervix	[243]
				Colon	[243]
				Liver	[243]
				Lung	[243]
				Rectal	[243]
				Skin	[243]
				Uterus	[243]
Polyhomeotic, Ph	RAE28	Not known	Loss of heterozygosity	Acute lymphoblastic leukemia	[215]

Besides aberrant overexpression in various cancers, genes encoding the PcG proteins are also amplified. For example, the BMI1 is found to be amplified in prostate cancer [211], mantle cell lymphoma [212, 213], and pituitary adenoma [212]. Similarly, EZH2 is amplified in prostate [211, 214], breast [156, 186], and also many other organ cancers [156]. To date, RAE28 is the only PcG protein found to be associated with loss of heterozygosity in cancers such as hematologic malignancy (acute lymphoblastic leukemia) and consequent loss of its expression [215].
Abbreviations: BMI1, B-cell-specific Moloney murine leukemia virus insertion site 1; EZH2, enhancer of zeste-2; PHD, plant homeodomain protein; PRC, Polycomb repressive complex; RAE28, polyhomeotic gene product isolated from retinoic acid differentiated murine embryonal carcinoma cells; SET, Su(var)3-9, Enhancer-of-zeste, Trithorax.

Rajasekhar, V. K. et al. Stem Cells 2007;25:2498-2510

STEM CELLS

BAP1 Driver Mutations in Mesothelioma and Melanoma



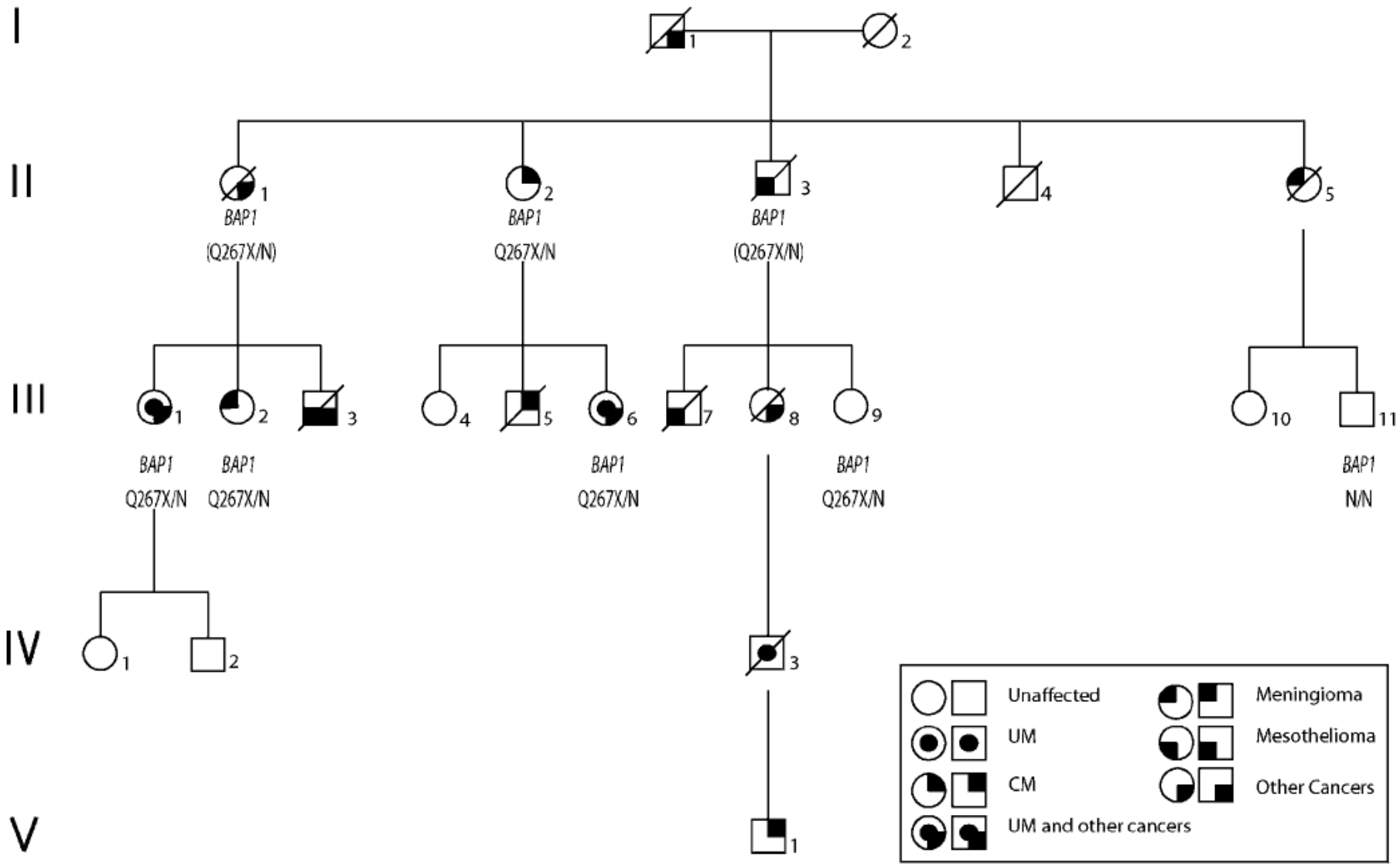


Figure 1 Pedigree of family FUM036 . Individuals III.1, II.2, III.2, III.6, III.9 were heterozygous for a truncating mutation (c. 799 C→T, p.Q267X) in BAP1 (designated Q267X/N in the figure). Individuals II.1 and II.3 are obligate carriers (inferred genotypes are shown in parentheses). Individual III.11 was negative for the mutation (designated N/N). No other individuals were tested. CM, cutaneous melanoma; UM, uveal melanoma.

