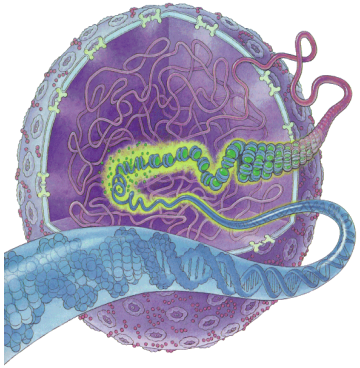
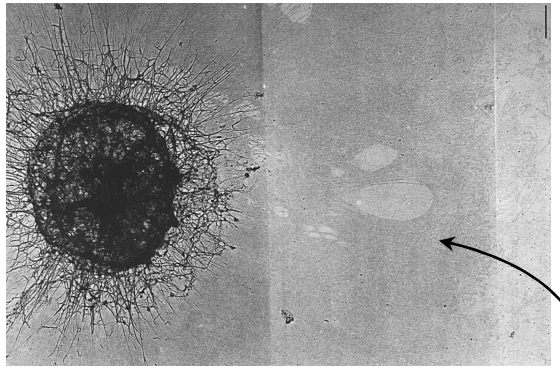
Compacting the eukaryotic genome

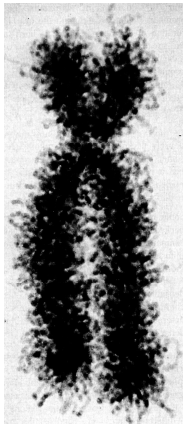


+ 2M NaCl

istoni



Mitosi

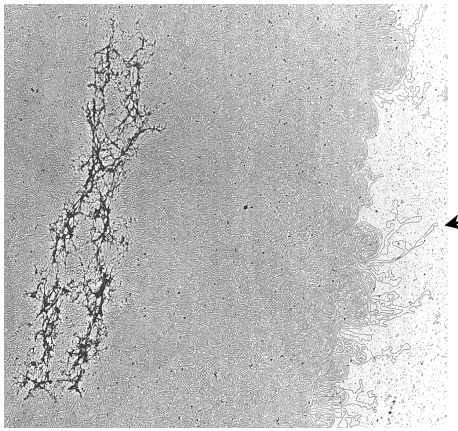


mitotic chromosome

chromatid

+ 2M NaCl

istoni

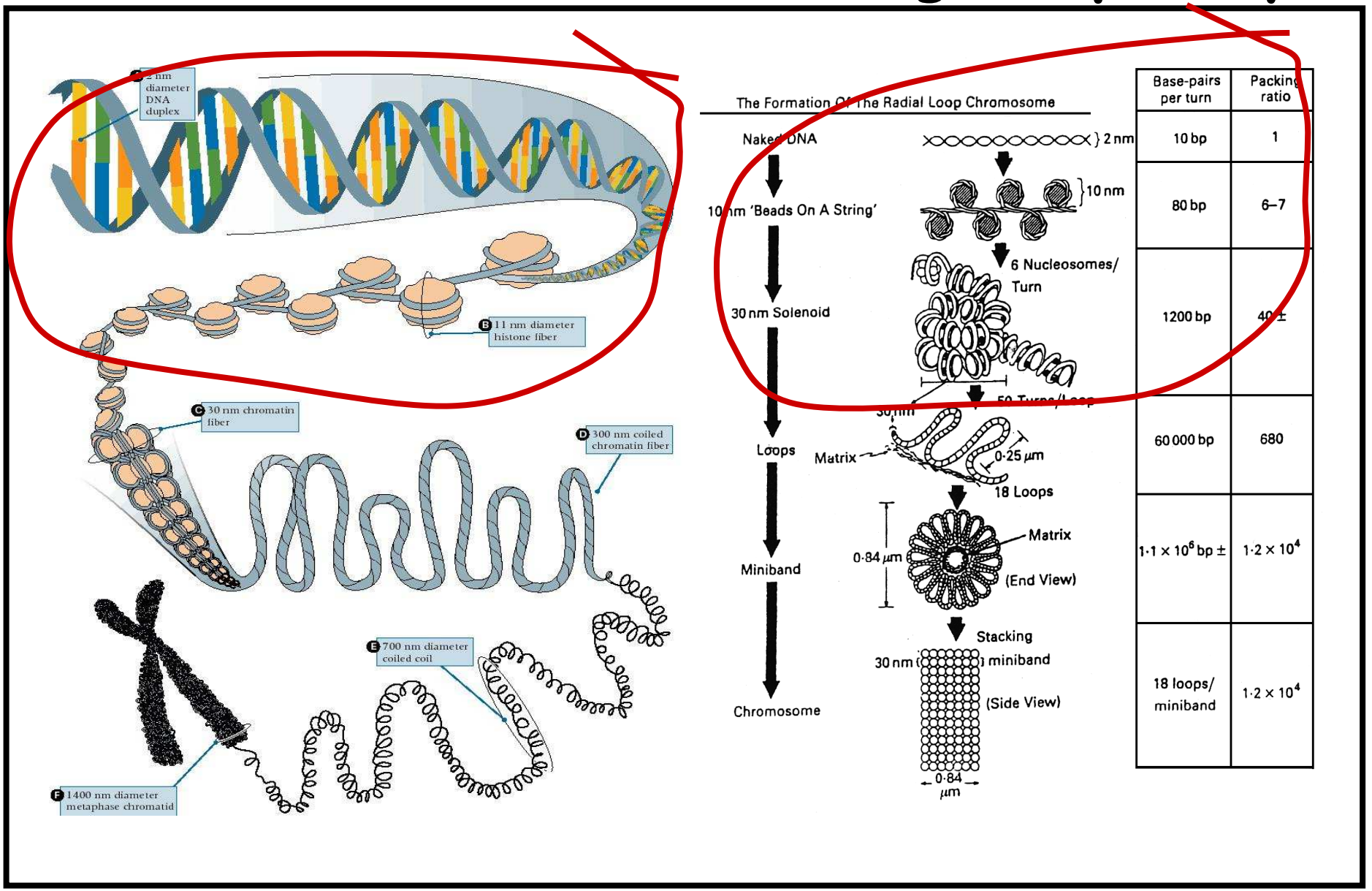


DNA loops

			compact size	DNA	length	compaction
nucleus (human)	2 x 23 = 46 chromosomes	92 DNA molecules	10 μm ball	12,000 Mbp	4 m DNA	**400,000 x**
mitotic chromosome	2 chromatids, 1 μm thick	2 DNA molecules	10 μm long X	2x 130 Mbp	2x 43 mm DNA	**10,000 x**
DNA domain	anchored DNA loop	1 replicon ?	60 nm x 0.5 μm	60 kbp	20 μm DNA	**35 x**

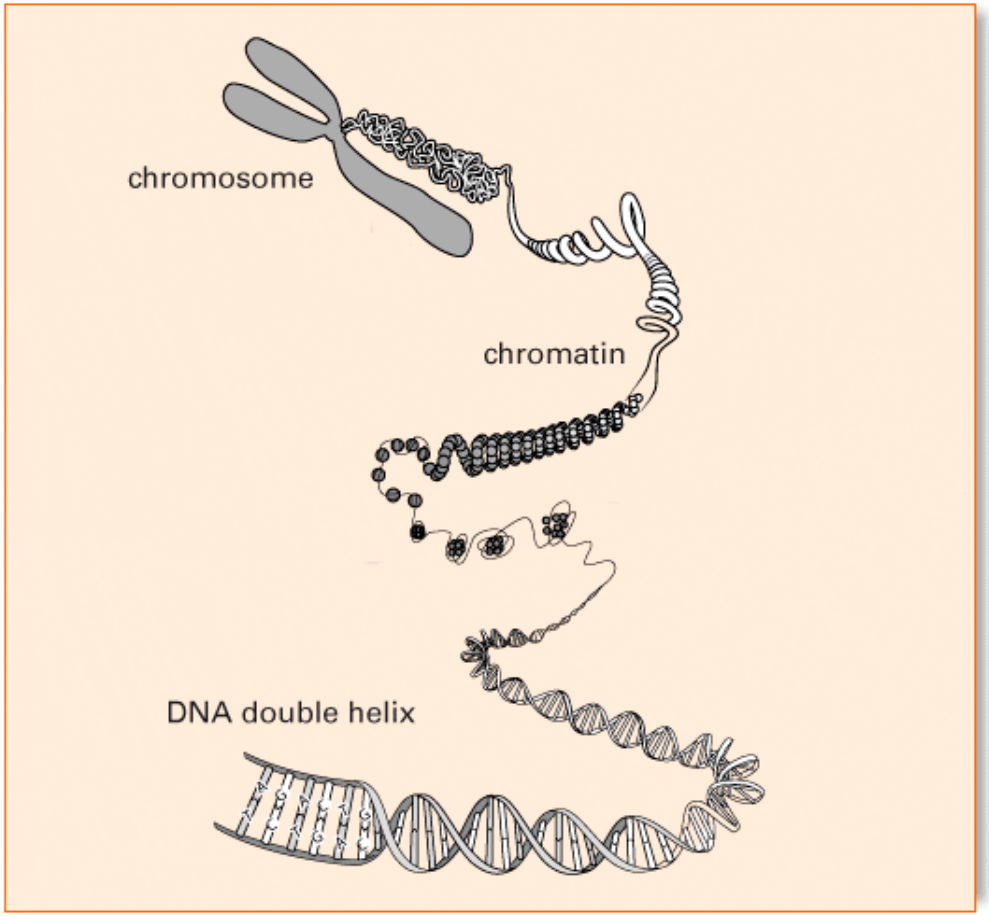
Compaction by chromosome scaffold / nuclear matrix

An architecture of increasing complexity

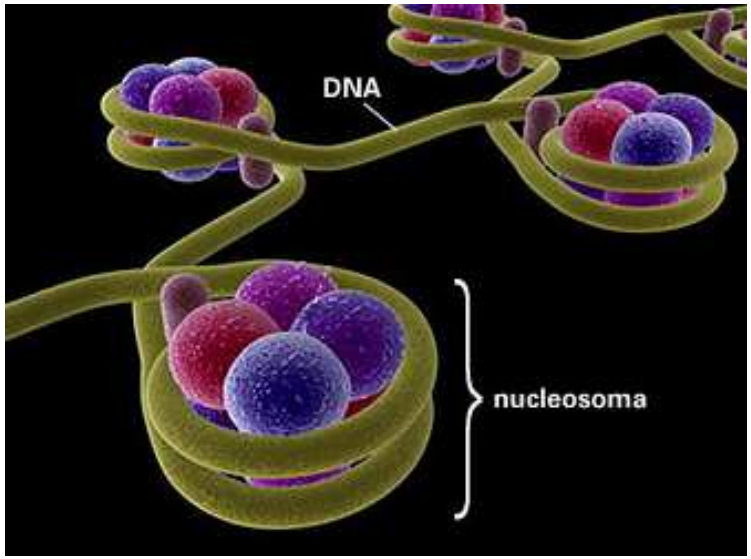


Base-pairs per turn	Packing ratio
10 bp	1
80 bp	6–7
1200 bp	40 ±
60 000 bp	680
$1{\cdot}1 \times 10^6$ bp ±	$1{\cdot}2 \times 10^4$
18 loops/ miniband	$1{\cdot}2 \times 10^4$

Chromatin structural organization has a key role in gene transcription regulation



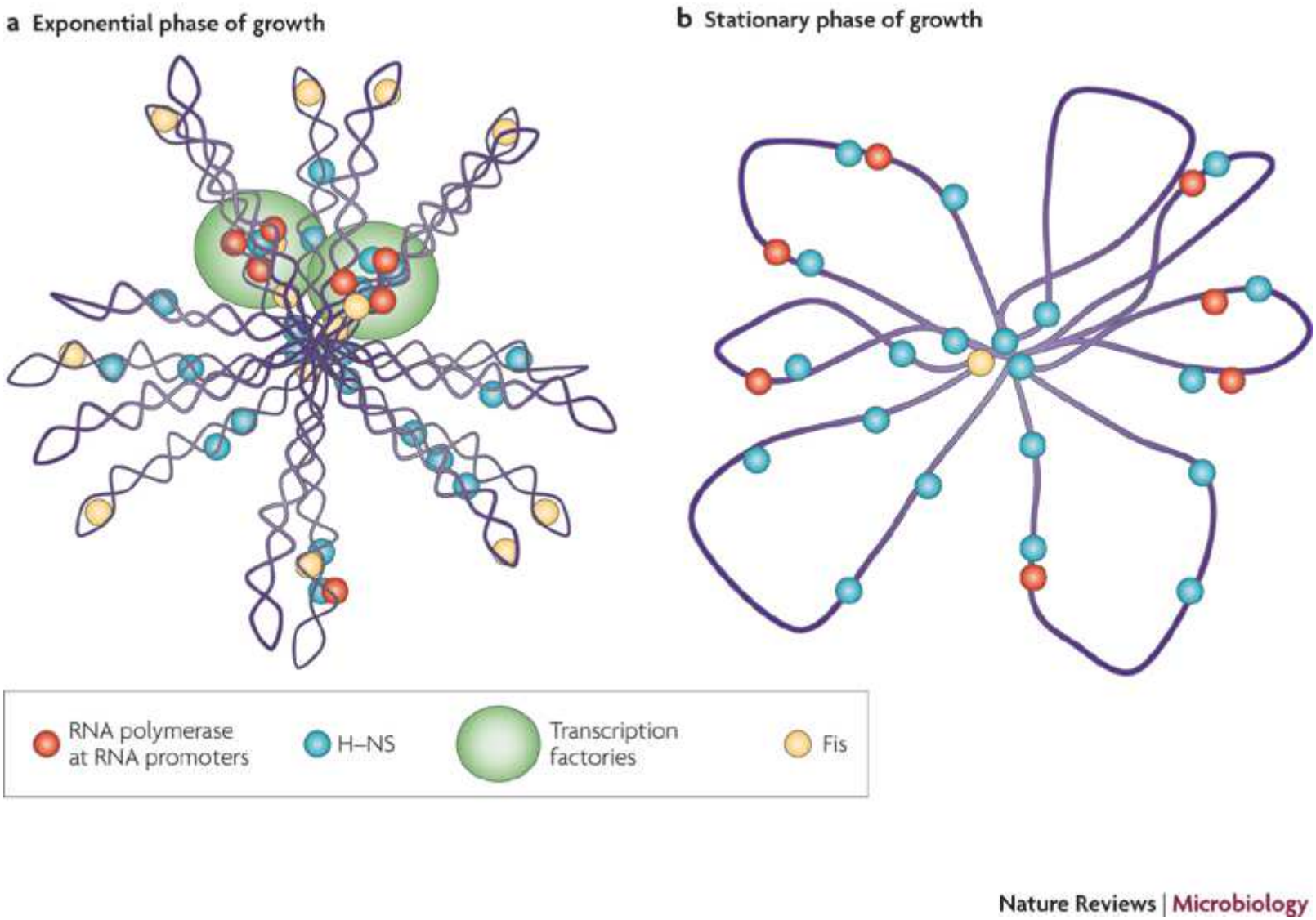
nucleosomes inhibit accessibility to promoter and binding of transcription factors to regulatory sequences



- Active promoters are generally nucleosomes depleted;
- Gene transcription activation is generally coupled to nucleosome eviction or displacement;
- Nucleosome occupancy in promoters is generally anti-correlated with transcription activity level.

The general idea is that nucleosomes obstacle transcription machinery. Chromatin remodeling, histone modifications and substitutions of canonical histones with variant isoforms are the strategy used to remove the obstacle.

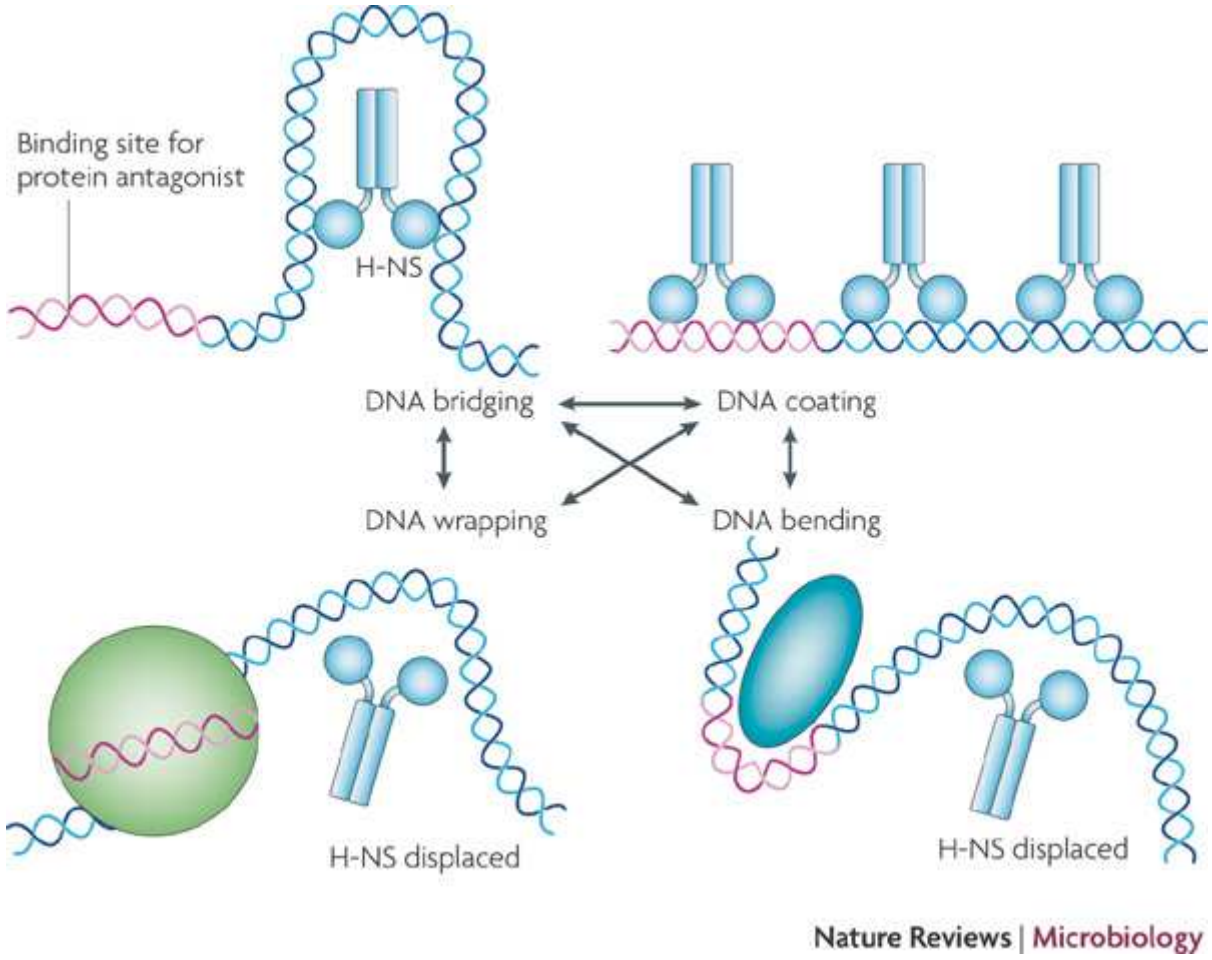
Histone-like proteins exist in bacteria

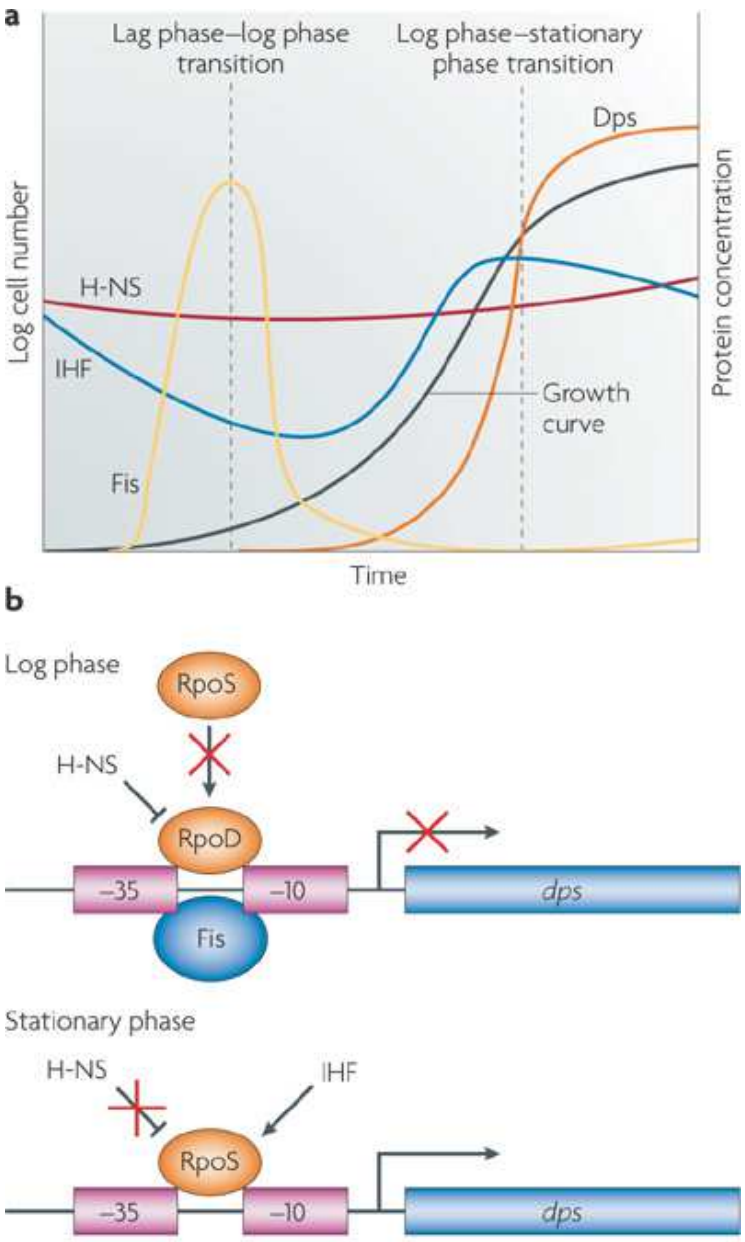


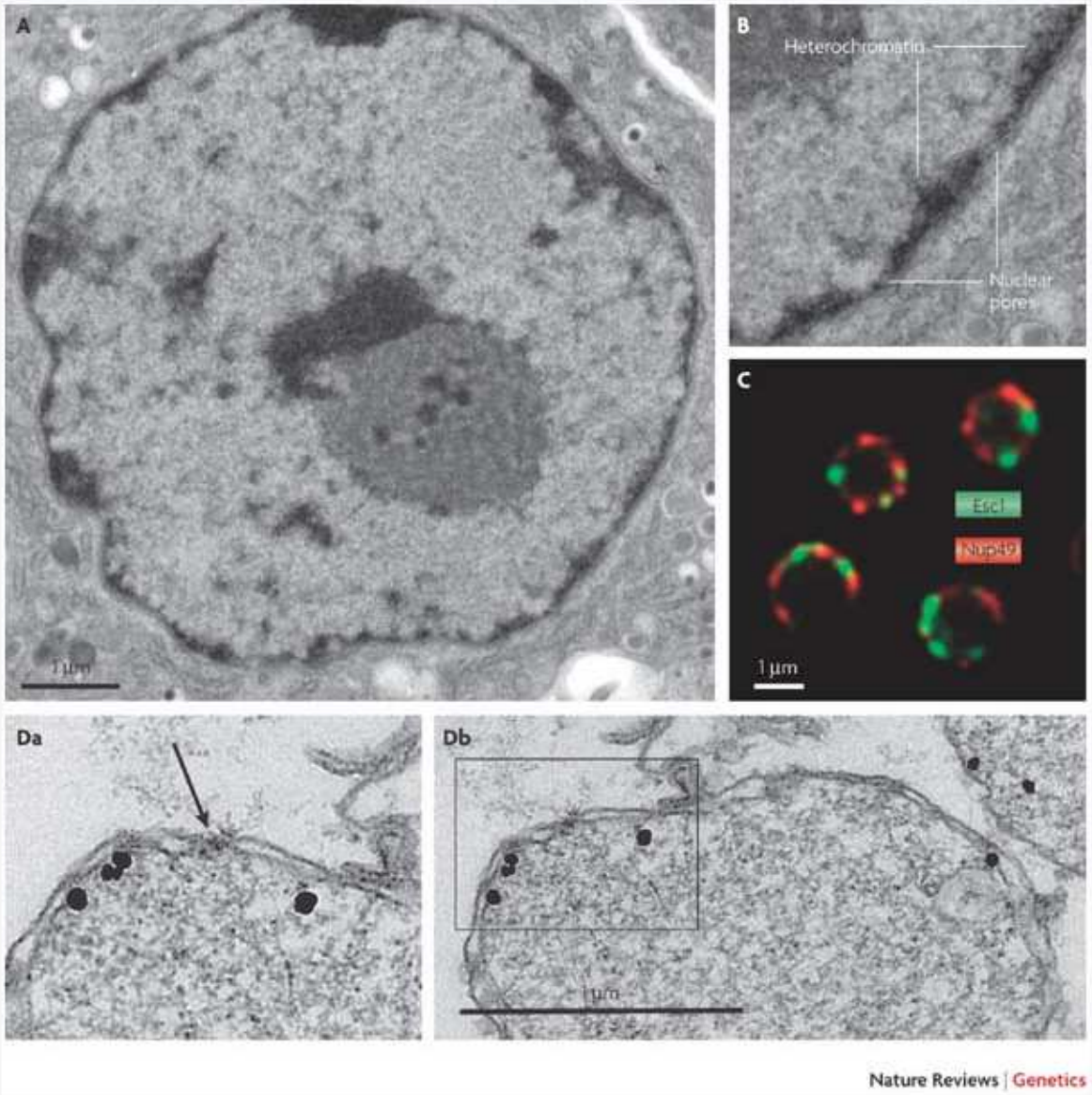
Protein or group of proteins	DNA wrapping	DNA bridging	DNA bending	Binding motif	Molecular mass	Native protomer	Refs
Eukaryotes							
Core histones, H2A, H2B, H3 and H4	Yes	ND	ND	A ~10 bp periodic oscillation of AA/TT/TA elements in-phase with each other and out-of-phase with ~10 bp periodic GCs	11–14 kDa	Homodimer	115
Linker histones, H1 and H5	ND	Yes	ND	AT-rich DNA	~21 kDa	Homodimer	116
Smc	ND	Yes	ND	AT-rich DNA able to form secondary structures	~140 kDa	Heterodimer (for example, SMC1–SMC3)	117
Hmg	ND	ND	Yes	AT-tract sites	11–38 kDa	Homodimer or heterodimer (for example, HMG1–HMG2)	118
Euryarchaeota							
Archaeal histones HMfA and HMfB	Yes	ND	ND	$(A/T)_3NN(G/C)_3NN$	~7.5 kDa	Homodimer or heterodimer	119
Lrp	Yes	Yes	ND	ND	15–22 kDa	Homodimer	120
Alba	ND	Yes	ND	ND	~10 kDa	Homotetramer	20
MC1	ND	ND	Yes	AT-rich DNA	~10 kDa	Homodimer	121
HU	ND	ND	Yes	ND	~10 kDa	Homodimer	122
SMC	ND	Yes	ND	ND	~135 kDa	Homodimer	123
Crenarchaeota							
Lrp	Yes	Yes	ND	ND	~18 kDa	Homodimer	124
Cren7	ND	ND	Yes	ND	~7 kDa	Monomer	125
Sul7d	ND	ND	Yes	ND	~7 kDa	Monomer	126
Alba	ND	Yes	ND	ND	~10 kDa	Homodimer or homotetramer	127
SMC	ND	Yes	ND	ND	~100 kDa	Homodimer	128
CC1	ND	ND	ND	ND	~6 kDa	Monomer	129
Gram-negative bacteria							
HU	Yes	ND	Yes	A DNA structural motif in dsDNA joined to either dsDNA or ssDNA, with a mild preference for AT-rich or curved DNA	~9 kDa	Heterodimer (for example, HUα–HUβ)	42, 130
Lrp	Yes	Yes	ND	(T/C)AG(A/T/C)A(A/T)ATT(A/T)T(A/T/G)CT(A/G)	~18 kDa	Homodimer	69
MukB	ND	Yes	ND	ND	~175 kDa	Homodimer	57
Fis	Yes (helically phased sites)	Yes	Yes	A_5 tracts and AT tracts	~11 kDa	Homodimer	77,79
H-NS	ND	Yes	ND	AT-rich DNA and TCGATAAATT	~15 kDa	Homodimer or heterodimer (H-NS–StpA)	19
IHF	ND	ND	Yes	(A/T)ATCAANNNNTT(A/G)	~11 kDa	Heterodimer (IHFα– IHFβ)	44,45
Dps	ND	ND	ND	ND	~19 kDa	Monomer or dodecamer	94
StpA	ND	Yes	ND	AT-rich DNA	~15 kDa	Homodimer or heterodimer (StpA–H-NS)	29
CbpA	ND	ND	ND	Curved DNA	~33 kDa	Homodimer or heterodimer (CbpA–CbpM)	92
CbpB	ND	ND	ND	Curved DNA	~33 kDa	Monomer	102
EbfC	ND	Suggested	ND	GTNAC	~11 kDa	Homodimer	93
MvaT	ND	Yes	ND	AT-rich DNA		Homodimer	26
Gram-positive bacteria							
MukB	ND	Yes	ND	Preference for ssDNA	~130 kDa	Homodimer	131
Lrp	Yes	Yes	ND	ND	~17 kDa	Homodimer	72
HU	ND	ND	Yes	ND	~10 kDa	Homodimer	37
Lsr2	ND	Yes	ND	AT-rich DNA	~12 kDa	Homodimer	24
Hlp	ND	ND	ND	ND	~21 kDa	Monomer	38
MrgA	ND	ND	ND	ND	~17 kDa	Monomer or dodecamer	132

Alba, acetylation lowers binding affinity; CbpA, curved-DNA-binding protein A; CbpB, curved-DNA-binding protein B (also known as Rob); CbpM, chaperone modulatory protein; Dps, DNA protection from starvation; dsDNA, double-stranded DNA; Fis, factor for inversion stimulation; Hlp, histone-like protein; Hmg, high mobility group; H-NS, histone-like nucleoid-structuring; IHF, integration host factor; Lrp, leucine-responsive regulatory protein; MrgA, metalloregulation DNA-binding stress protein; ND, not determined; Smc, structural maintenance of chromosome; ssDNA, single-stranded DNA.

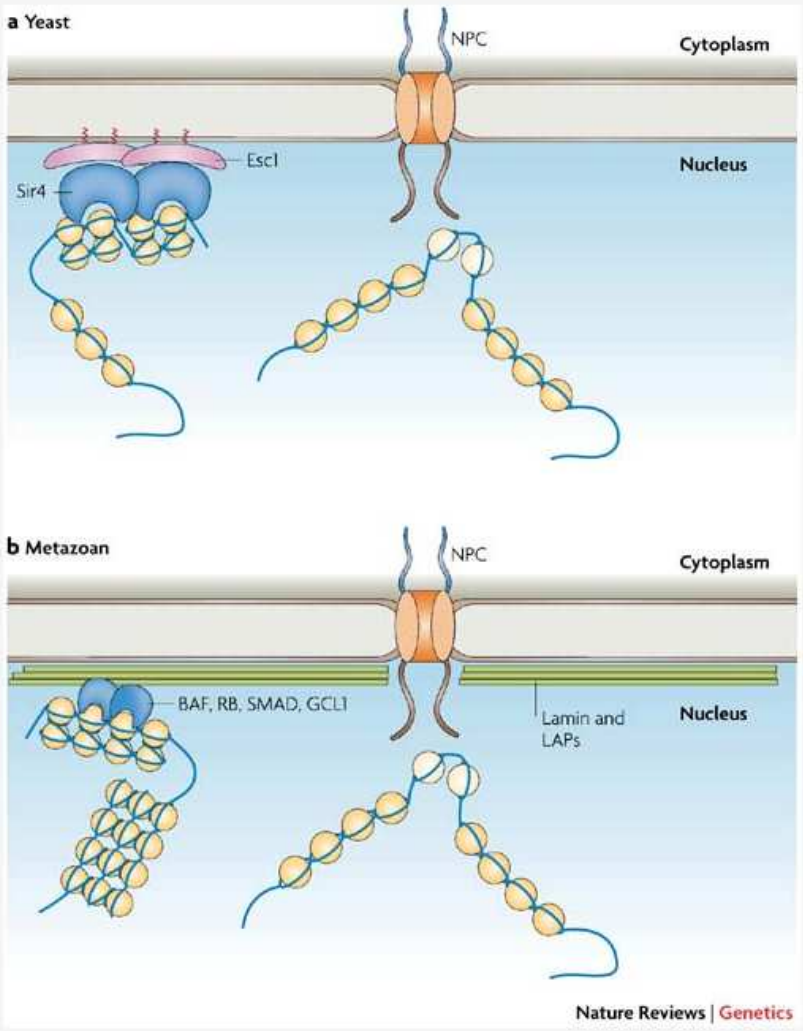
Histone-like



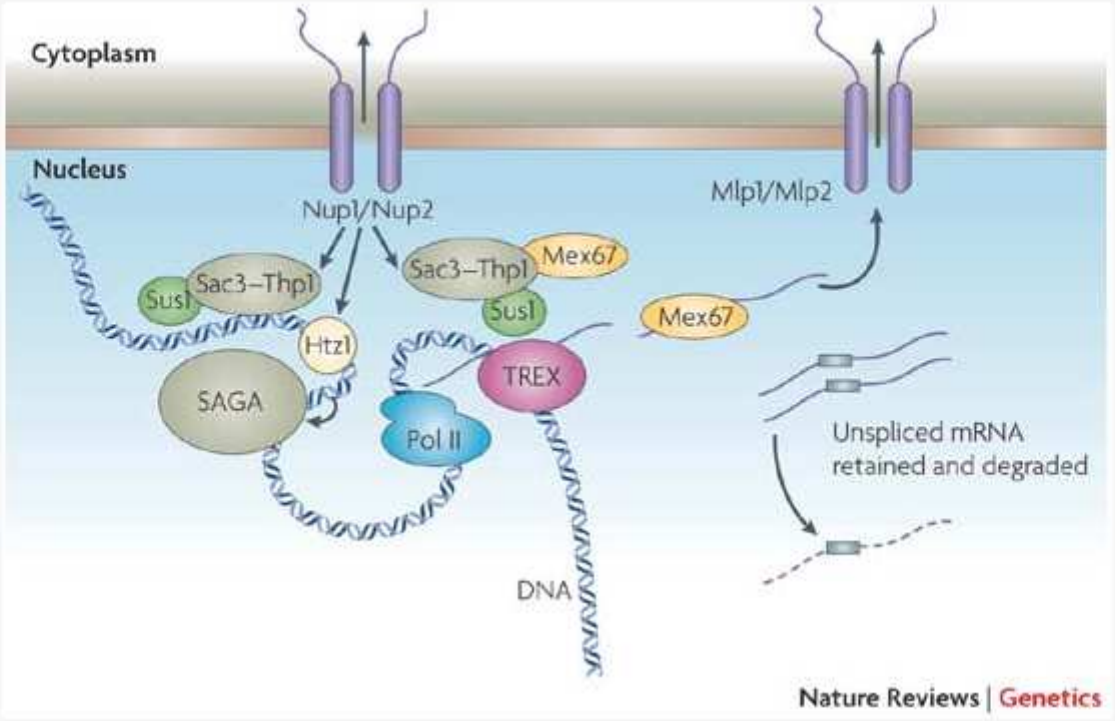

a
Lag phase–log phase transition
Log phase–stationary phase transition
Dps
H-NS
IHF
Fis
Growth curve
Log cell number
Protein concentration
Time
b
Log phase
RpoS
H-NS
RpoD
–35
–10
dps
Fis
Stationary phase
H-NS
IHF
RpoS
–35
–10
dps