Abdel-Rahman et al. (2011) J. Med. Gen. doi:10.1136/jmedgenet-2011-100156

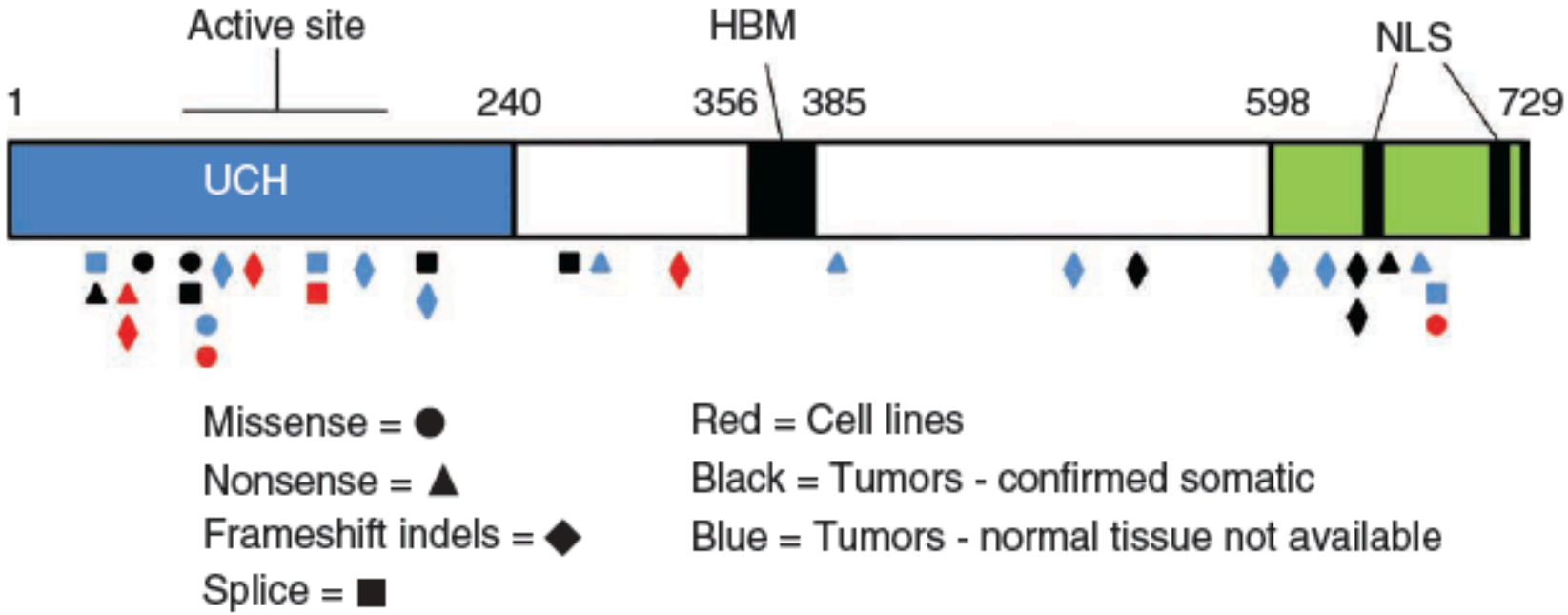


Figure 3 Distribution of *BAP1* mutations relative to functional domains. Shown are the N-terminal ubiquitin hydrolase domain (blue), the HCF1-binding domain (HBM) and the C-terminal protein interaction domain (green) containing two nuclear localization signals (black boxes).