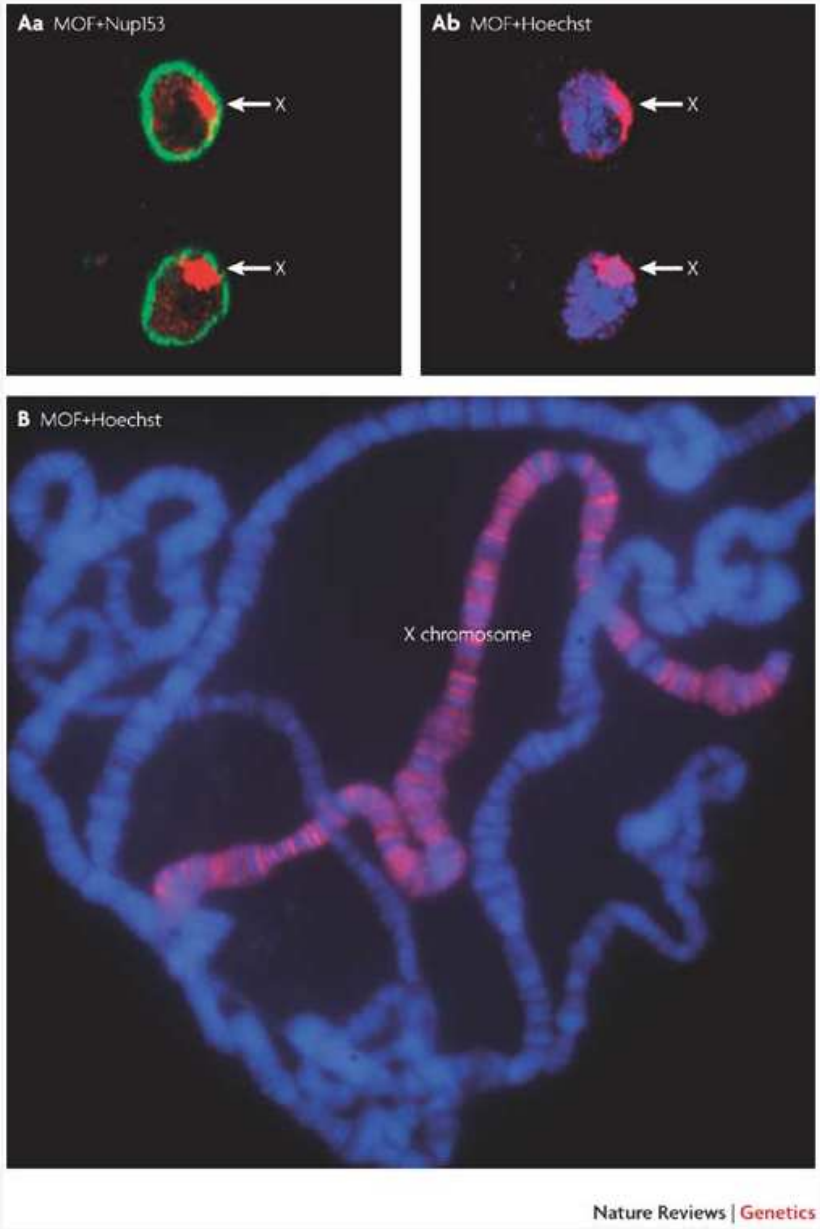
A | An electron micrograph of the mammalian liver nucleus (with an enlarged section shown in part **B**), showing dense-staining heterochromatin located around the nucleolus and against the nuclear envelope. Nuclear pores open onto lighter-staining open chromatin. **C** | In budding yeast, heterochromatin binds the nuclear envelope through Esc1 (enhancer of silent chromatin 1; labelled green), which forms distinct foci alternating with nuclear pores (visualized in red through labelling of Nup49 (nucleoporin 49)). **D** | An electron micrograph showing Esc1 at non-pore sites along the yeast inner nuclear envelope. An arrow indicates the nuclear pore, and black dots represent the labelling of Myc-epitope-tagged Esc1 using fluoronanogold Alexa^{488} anti-mouse antibody[18]. The image in part c is reproduced with permission from *EMBO Journal* (Ref. 18) © (2004) Macmillan Publishers Ltd.



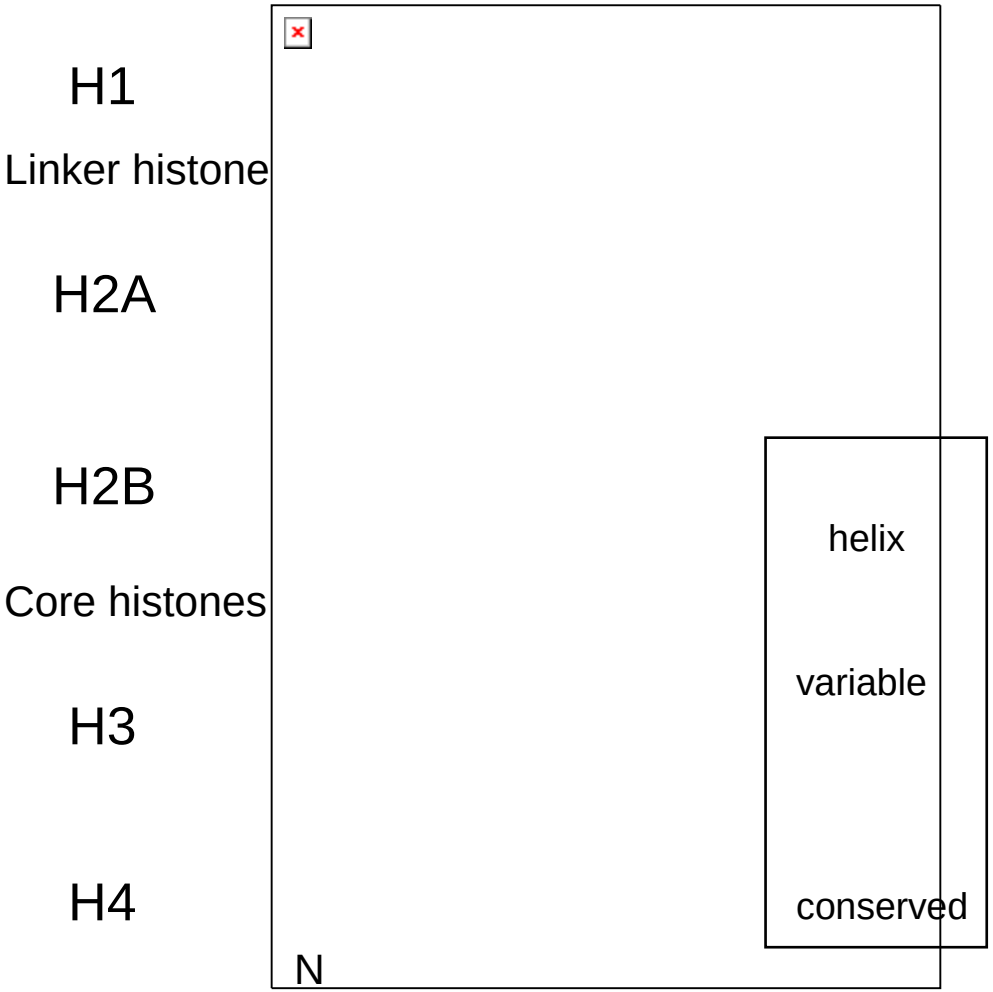
In eukaryotic cells, the nuclear compartment is separated from the cytoplasm by the inner and outer nuclear membranes. This membrane bilayer is perforated by nuclear pores, which are constituted by a large multiprotein complex (the nuclear pore complex (NPC)) that is composed of about 30 proteins (reviewed in Ref. 94). This nuclear membrane, together with the pores, is commonly referred as the 'nuclear envelope' (NE). **a** | In yeast nuclei, envelope-associated proteins such as Esc1 (enhancer of silent chromatin 1) are present in foci at the periphery; however, they do not coincide with the pores. Esc1 binds Sir4 (silent information regulator 4), which is an integral component of repressed heterochromatin in yeast[18, 19, 22]. **b** | In metazoan nuclei, the nuclear envelope is underlaid by a continuous meshwork of lamins and lamin-associated proteins (LAPs), which preferentially associate with inactive chromatin regions[37, 38]. Increasing evidence implicates interactions of chromatin with various nuclear-envelope components in gene repression as well as gene activation. BAF, barrier to autointegration factor; GCL1, germ-cell-less homologue; RB, retinoblastoma 1.



It is becoming increasingly clear that a number of transcription-coupled processes converge at nuclear pores, and by this virtue nuclear-pore complexes (NPCs) are likely to contribute, directly or indirectly, to transcriptional regulation. Although not all transcriptional activity in the nucleus will be subject to this mode of regulation, the budding yeast NPC seems to work together with transcriptional activation mechanisms to fine-tune gene activity, as proposed in this model. The SAGA chromatin-remodelling complex in yeast has been shown to contain Sus1; this protein is also present in the mRNA-export complex TREX, which interacts with the nucleoporin Nup1 (Refs 59, 60). Furthermore, Nup2 has been shown to interact with the promoters of active genes[16], and the NPC-associated protein Mlp1 (myosin-like protein 1) accumulates at the 3' end of active genes, where it contributes to an RNA surveillance mechanism[54, 80, 95]. Studies involving individual loci have shown that optimal activation (resulting in a twofold transcriptional effect) can require both localization of the induced gene at the NPC as well as at the 3' UTR[46, 47, 56]. Our model suggests that gene looping, which results from the coincident NPC-tethering of an initiation complex and mRNA-processing complexes that are associated with the 3' UTR, will help to fine-tune the expression of certain genes[62, 63, 64, 65]. NPC factors can facilitate efficient transcription elongation and/or efficient mRNA-processing events, or the coordination of the two. If aberrantly processed mRNAs are near the site of transcriptional initiation, their retention or rapid degradation can provide an immediate signal for the production of additional transcripts. Such a mechanism might be important for genes that require immediate high-level induction, such as heat-shock- or galactose-inducible genes. Finally, the pore protein Nup2 was found to tether genes through a histone variant H2A.Z (Htz1) in yeast[55]. This could reflect a heritable localization that contributes to forms of epigenetic control. This might be critical during dosage compensation of X-linked genes in male flies, where a twofold upregulation is crucial. Mex67; mRNA export factor 67; Pol II, RNA polymerase II.



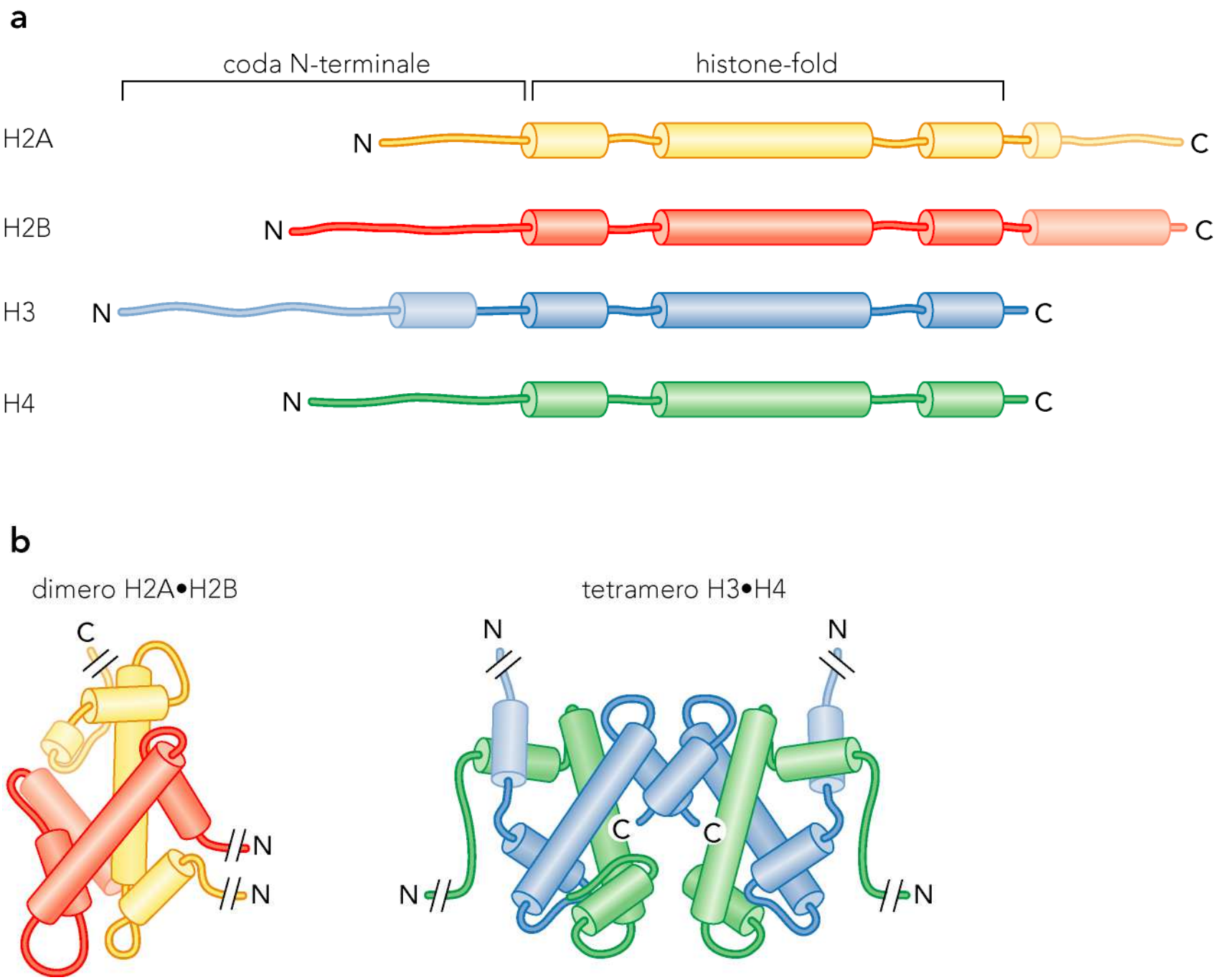
Dosage compensation of the male X chromosome requires approximately twofold transcriptional activation of X-linked genes in comparison with the female X chromosome, a process that is mediated by the male-specific lethal (MSL) complex (reviewed in Refs 71–73). The X chromosome is immunostained red with an antibody against MOF (Males on absent first), NPC is immunostained green with an antibody against the nucleoporin Nup153, and DNA is stained blue with Hoechst322. **A** | The nuclei of Schneider (SL-2) cells, when immunostained with antibodies against members of the MSL complex (in this case MOF (Males absent on first)), show a distinct X-chromosomal territory within the nucleus. Using confocal microscopy, parts of this X-chromosomal territory appear juxtaposed at or