Bott et al. (2011). Nature Genetics 43:668-672

The epigenetic progenitor origin of human cancer

Andrew P. Feinberg, Rolf Ohlsson‡ and Steven Henikoff§*

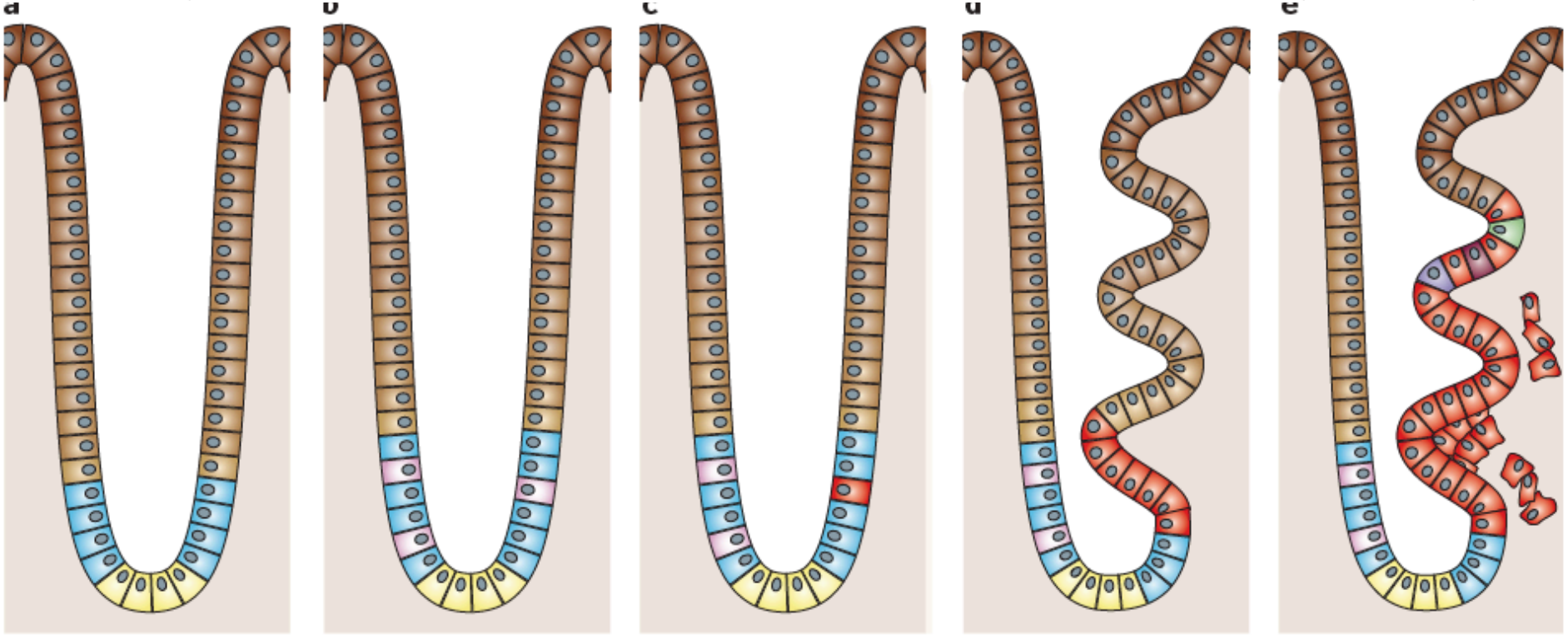


Figure 3 | **The epigenetic progenitor model in the context of a stem cell niche.** Normal colonic epithelium (first panel) includes a proliferative zone that contains stem cells (blue), which give rise to differentiated cells further up the crypt (shades of brown represent differentiation stages) (**a**). The epigenetic progenitor model suggests that the stem cell compartment is altered epigenetically (**b**), which can involve an expansion of the progenitor compartment or other epigenetic changes in gene expression (pink), followed by genetic mutation (**c**, red). Subsequent evolution of the tumour involves genetic and epigenetic plasticity; the latter allows expression of phenotypic features (invasion, metastasis and drug resistance, the last of which is denoted by altered colour) that are inherent properties of the stem cell progenitor (**d** and **e**).

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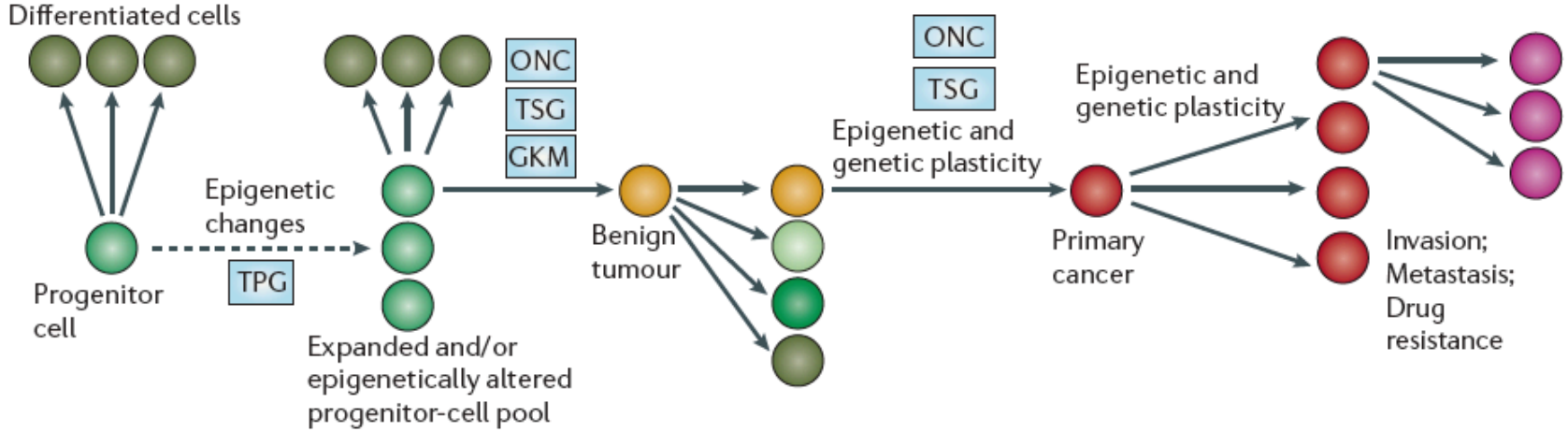

Figure 2 | **The epigenetic progenitor model of cancer.** According to this model, cancer arises in three steps. First is an epigenetic alteration of stem/progenitor cells within a given tissue, which is mediated by aberrant regulation of tumour-progenitor genes (TPG). This alteration can be due to events within the stem cells themselves, the influence of the stromal compartment, or environmental damage or injury. Second is a gatekeeper mutation (GKM) (tumour-suppressor gene (TSG) in solid tumours, and rearrangement of oncogene (ONC) in leukaemia and lymphoma). Although these GKMs are themselves monoclonal, the expanded or altered progenitor compartment increases the risk of cancer when such a mutation occurs and the frequency of subsequent primary tumours (shown as separately arising tumours). Third is genetic and epigenetic instability, which leads to increased tumour evolution. Note that many of the properties of advanced tumours (invasion, metastasis and drug resistance) are inherent properties of the progenitor cells that give rise to the primary tumour and do not require other mutations (highlighting the importance of epigenetic factors in tumour progression).

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Table 1 | **Hypomethylation and hypermethylation in cancer**

	Hypomethylation	Hypermethylation
Frequency	Ubiquitous even in the earliest benign tumours	Some early hypermethylation, with increasing frequency with tumour progression
Targets	Repetitive sequences, coding regions, promoters	Promoters
Primary/secondary change	Primary?	Can be secondary to gene silencing, chromatin changes
Possible effects in humans	Chromosomal instability, loss of imprinting, oncogene activation	Maintenance of tumour-suppressor-gene silencing
Effects in animal models	Lymphoma, increased intestinal tumour initiation, liver cancer	Increased intestinal tumour progression
Variation in the age of onset	Yes	Yes
Therapy	Inhibitor side effect?	Inhibitor therapy

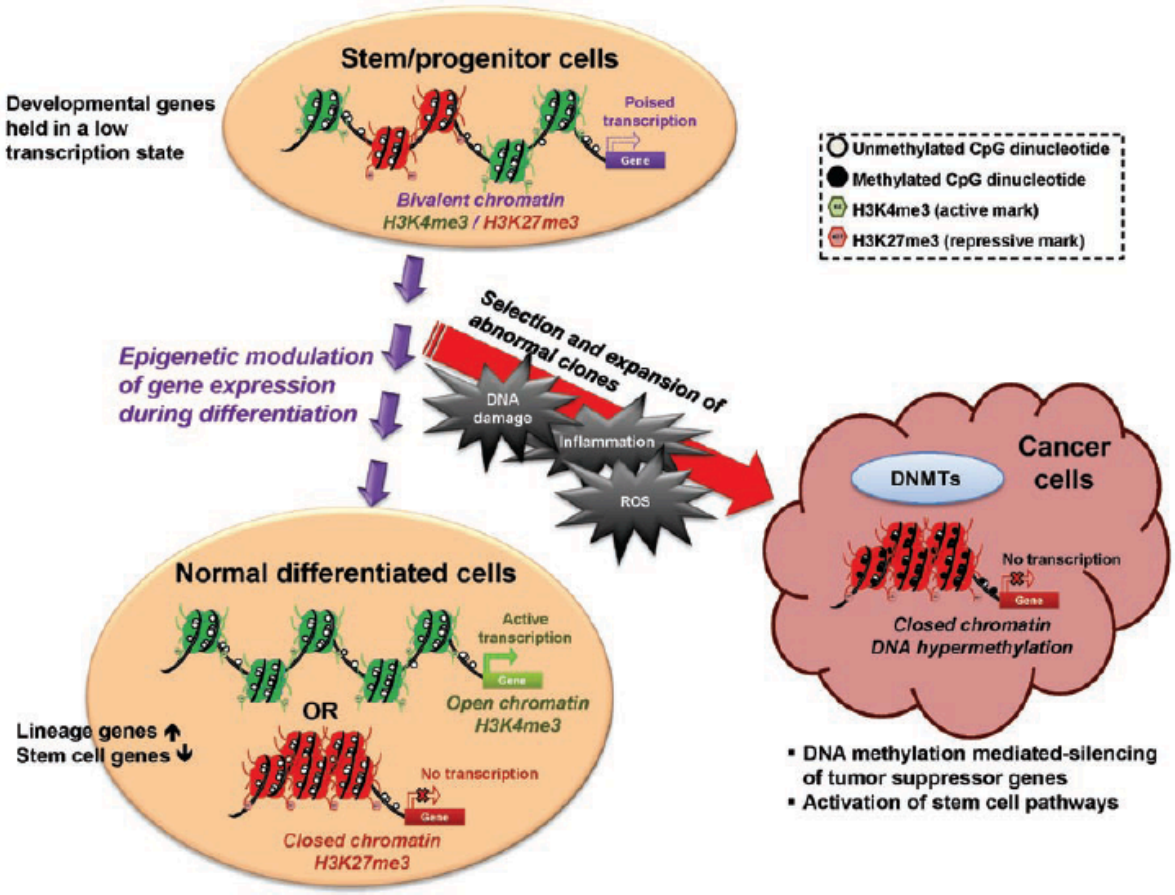

Figure 1 In normal stem/progenitor cells, the promoter regions of many CpG island-containing developmental genes are marked by both active (trimethylated histone H3 lysine 4; H3K4me3) and repressive marks (trimethylated histone H3 lysine 27; H3K27me3), termed "bivalent chromatin" by Bernstein *et al.* [91]. This chromatin pattern holds these genes in a low, poised transcription state to prevent premature lineage commitment. When the stem/progenitor cells respond to environmental cues and start to differentiate, a shift of the balance between the active and repressive epigenetic marks takes place with corresponding changes in chromatin architecture, leading to the silencing of stemness genes and upregulation of lineage-specific genes. However, repeated environmental stress such as chronic inflammation or accumulating reactive oxygen species (ROS) may promote clonal expansion of cells with genetic or epigenetic abnormalities, which then contribute to tumor initiation and progression. During this course of oncogenesis, the repressive marks in the promoter regions of tumor suppressor genes may recruit DNA methylation machinery to impose abnormal CpG island methylation on these genes leading to permanent gene silencing. At the same time, these epigenetic abnormalities may also contribute to activation of stem cell pathways, such as the Wnt pathway, and bestow self-renewing properties on cancer cells.