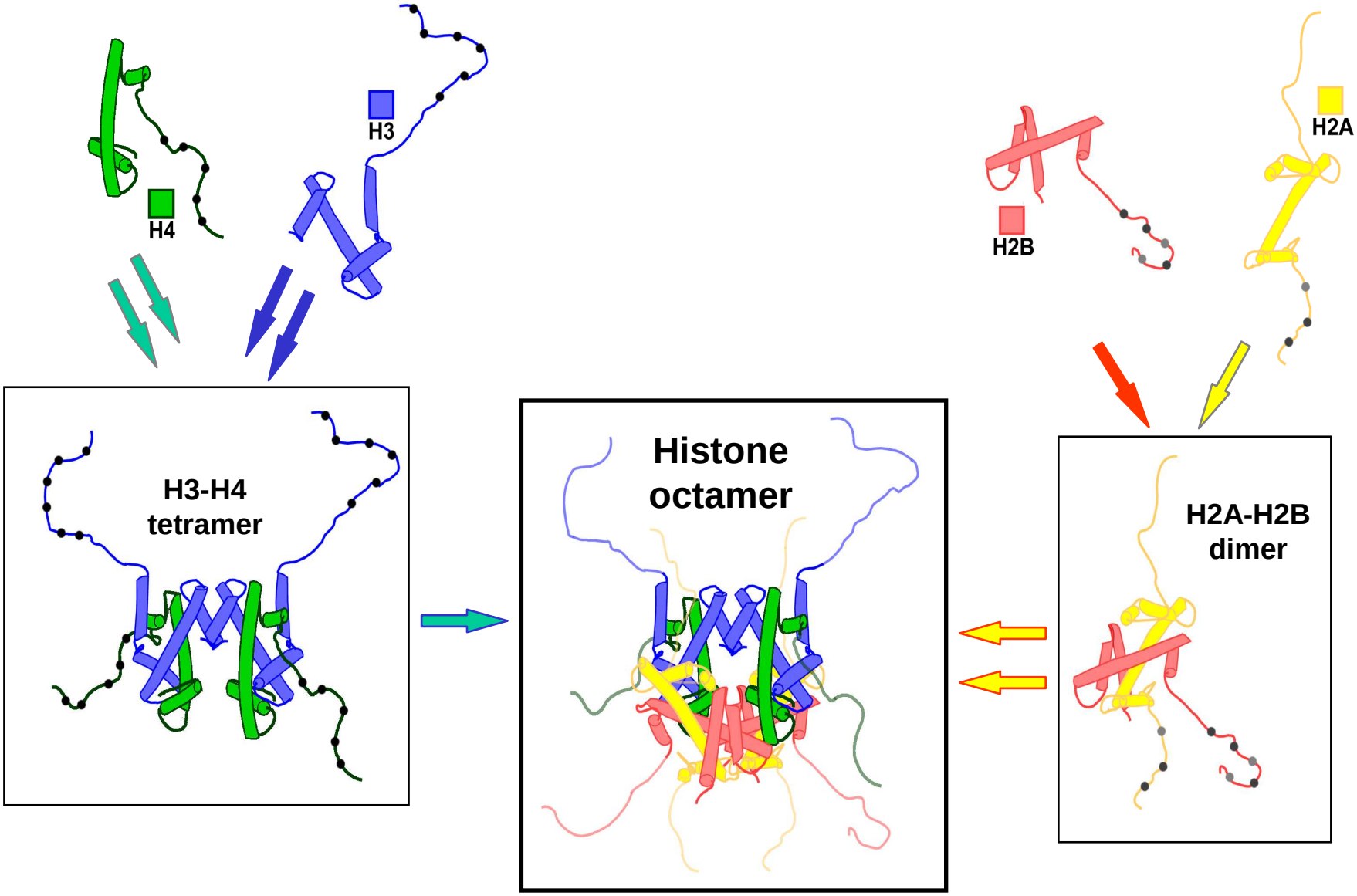
Histones

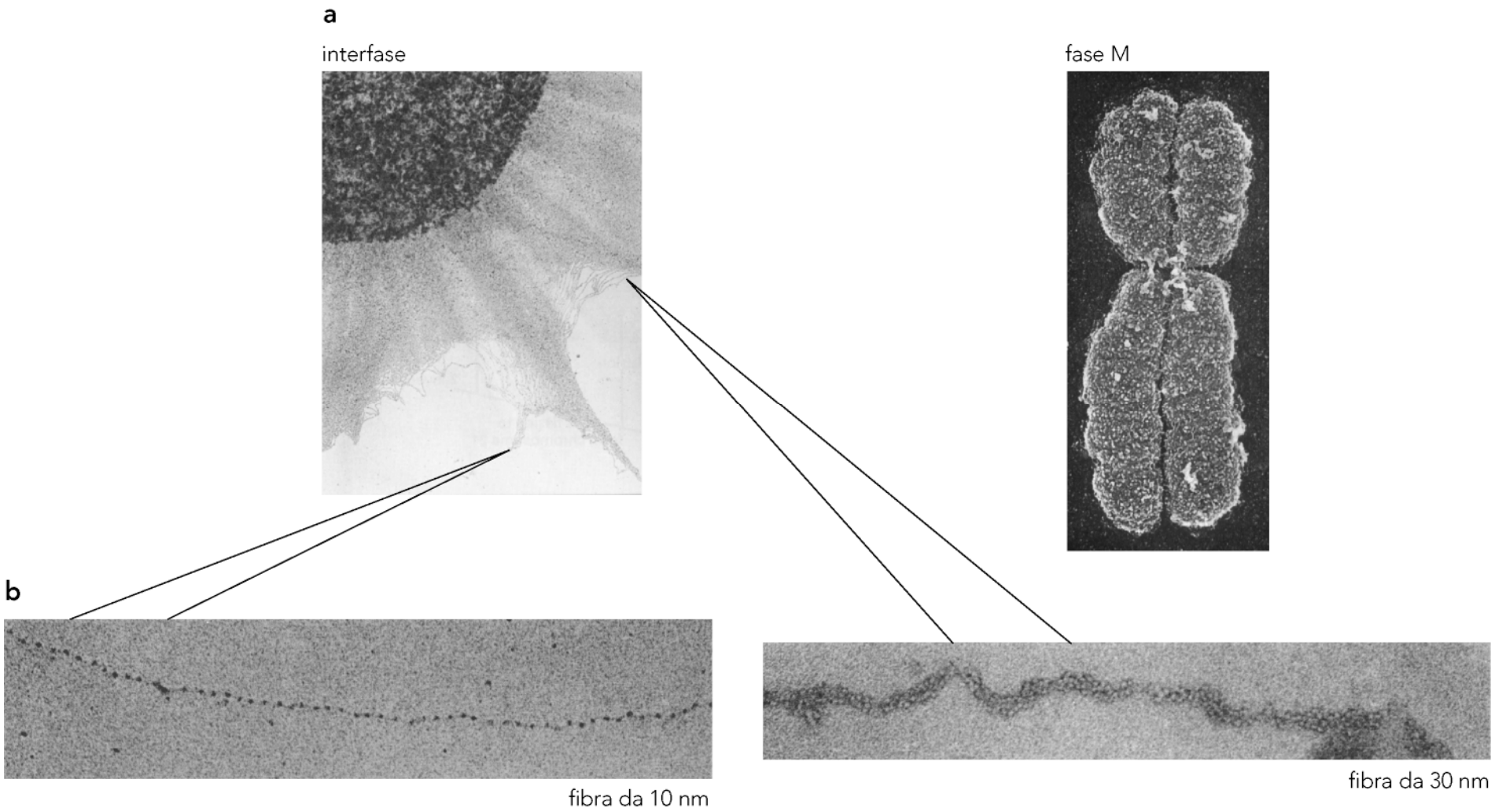
Highly conserved small basic proteins

Histone Type	Molecular Weight	Number of Amino Acids	Approx. Content of Basic Amino Acids
H1	17,000–28,000	200–265	27% lysine, 2% arginine
H2A	13,900	129–155	11% lysine, 9% arginine
H2B	13,800	121–148	16% lysine, 6% arginine
H3	15,300	135	10% lysine, 15% arginine
H4	11,300	102	11% lysine, 4% arginine

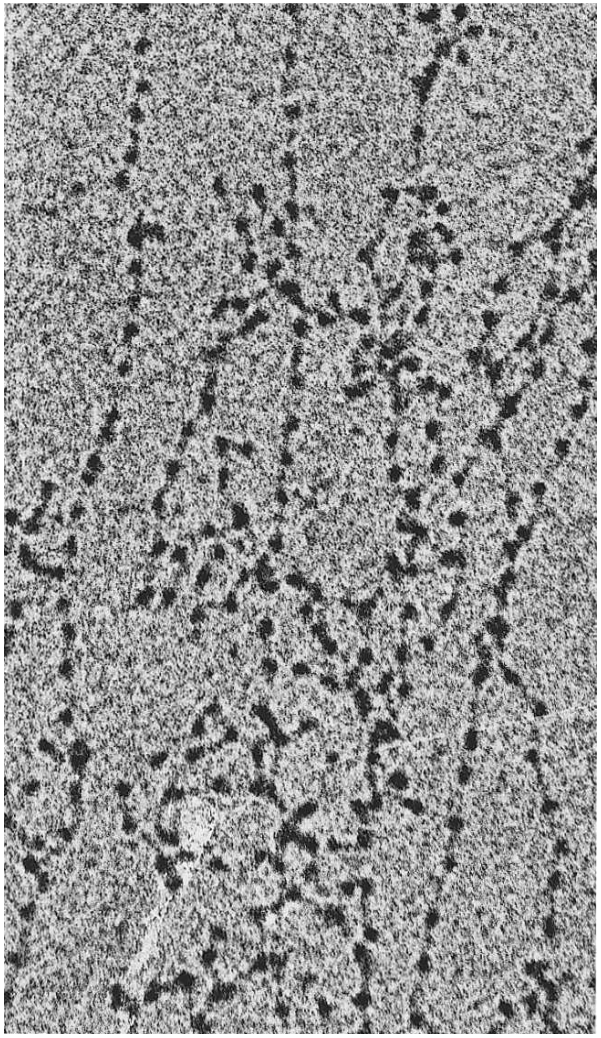

a
coda N-terminale
histone-fold
H2A
H2B
H3
H4
N
C
b
dimero H2A•H2B
tetramero H3•H4

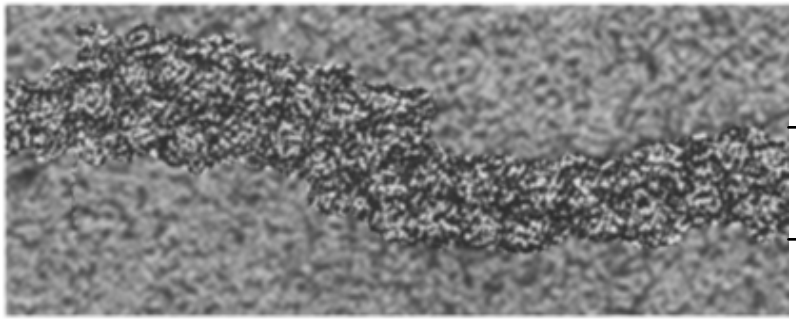
Histone octamer assembling



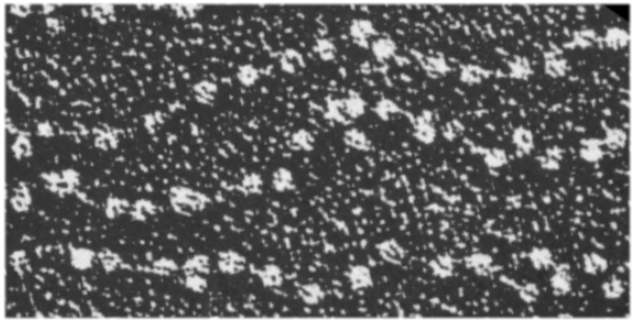

a
interfase
fase M
b
fibra da 10 nm
fibra da 30 nm

Pearl necklace

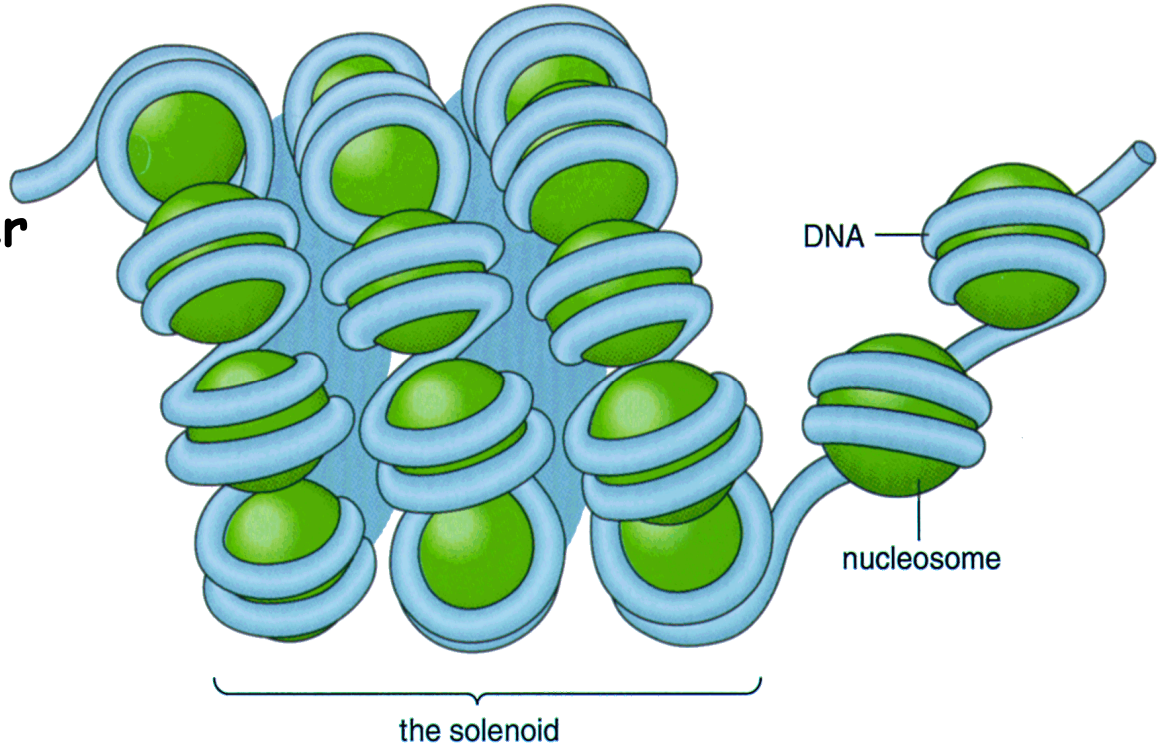




Chromatin fibers

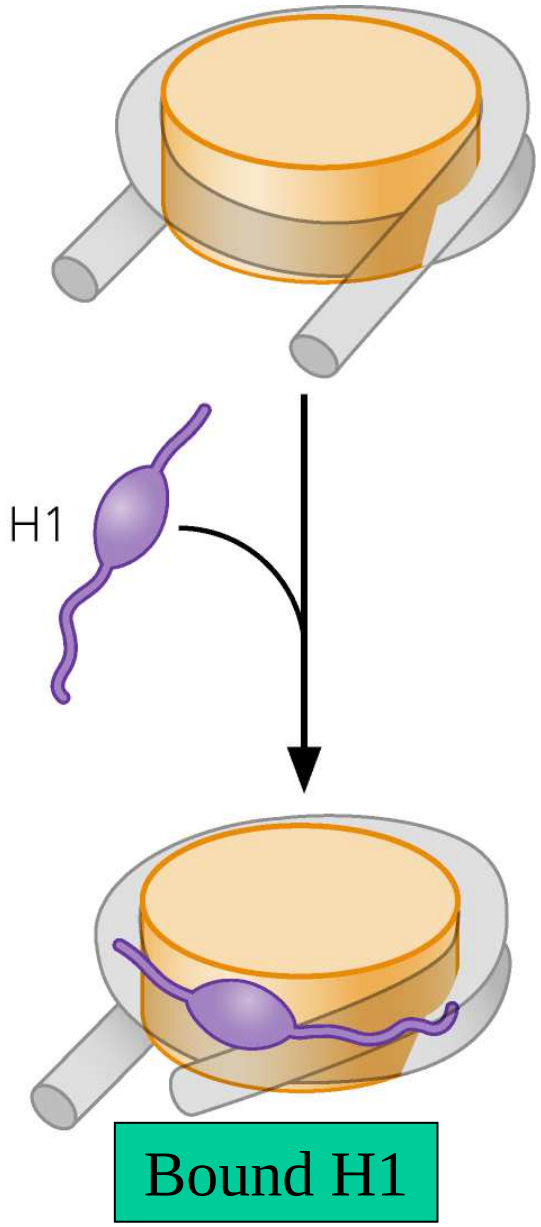


30 nm chromatin fiber



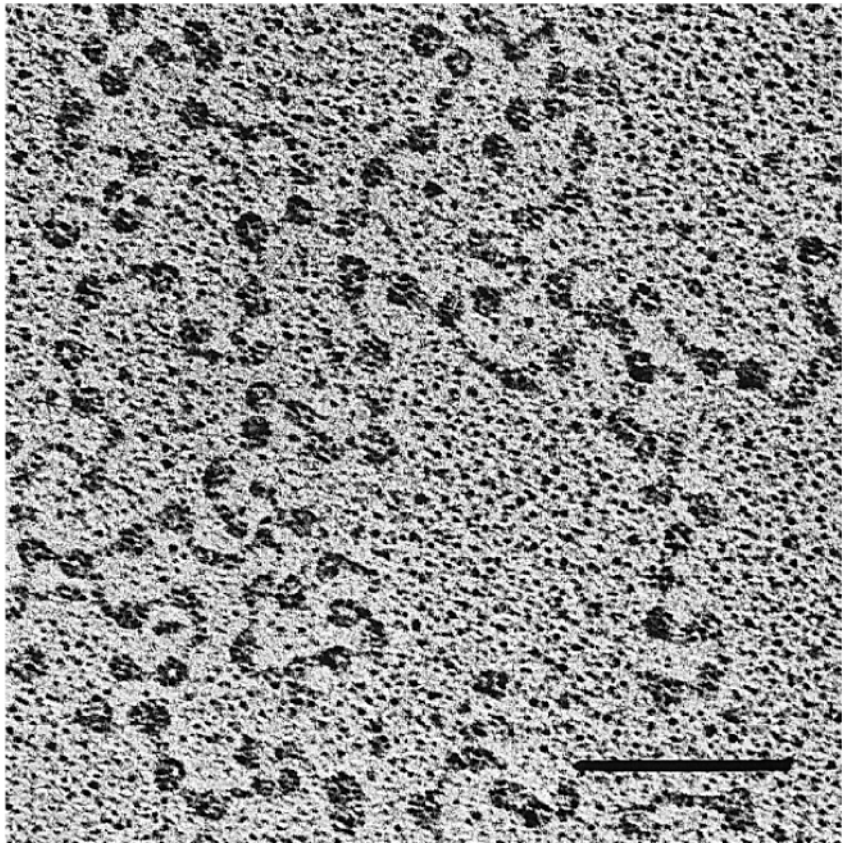
11 nm (beads)