Tsai and Baylin (2011) Cell Research 21:502-517

Box 1 | **Evidence in support of an epigenetic progenitor model**

The epigenetic progenitor model states that cancer has a fundamentally common basis that is grounded in a polyclonal epigenetic disruption of stem/progenitor cells, mediated by tumour-progenitor genes. A second step involves monoclonal genetic mutation of gatekeeper genes (or characteristic chromosomal rearrangements in leukaemia or lymphoma), followed by a third step that involves acquisition of genetic and epigenetic plasticity.

The epigenetic progenitor model includes a key step before commonly recognized neoplasia, which can help to explain the late onset of most adult cancers, recurrent disease, environmental effects, tumour heterogeneity and the genetics of cancer risk.

Evidence for the epigenetic progenitor model

- *In vitro* studies of tumour cells demonstrate reversibility of phenotype in both leukaemia and solid tumour development[74–76].
- Global epigenetic changes precede the initial mutations in cancer; the changes involve widespread DNA hypomethylation in all tumours examined[33,34], and promoter hypermethylation in many cases[73,78,79]. These changes must precede the earliest genetic alterations as the epigenetic alterations are always found, even in benign neoplasms.
- Cloned mouse melanoma nuclei can differentiate into normal mice, which indicates that most of the properties of tumour cells can be reprogrammed to normal development — that is, they are epigenetically controlled[80].
- Serial grafting of tumour tissue. Daughter cells retain a diverse range of primary tumour markers, which indicates that a subpopulation of tumour cells possesses a self-renewal mechanism that is similar or identical to stem cells[72,81,82]. Imatinib resistance might be largely due to *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral (*v-abl*) oncogene homologue) mutations in chronic myelocytic leukaemia, but an important contribution seems to be the clonal expansion of progenitor cells[85].
- Loss of imprinting of *IGF2* (insulin-like growth factor 2) is common in the normal colonic epithelium of patients that are at risk of colorectal cancer, and mouse studies show that this epigenetic change shifts the balance of the intestinal epithelium towards an expanded progenitor-cell population[46,47,65]. Altered methylation is also found in the stroma of cancer patients[88,146].

How is epigenetics being exploited in the clinic?

1) Non-invasive detection of cancers (lung, colon, bladder)
2) Predicting risk of cancer (lung, colon)
3) Predicting the response to chemotherapy (e.g., MGMT)
4) Treatment of cancer

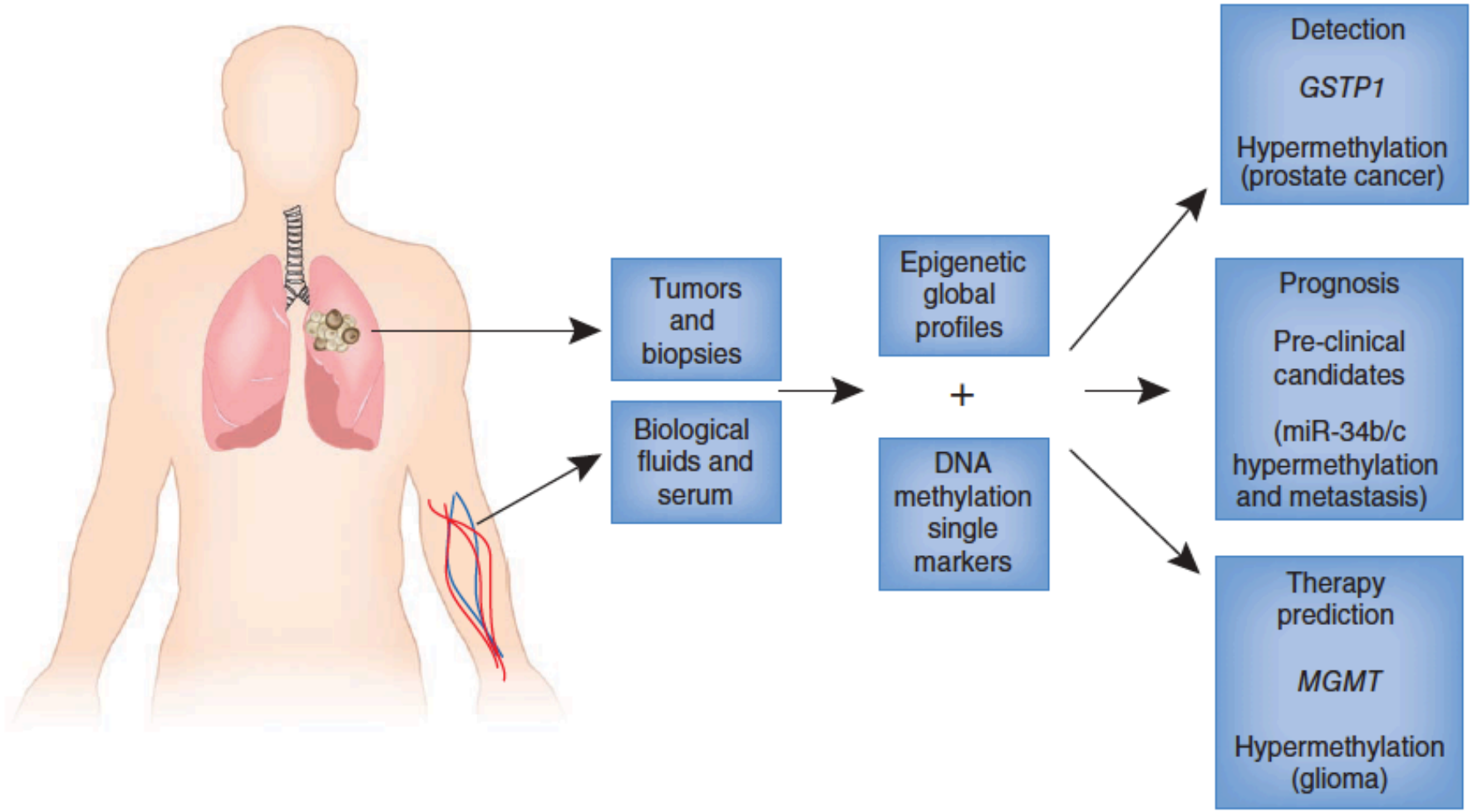

Figure 4 Epigenetic biomarkers in oncology. From all types of samples obtained from individuals with cancer, single and global epigenetic screenings have been developed to identify new molecular markers to manage the disease. To predict malignancy in prostate tumorigenesis and response to temozolomide in gliomas, the study of hypermethylation events in *GSTP1* and *MGMT*, respectively, is reaching the clinical stage.

Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330

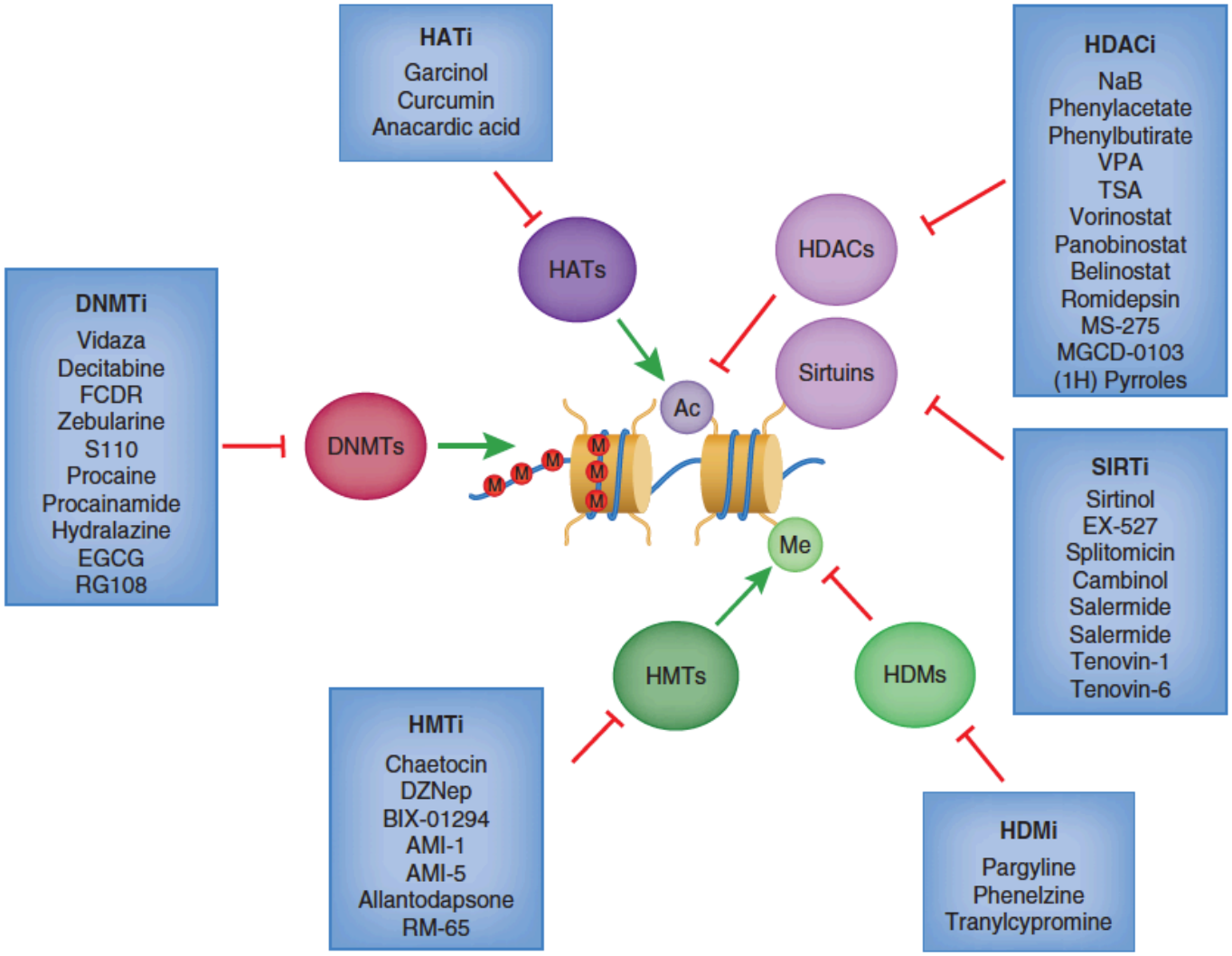

Figure 5 Epigenetic drugs for cancer therapy. Numerous compounds have been reported to be effective against cancer cells by inhibiting components of the epigenetic machineries. This figure shows the most important epigenetic drugs classified depending on their particular epigenetic targets.

Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330

Table 1 HDAC inhibitors

Chemical class	Selected members	Comments	References
Short-chain fatty acids	Sodium *n*-butyrate (NaB) Phenylacetate Phenylbutyrate Valproate	Butyrates such as NaB inhibit proliferation of colon, prostate, endometrial and cervical carcinomas at high millimolar concentrations. 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.	164–166
Hydroxamic acids	Trichostatin A Vorinostat (SAHA) Panobinostat Belinostat	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 is FDA-approved for hematological malignancies. 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 is quite active against HDACs 1–10. It is in clinical trials for the treatment of hematological malignancies and solid tumors.	112,167–170
Cyclic peptides	Romidepsin (formerly FK-228)	A natural, stable prodrug that, once converted to its active form (redFK) by cellular reducing activity, 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.	110,113,171
Benzamide derivatives	MS-275 (or entinostat) MGCD-0103	MS-275 inhibits HDACs 1–3 and 9 and has also been used in clinical trials in conjunction with other agents. 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.	168,172–174

CML, chronic myeloid leukemia.

Rodriguez-Paredes and Esteller (2011). Nat. Medicine 17:330-330

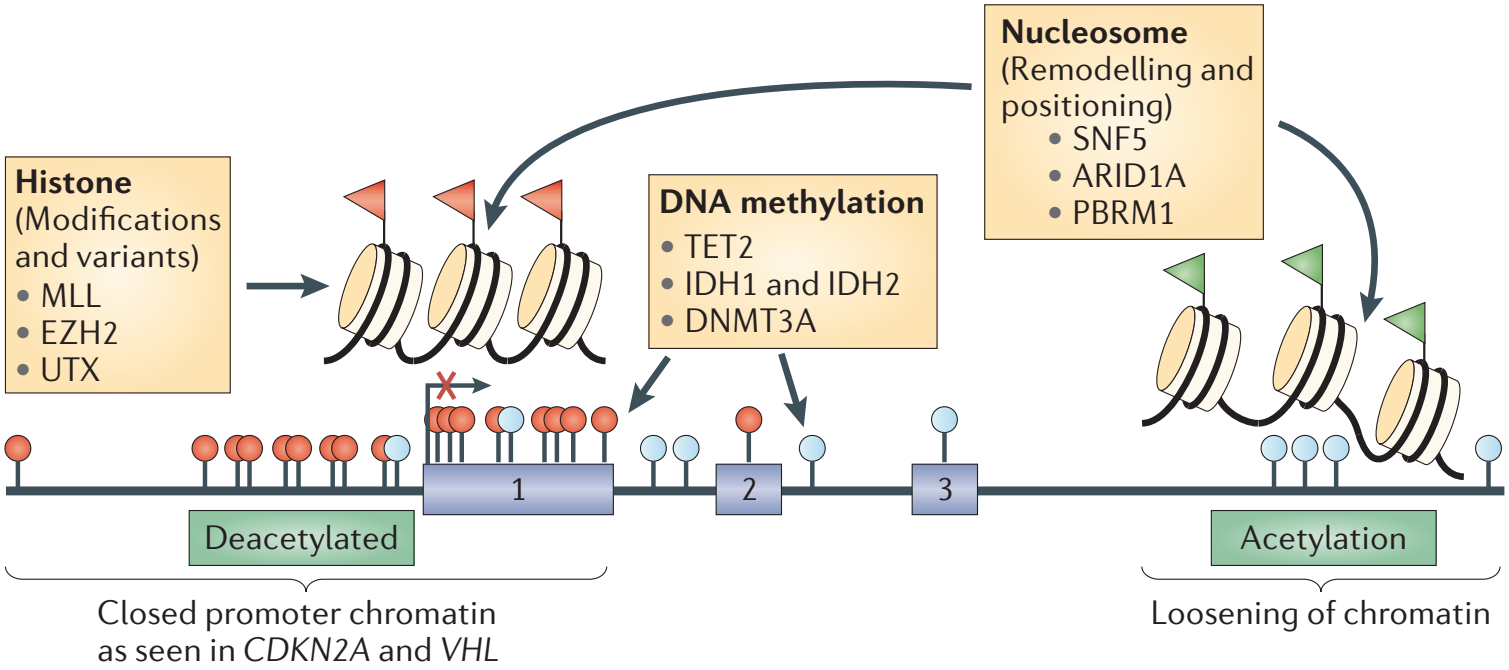

Figure 2 | **The cancer epigenome and relevant gene mutations.** The cancer epigenome is characterized by simultaneous global losses in DNA methylation (indicated by pale blue circles) with hundreds of genes that have abnormal gains of DNA methylation (indicated by red circles) and repressive histone modifications (indicated by red flags) in promoter region CpG islands. The hypomethylated regions have an abnormally open nucleosome configuration and abnormally acetylated histone lysines (indicated by green flags). Conversely, abnormal DNA hypermethylation in promoter CpG islands is associated with nucleosomes positioned over the transcription start sites