H1 seals the nucleosome

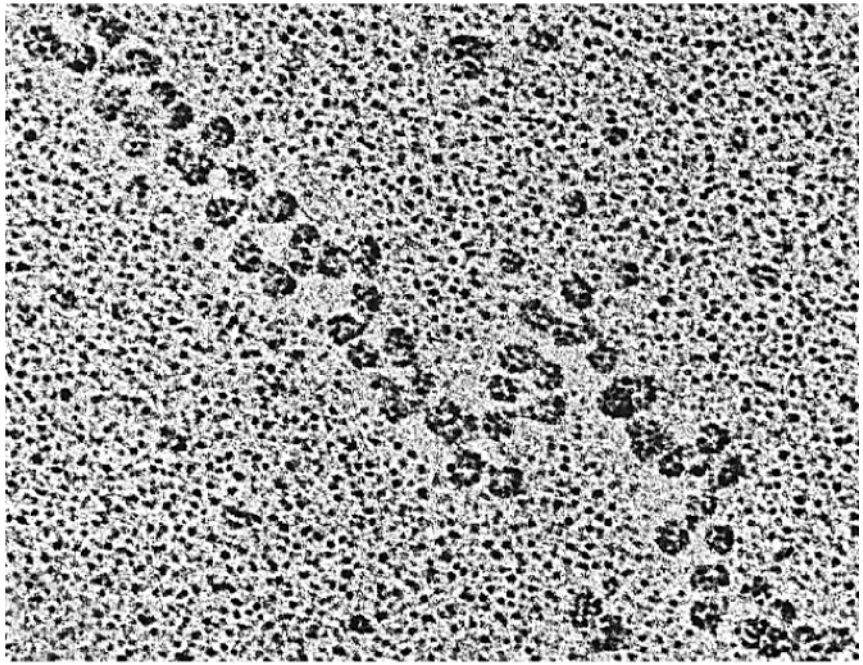


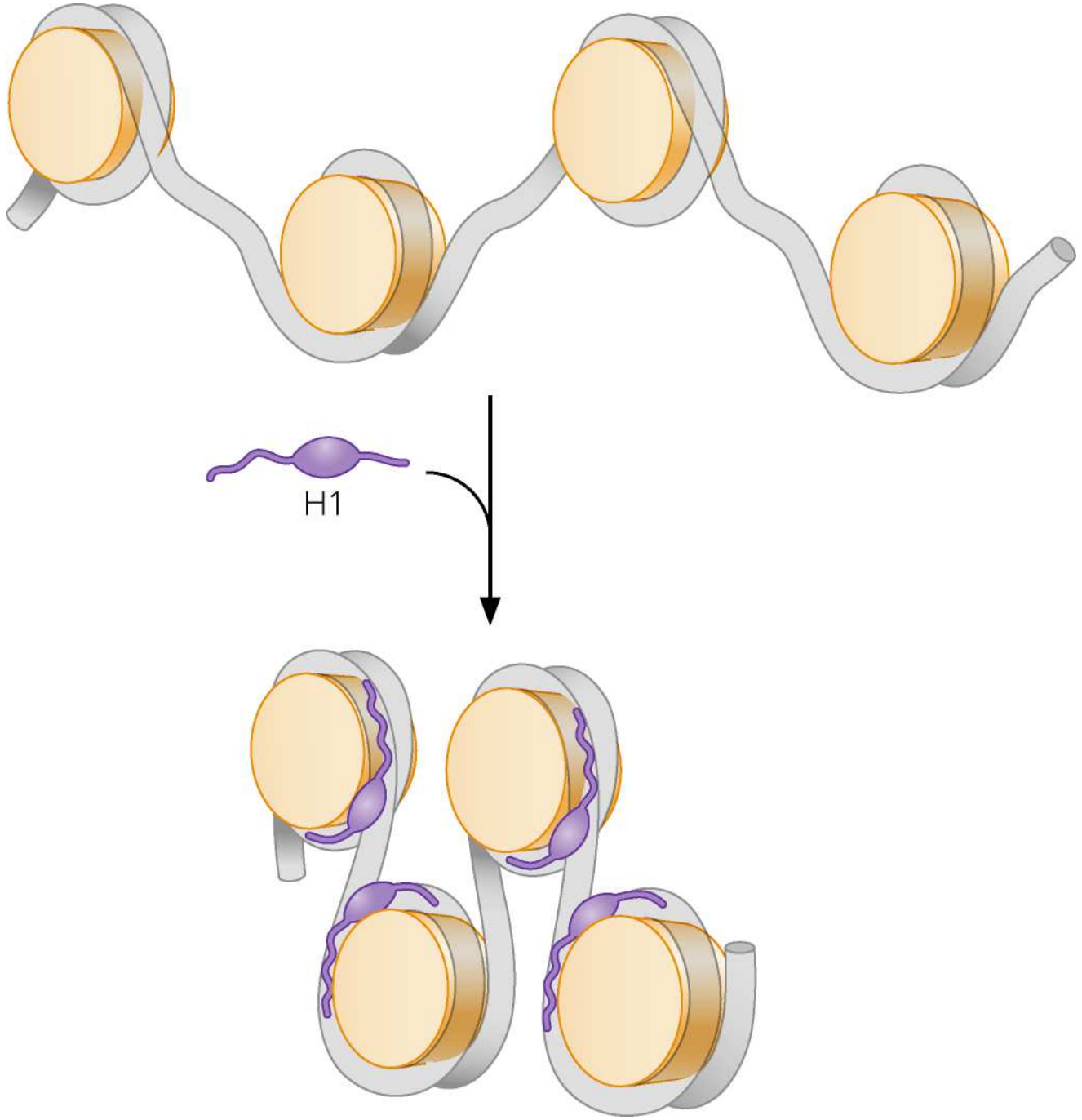
Pearl necklace vs 30nm fiber

a

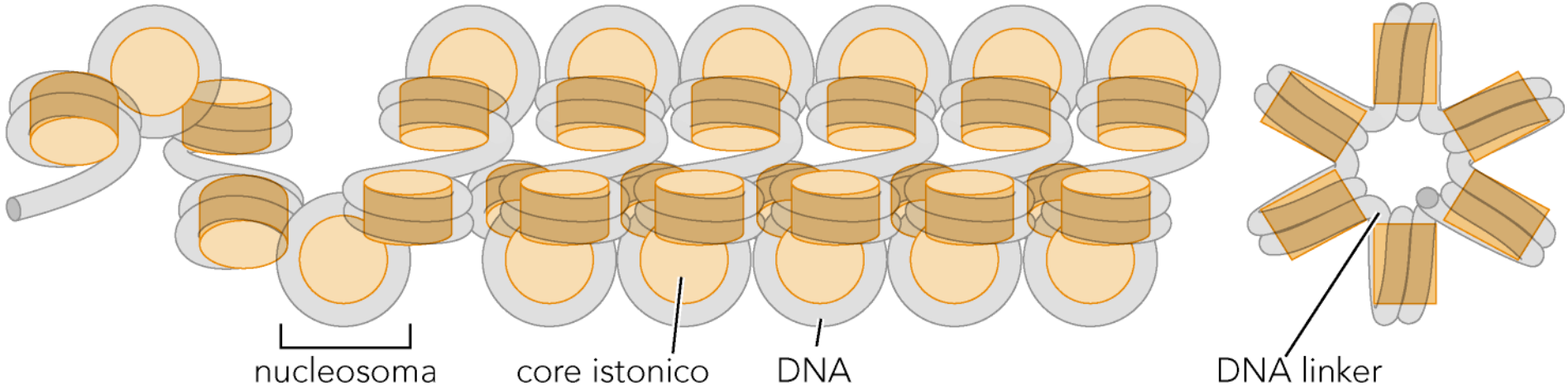


b

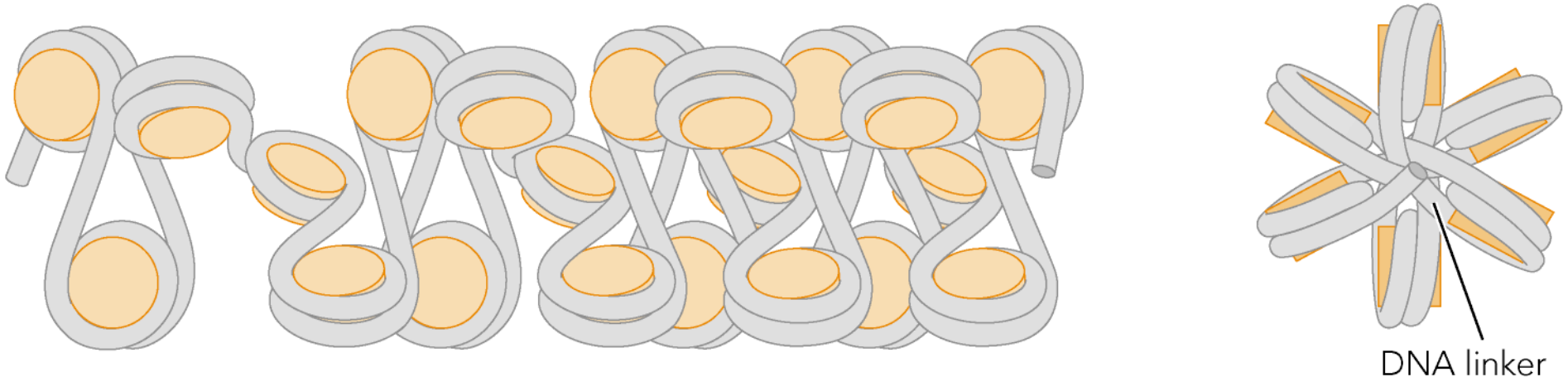


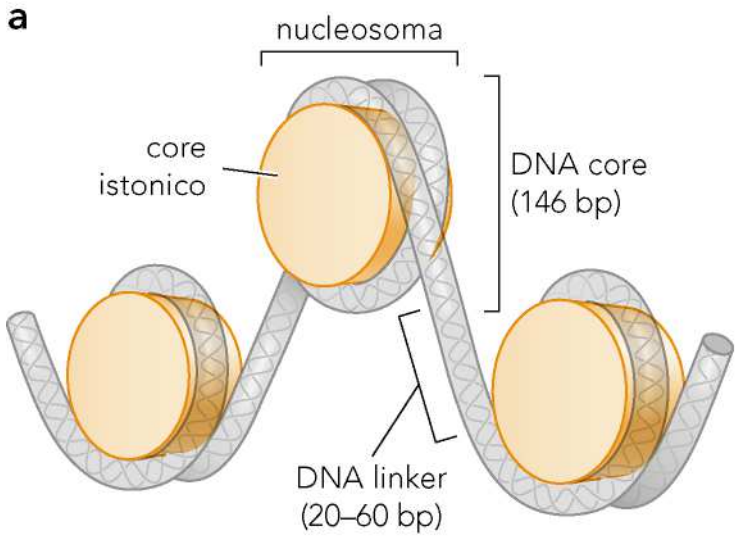

H1

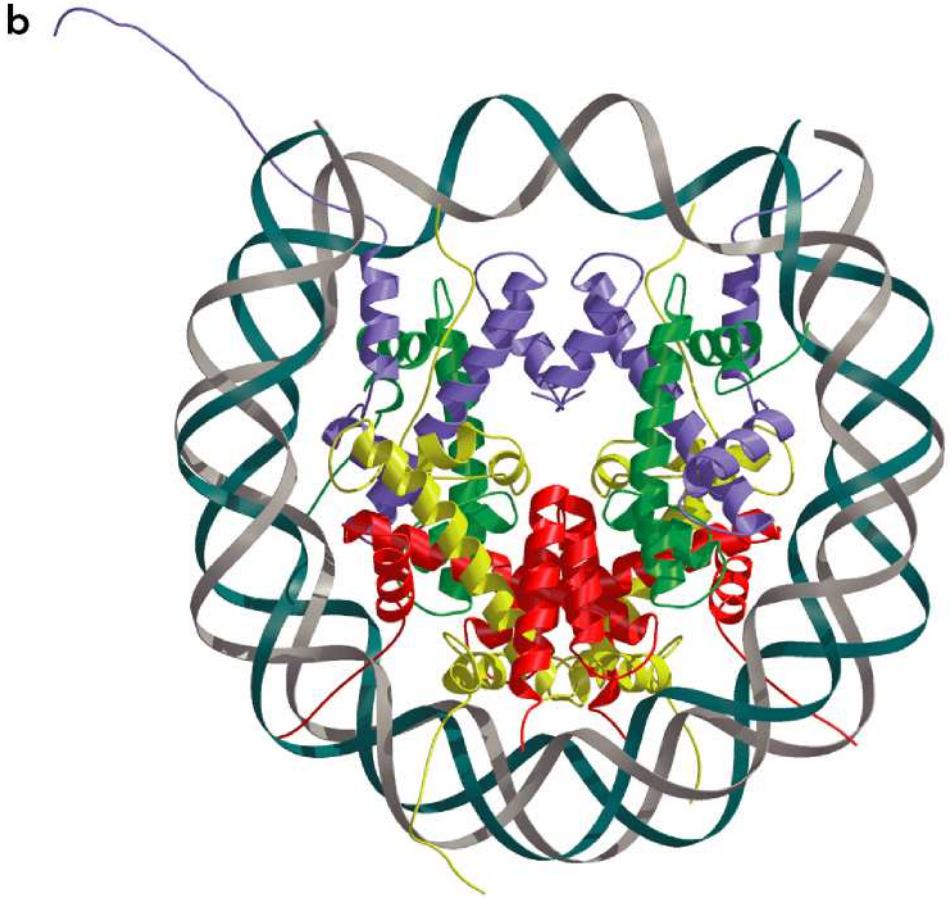
a solenoide



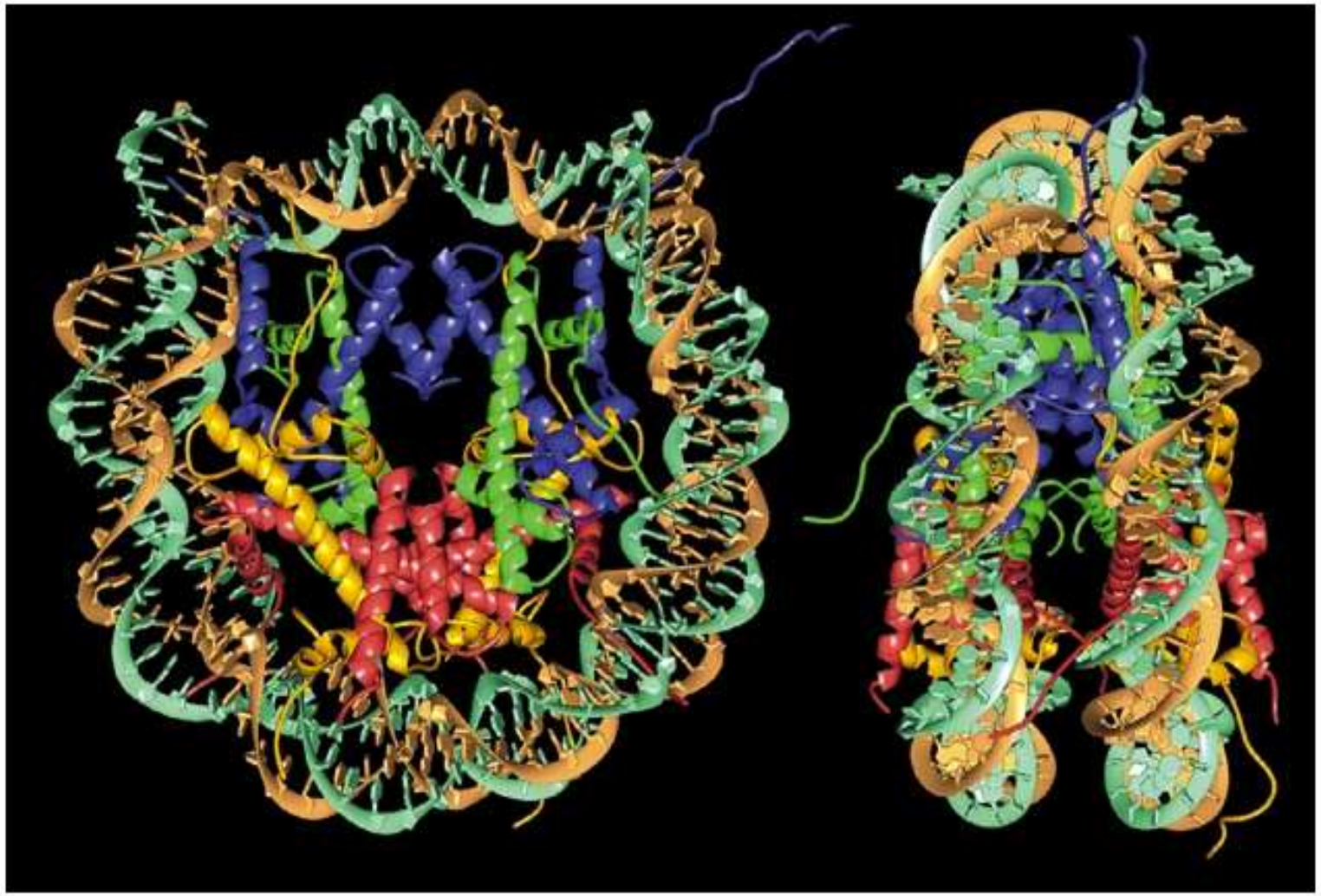
b zig-zag




a
nucleosoma
core
istonico
DNA core
(146 bp)
DNA linker
(20–60 bp)


b

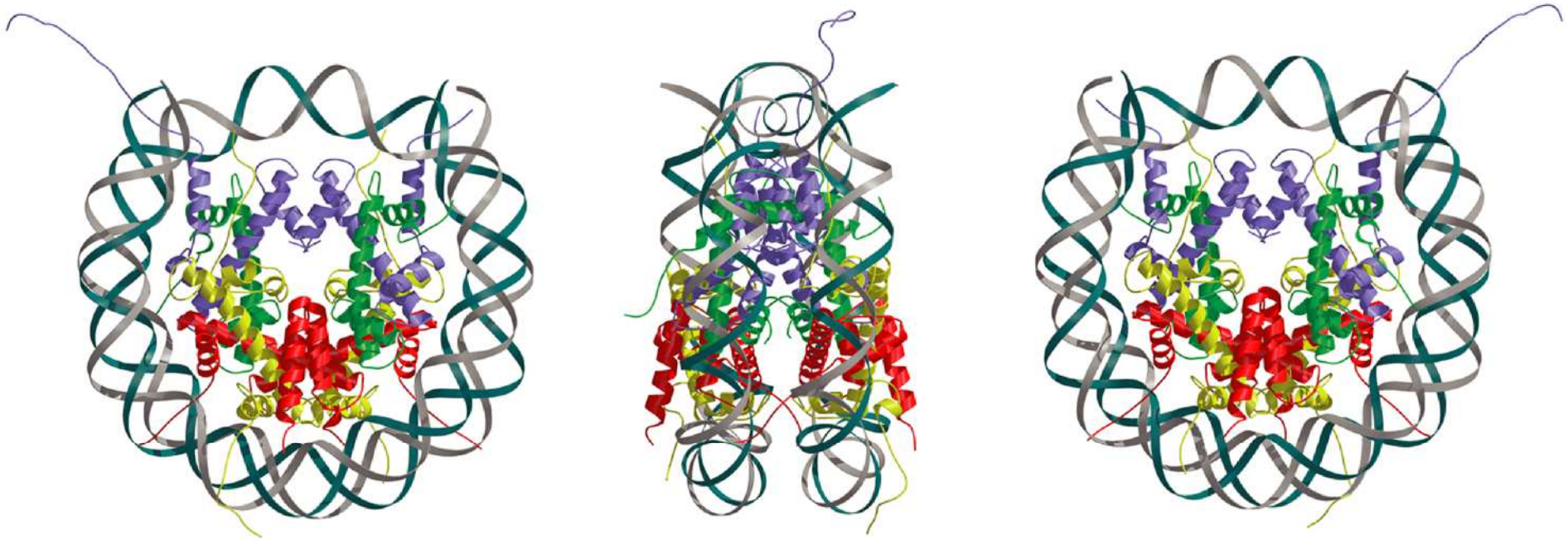
High resolution cristallographic structure