of the associated silenced genes (indicated by an arrow with a red X). Recent whole-exon sequencing of human cancers has shown a high proportion of mutations in genes in leukaemias, lymphomas, and ovarian, renal and pancreatic cancers, and rhabdomyosarcoma[109–111,154–156] (indicated in yellow boxes), which are depicted as helping to mediate either abnormal DNA methylation, histone modifications and/or nucleosome remodelling[100,107,108,118,155,157–165]. ARID1A, AT-rich interactive domain-containing protein 1A; DNMT3A, DNA methyltransferase 3A; EZH2, ehancer of zeste 2; IDH1, isocitrate dehydrogenase 1; MLL, mixed lineage leukaemia; PBRM1, protein polybromo 1; SNF5, SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin, subfamily B, member 1; VHL, Von Hippel–Lindau.

Baylin and Jones (2011). Nature Reviews Cancer 11:726-734

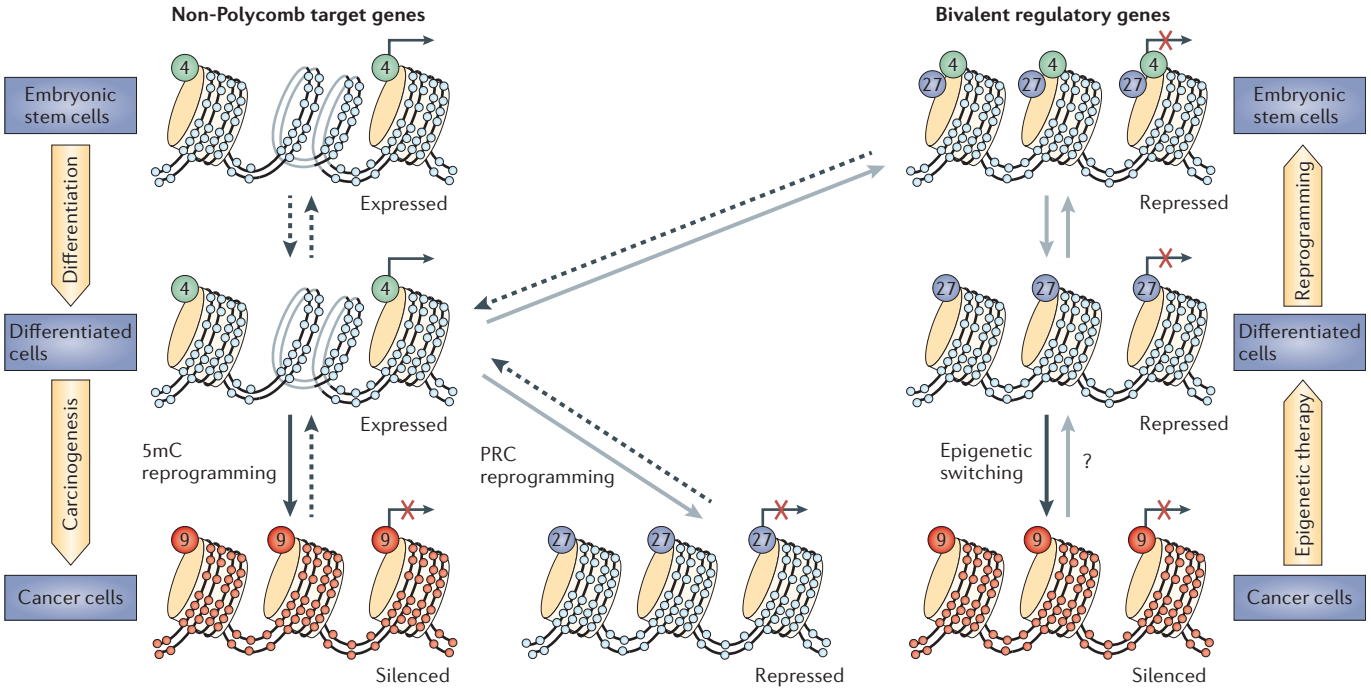

Figure 3 | **Modes of abnormal gene silencing in cancer.** The currently suggested routes to abnormally silenced genes in cancer are shown. Genes that are active in cells throughout development and adult cell renewal initially have active promoter chromatin that is characterized by the presence of the histone modification, H3K4me (indicated by green circles and dashed arrows), and a lack of DNA methylation (indicated by pale blue circles). Genes that become silenced (indicated by a red X) can do so either by the acquisition of DNA methylation (indicated by red circles) and the presence of the repressive mark, H3K9me (indicated by orange circles and black arrows), or by the presence of Polycomb-mediated repressive chromatin (PRC) marks, H3K27me (purple circles and grey arrows). DNA methylation and H3K9me marks during tumour progression are shown. The wide yellow arrows at the sides of the figure depict movements that link stem and progenitor cells and differentiated cells and which can be impeded by epigenetic abnormalities in cancer or which can be corrected by epigenetic therapy.

Baylin and Jones (2011). Nature Reviews Cancer 11:726-734