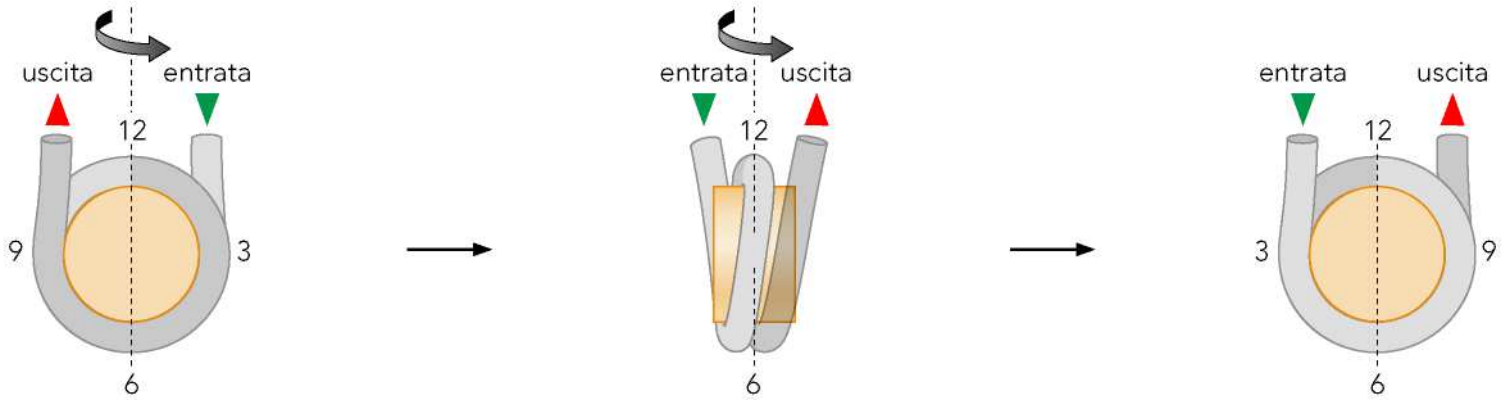
Luger, K., Maeder, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J.

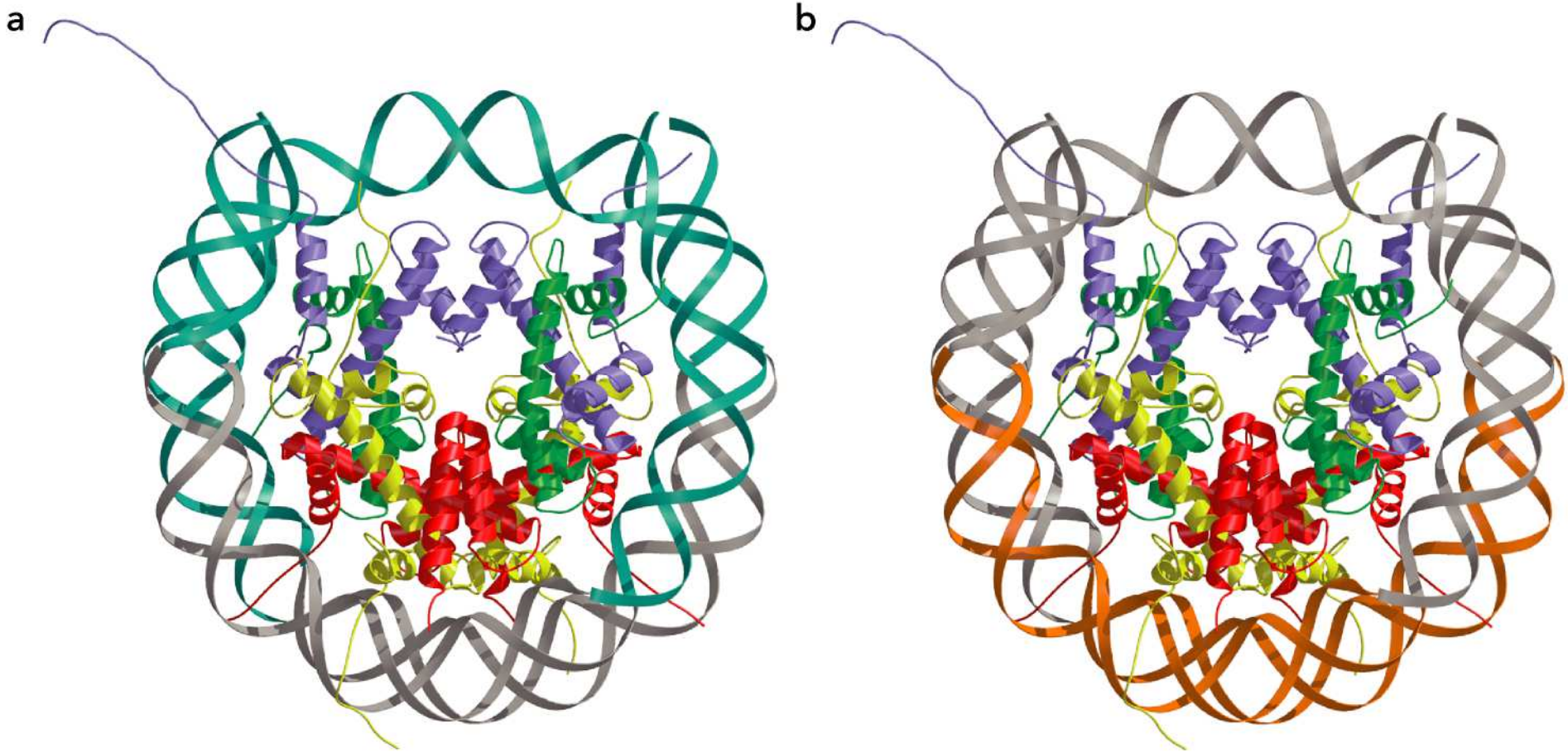
Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251-260 (1997)

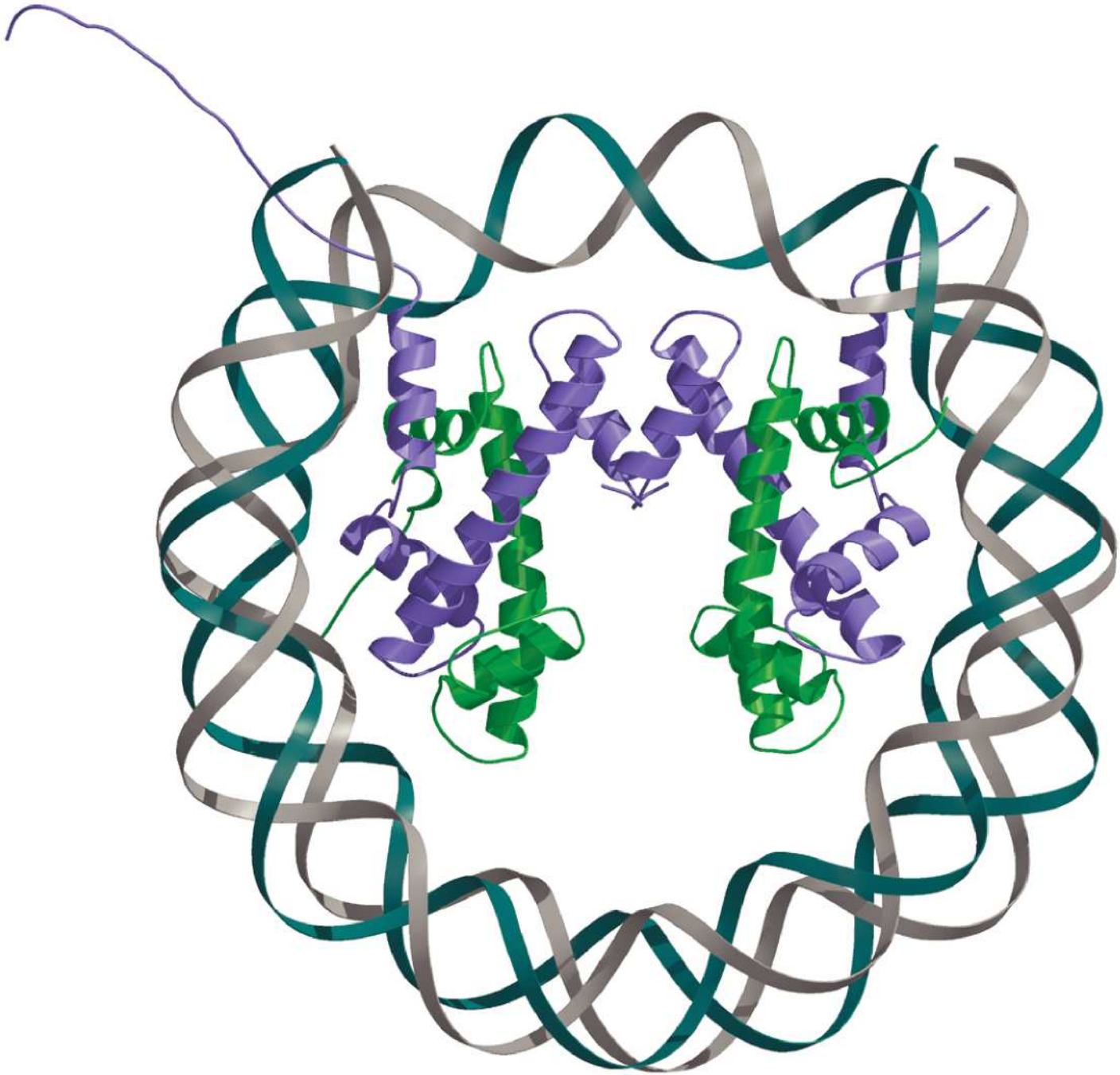
a

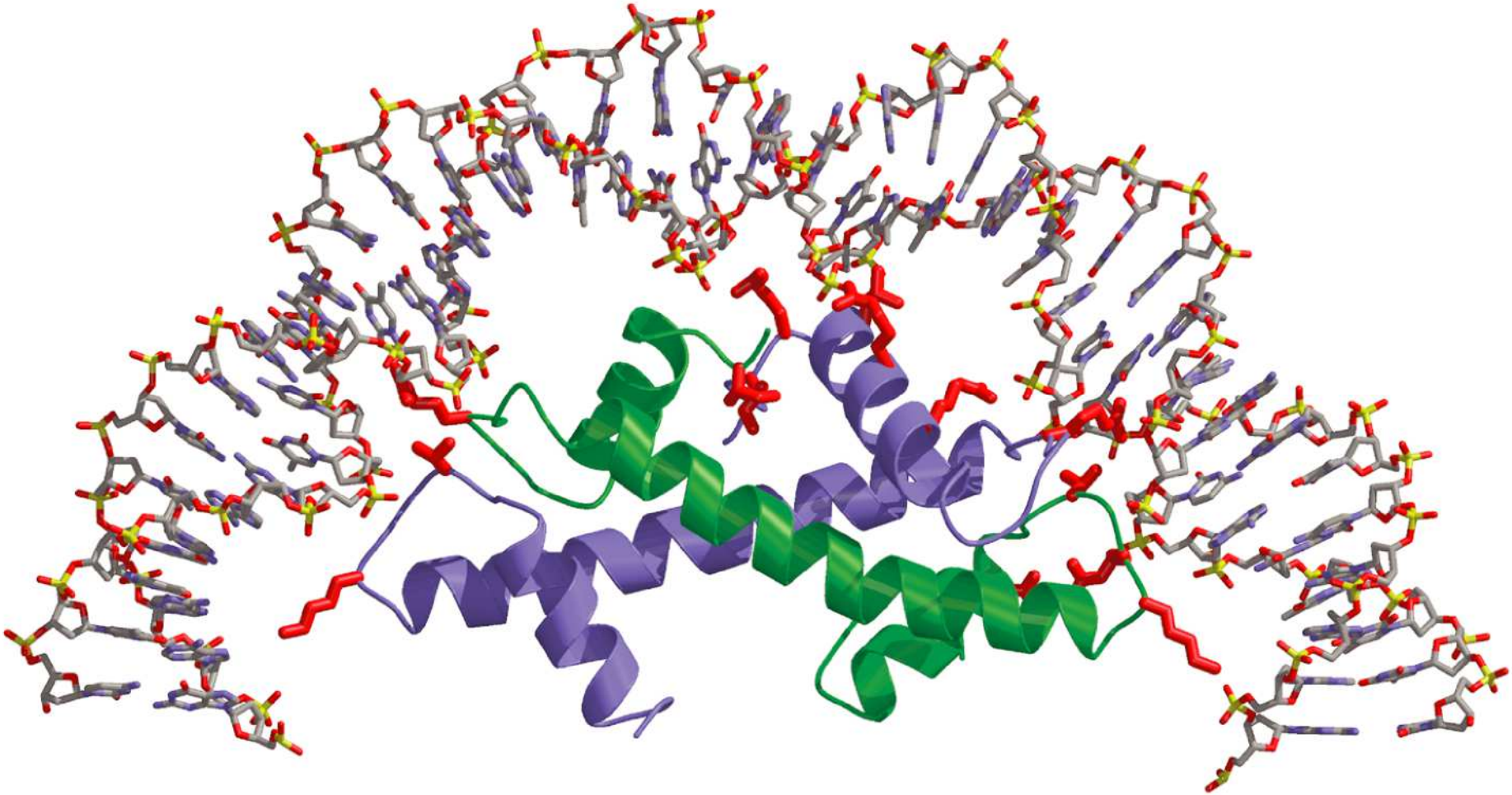


b


uscita
entrata
12
9
3
6
entrata
uscita
12
6
entrata
uscita
12
3
9
6


a
b





Mechanismsof nucleosome positioning

- Replication
- DNA/histone interactions
- Positioning proteins
- Bboundaries
- Chromatin Folding

DNA/Histone interactions

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 276, No. 37, Issue of September 14, pp. 35209–35216, 2001
Printed in U.S.A.

DNA Sequence Plays a Major Role in Determining Nucleosome Positions in Yeast *CUP1* Chromatin*

Received for publication, May 23, 2001, and in revised form, July 17, 2001
Published, JBC Papers in Press, July 18, 2001, DOI 10.1074/jbc.M104733200

Chang-Hui Shen and David J. Clark‡

From the Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

Histones associate with a great variety of genomic sequences but …

…different sequences can present the same structural arrangement

Conformational flexibility of sequences families

Their localization in the nucleosomal particle

DNA/Histone interactions

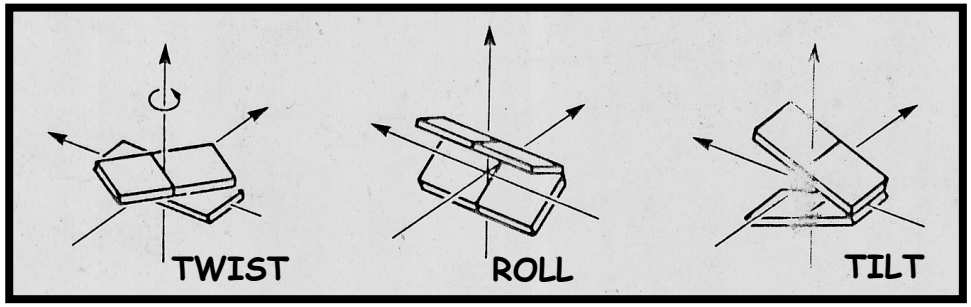
ROTATIONAL parameters:
Sequences that influence the rotational DNA arrangement on the nucleosome surface. Sequence periodicity is crucial

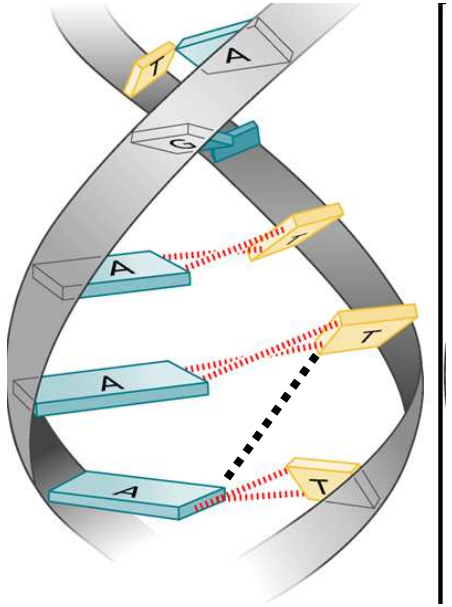
TRASLATIONAL parameters:
Sequences that influence the translational position of the nucleosomal dyad

They are inter-dependent

DNA/Histone interactions

ROTATIONAL parameters **10 bp periodicity**

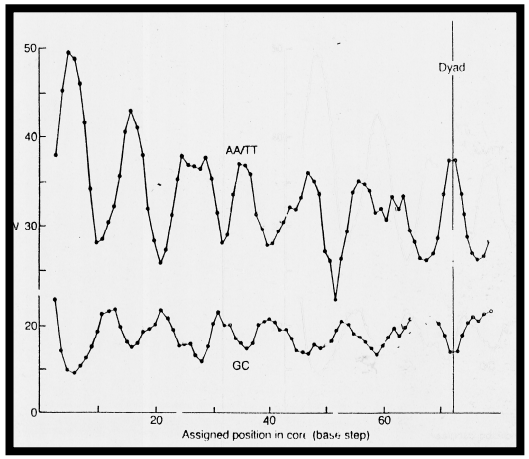




Seq d(AA/TT)
ROLL angle Close to 0

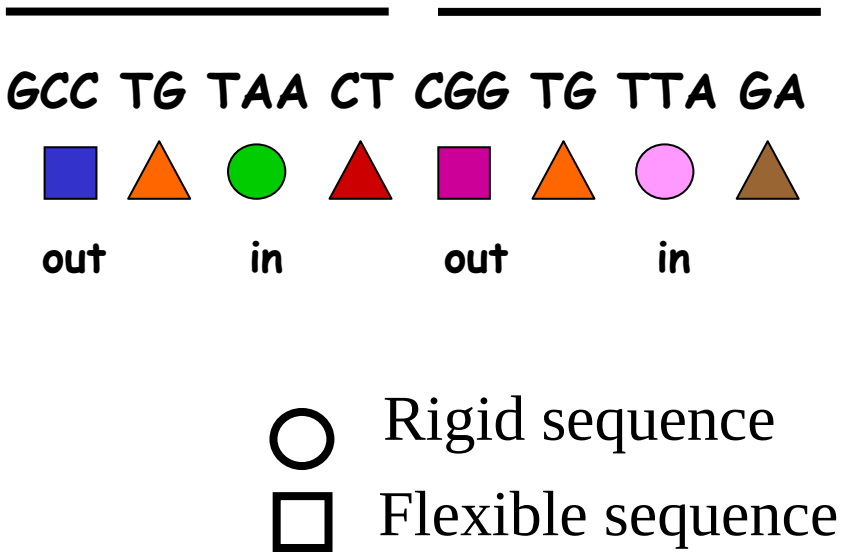
propeller twist > Large with crossbond

Seq d(GC/CG)
ROLL angle Negative (-20°)
opens the major groove



DNA/Histone interactions

High affinity nucleosomal sequences can be predicted



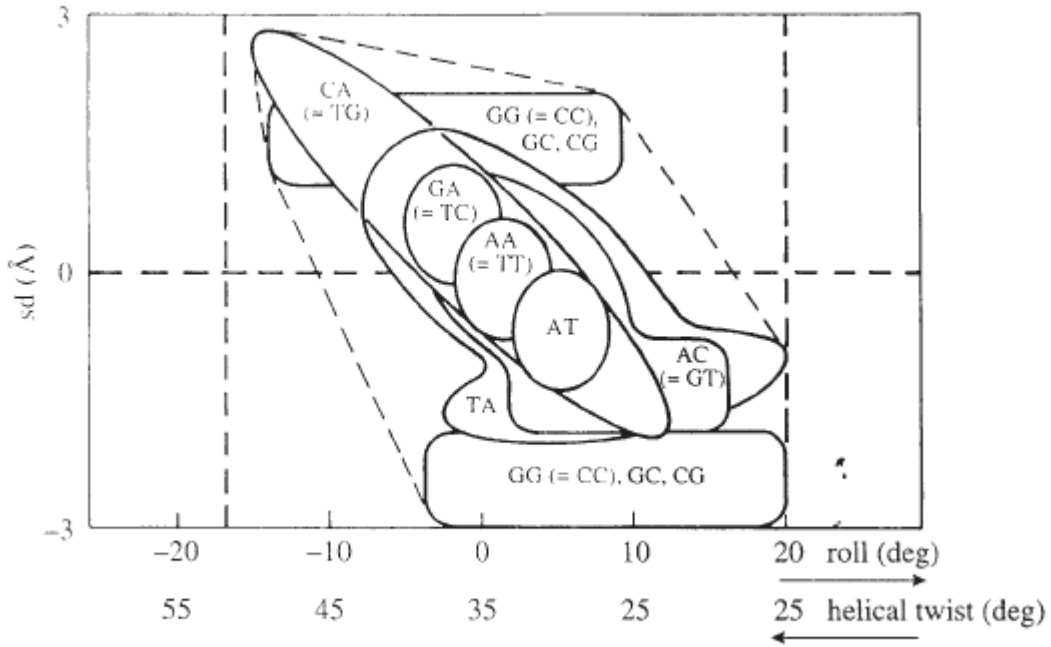
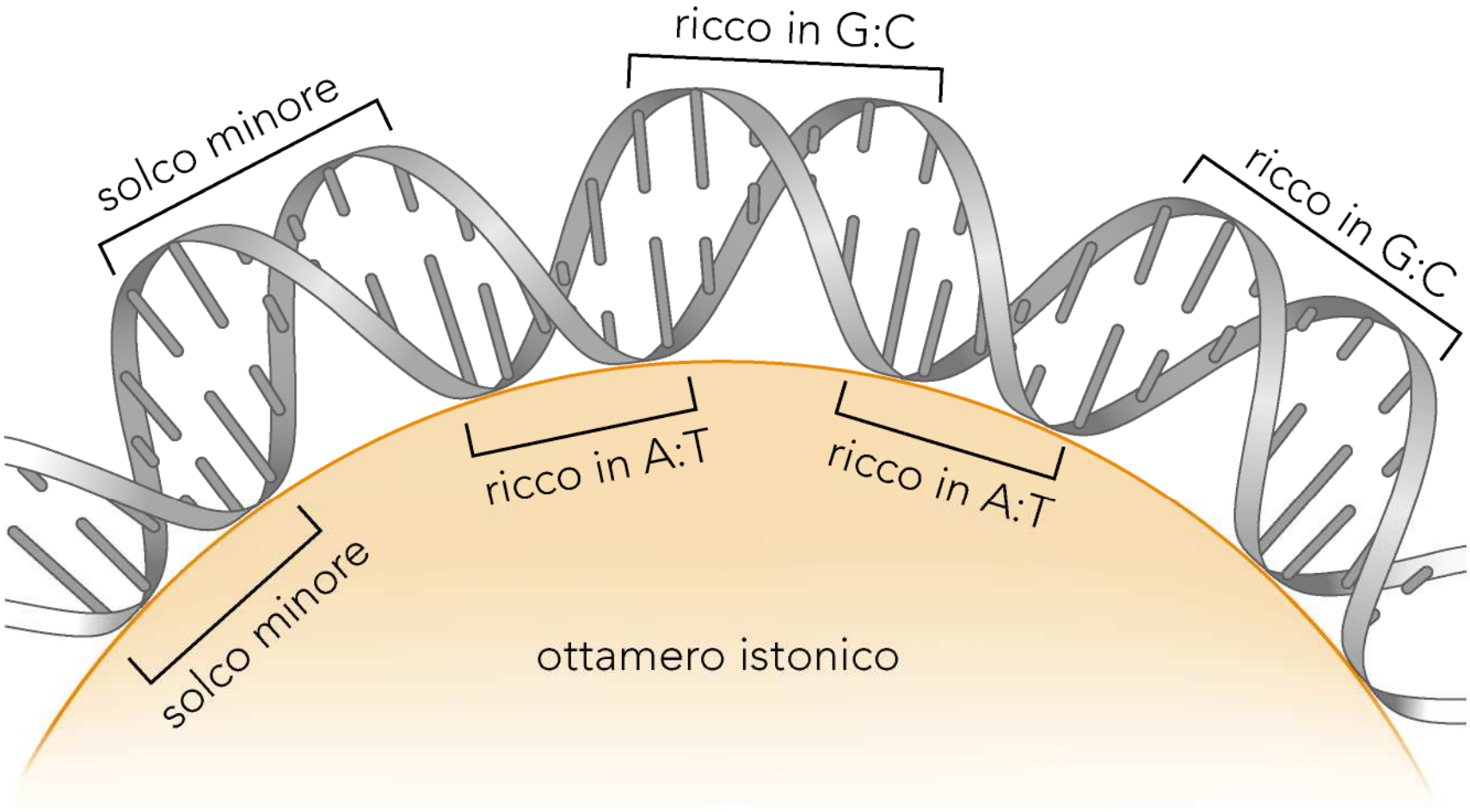


FIGURE 2 Ranges of conformational space occupied by different dinucleotide steps in crystal structures of DNA oligomers. Reproduced with permission from Ref. 5.


ricco in G:C
solco minore
ricco in G:C
ricco in A:T
ricco in A:T
solco minore
ottamero istonico

1034–1047 *Nucleic Acids Research, 2010, Vol. 38, No. 3* *Published online 17 November 2009*
doi:10.1093/nar/gkp962

Intrinsic flexibility of B-DNA: the experimental TRX scale

Brahim Heddi[1,2], Christophe Oguey[3], Christophe Lavelle[4], Nicolas Foloppe[5,*] and Brigitte Hartmann[1,2,*]

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Received June 10, 2009; Revised and Accepted October 13, 2009

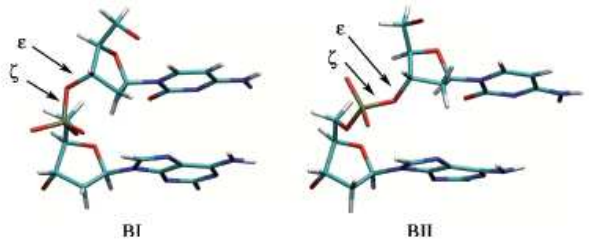


Figure 1. Illustration of the BI (left) and BII (right) phosphate linkage conformations with a CpA dinucleotide. BI and BII backbone conformations differ in the torsion angles ε and ζ which are respectively *trans*/*g*- in BI ($\varepsilon - \zeta = -90°$) and *g*-/*trans* in BII ($\varepsilon - \zeta = +90°$).

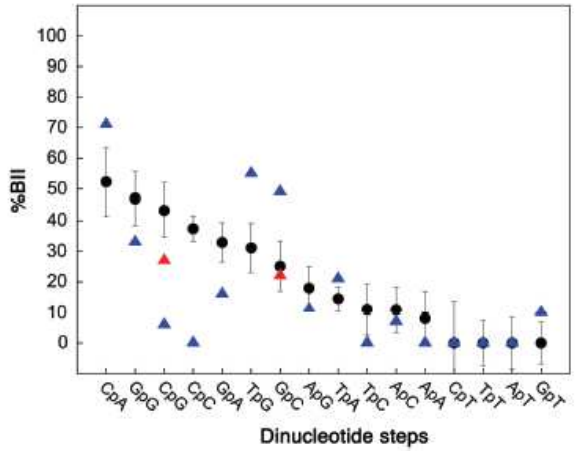


Figure 2. Influence of the dinucleotide sequences on BII percentages in B-DNA. BII percentages (%BII) of free B-DNA inferred from δP in solution (black circles, with vertical bars for standard deviations) compiled from data published in the literature (see Supplementary Table S1). The conversion of δP in terms of BII percentage, using a published procedure (48), is detailed in 'Materials and Methods' section. BII percentages in solution are compared with those extracted from X-ray structures (triangles; blue: all structures, red: decamers only).

RESULTS

BI/BII dinucleotide sequence effect in B-DNA

Table 1. Influence of DNA base sequence on the BII percentages in free DNA in solution

	N	%BII	TRX score
CpG•CpG	25	**43•43**	**43**
CpA•TpG	28	**52•31**	**42**
GpG•CpC	11	**47•37**	**42**
GpC•GpC	22	**25•25**	**25**
GpA•TpC	25	**33•11**	**22**
TpA•TpA	12	14•14	14
ApG•CpT	19	18•0	9
ApA•TpT	17	11•0	5
ApC•GpT	23	8•0	4
ApT•ApT	22	0•0	0

The DNA sequence is expressed in terms of the 10 complementary dinucleotides, of frequency N in the NMR data collected from the literature. The BII percentages (%BII) are given for each partner in a complementary dinucleotide. The average standard deviation of %BII is ± 8. The TRX scores are the half-sums of the %BII observed for two facing phosphates in a complementary dinucleotide. The %BII and TRX scores higher (in bold) and lower than average (which is 21) correspond to enhanced and restricted flexibilities, respectively. A maximal flexibility on this scale corresponds to a TRX score of 50.

from our data set to BII percentages with an established method (48) (see 'Materials and Methods' section). This analysis shows that (i) the averaged BII percentage in solution is 21% and (ii) 7 out of the 16 dinucleotides exhibit BII populations higher than average. These steps can thus be regarded as BII-rich steps. The maximal flexibility is observed for the phosphates of CpG, CpA and GpG, with a BI/BII ratio close to 1 (50% BI, 50% BII).

Considering the BII propensities of the 10 possible complementary dinucleotide sequences $N_ipN_{i+1}•N_jpN_{j+1}$ (Table 1; N: any base) highlights that the facing phosphates tend to exhibit similar behaviors overall. In a first group containing ApN•NpT and TpA•TpA, the phosphates are very rarely in BII. This group, mainly confined in BI•BI, is thus characterized by a restricted backbone flexibility. In another group of dinucleotide steps (GpG•CpC, CpG•CpG, GpC•GpC and CpA•TpG) the facing phosphates can adopt BI and BII conformations, with a higher-than-average BII percentage. This family exhibits an enhanced backbone flexibility, potentially able to explore the three possible

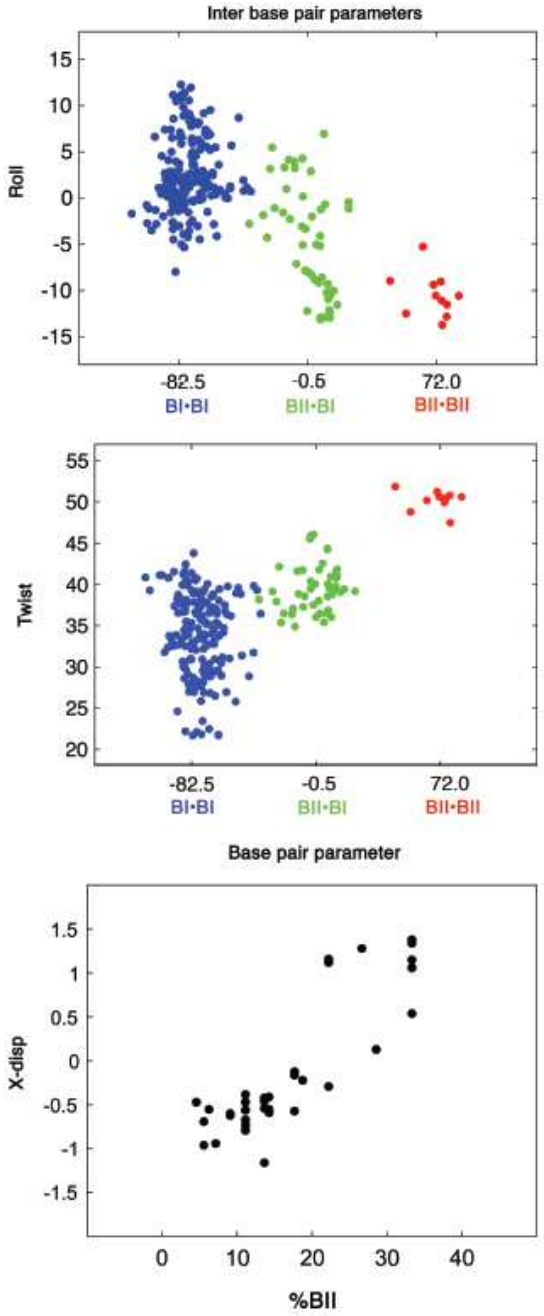


Figure 4. Influence of BII phosphates on helical descriptors. Inter base pair parameters: Twist (°) and roll (°) plotted versus $(\varepsilon-\zeta)_{av}$, averaged on the two facing phosphates. BI•BI (blue, $(\varepsilon-\zeta)_{av} = -82.5°$), BI•BII (green, $(\varepsilon-\zeta)_{av} = -0.5°$) and BII•BII (red, $(\varepsilon-\zeta)_{av} = +72°$) are the

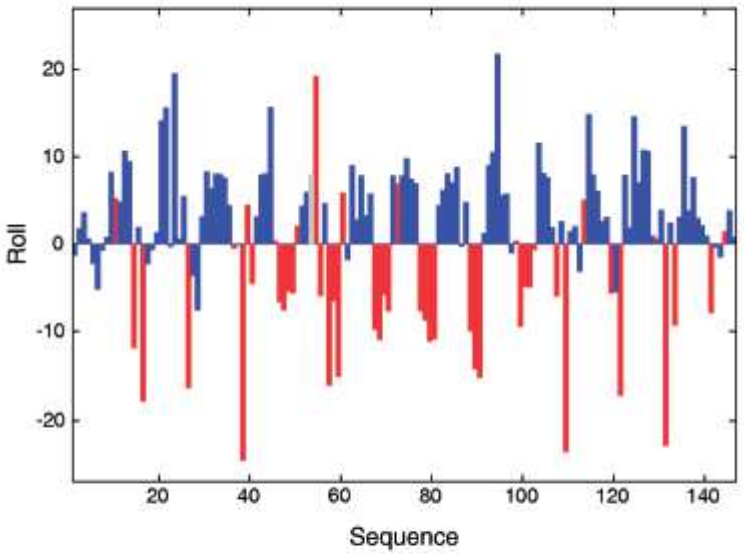
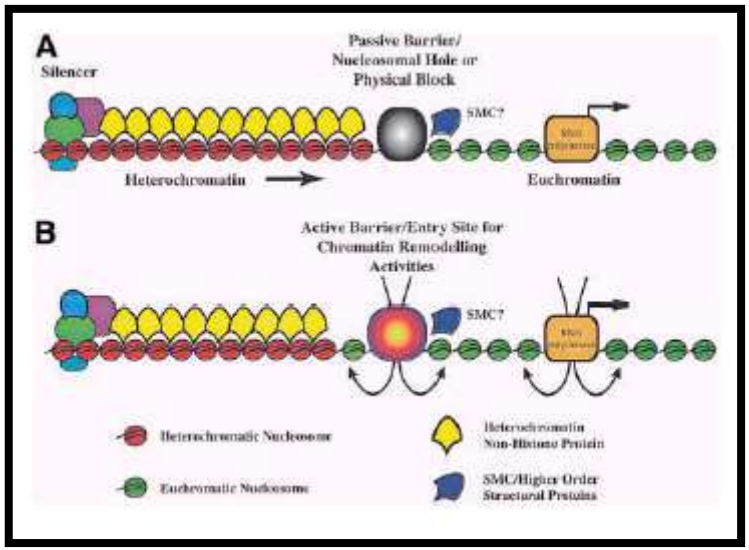


Figure 5. Relationship between roll and backbone conformations in NCP X-ray structures. Roll (°) profile along the DNA sequence in nucleosome X-ray structure 1KX5. The bars giving the roll values are colored according to the state of the facing phosphates in the complementary dinucleotide steps: blue for BI•BI and red for BI•BII or BII•BII. The grey bar represents an unclassifiable step (BI on strand 1 and $(\varepsilon-\zeta)\sim170°$, i.e. neither BI nor BII, on strand 2). Structures 1KX3 and 1M19 give similar patterns.

Boundaries

Example: protein complex blocking chromatin spreading



Example: cruciform structures

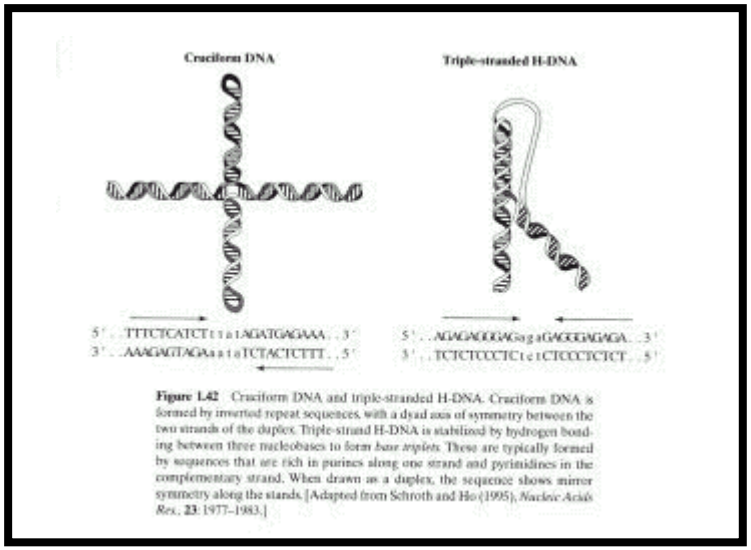


Figure 1.42 Cruciform DNA and triple-stranded H-DNA. Cruciform DNA is formed by inverted repeat sequences, with a dyad axis of symmetry between the two strands of the duplex. Triple-strand H-DNA is stabilized by hydrogen bonding between three nucleobases to form *base triplets*. These are typically formed by sequences that are rich in purines along one strand and pyrimidines in the complementary strand. When drawn as a duplex, the sequence shows mirror symmetry along the stands. [Adapted from Schroth and Ho (1995), *Nucleic Acids Res.* **23**:1977–1983.]

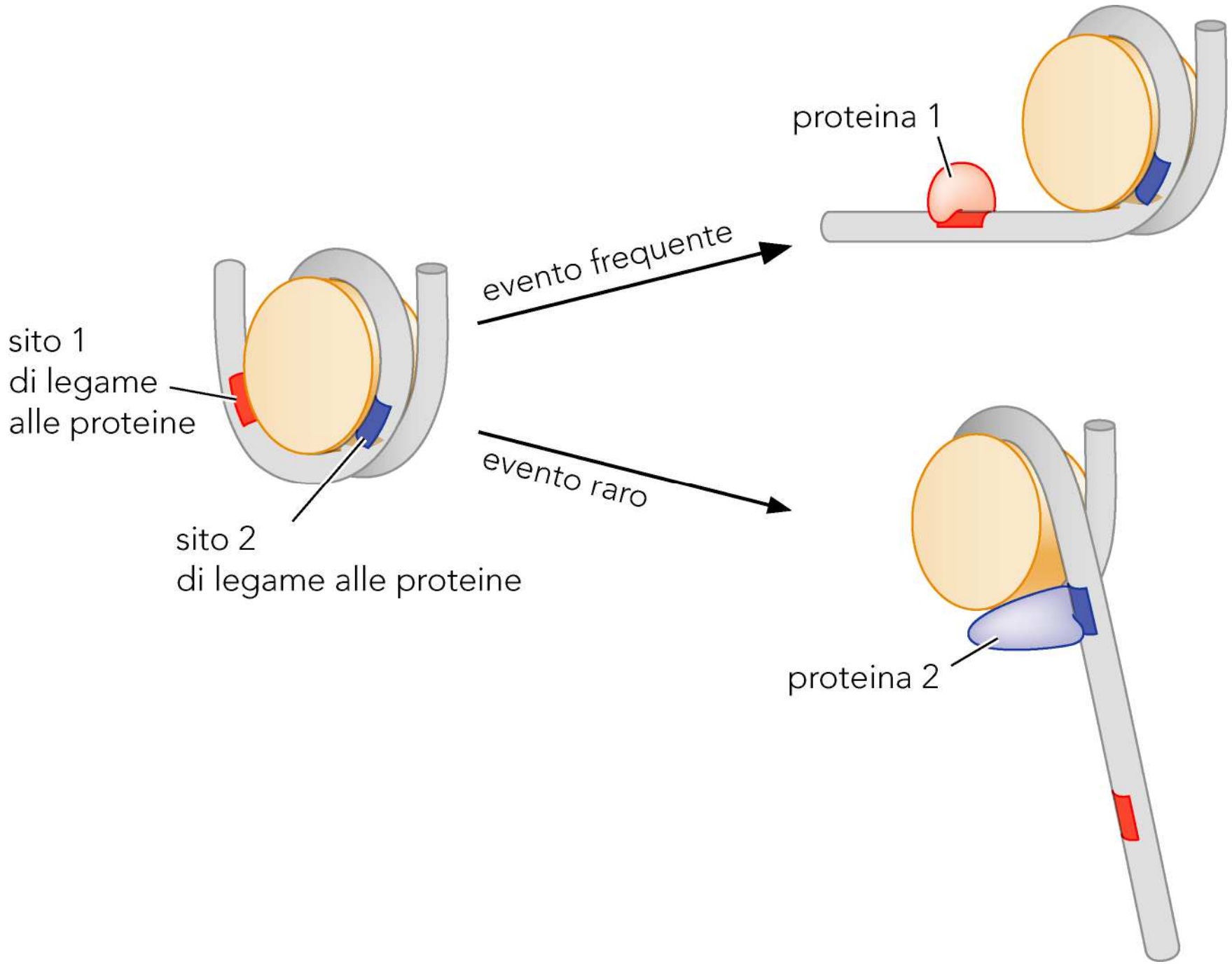
J Mol Biol 2003 Jan 31;325(5):873-87

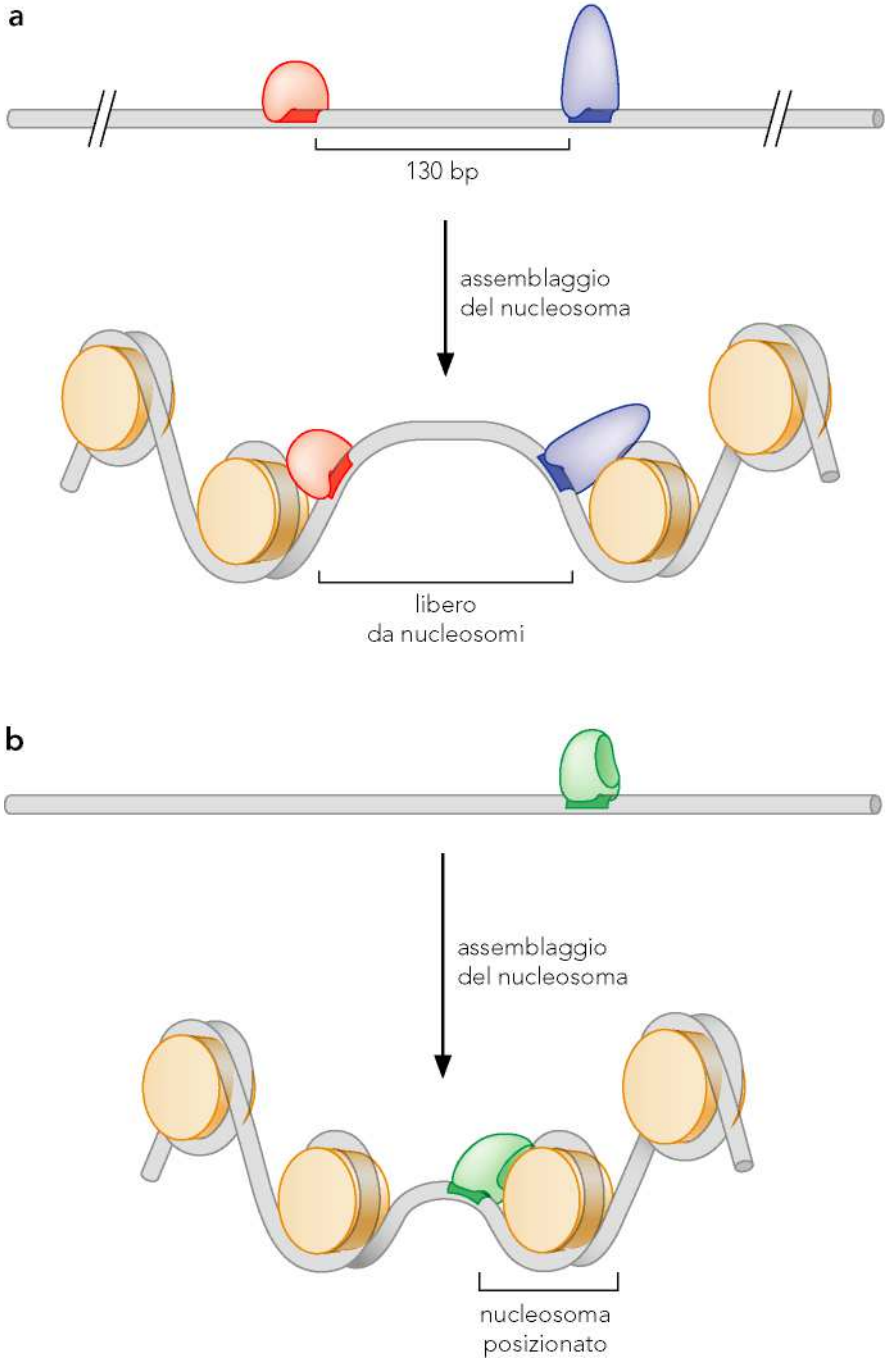
Related Articles, Links

ELSEVIER SCIENCE
FULL-TEXT ARTICLE

Nucleosome positioning signals in the DNA sequence of the human and mouse H19 imprinting control regions.

Davey C, Fraser R, Smolle M, Simmen MW, Allan J.


proteina 1
evento frequente
sito 1
di legame
alle proteine
evento raro
sito 2
di legame alle proteine
proteina 2


a
130 bp
assemblaggio
del nucleosoma
libero
da nucleosomi
b
assemblaggio
del nucleosoma
nucleosoma
posizionato

Tridimentional chromatin architecture

Tridimentional folding impone imposes strong costraints to nucleosome positioning…

…and viceversa

Experiments with circular minichromosomes in yeast

1: Simpson RT, Thoma F, Brubaker JM. — Related Articles

Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure.
Cell. 1985 Oct;42(3):799-808.
PMID: 2996776 [PubMed - indexed for MEDLINE]

2: Thoma F, Simpson RT. — Related Articles

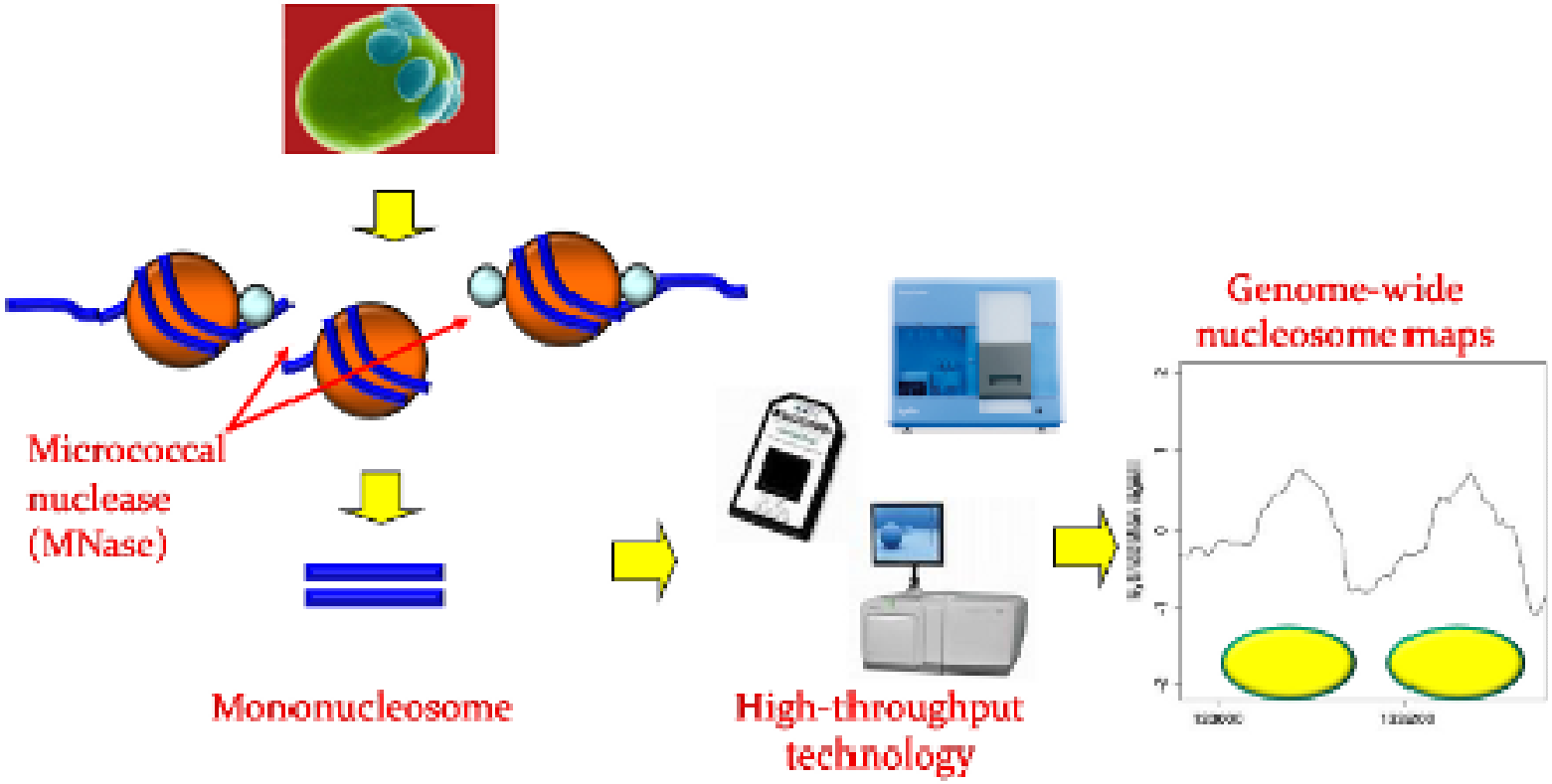
Local protein-DNA interactions may determine nucleosome positions on yeast plasmids.
Nature. 1985 May 16-22;315(6016):250-2.
PMID: 3889654 [PubMed - indexed for MEDLINE]

FEBS Lett 2002 Jul 17;523(1-3):7-11
ELSEVIER SCIENCE
FULL-TEXT ARTICLE

What positions nucleosomes?--A model.

Kiyama R, Trifonov EN.

From 2004 several nucleosome genomic maps have been generated in several cellular systems



ChIP on chip
ChIP-Seq

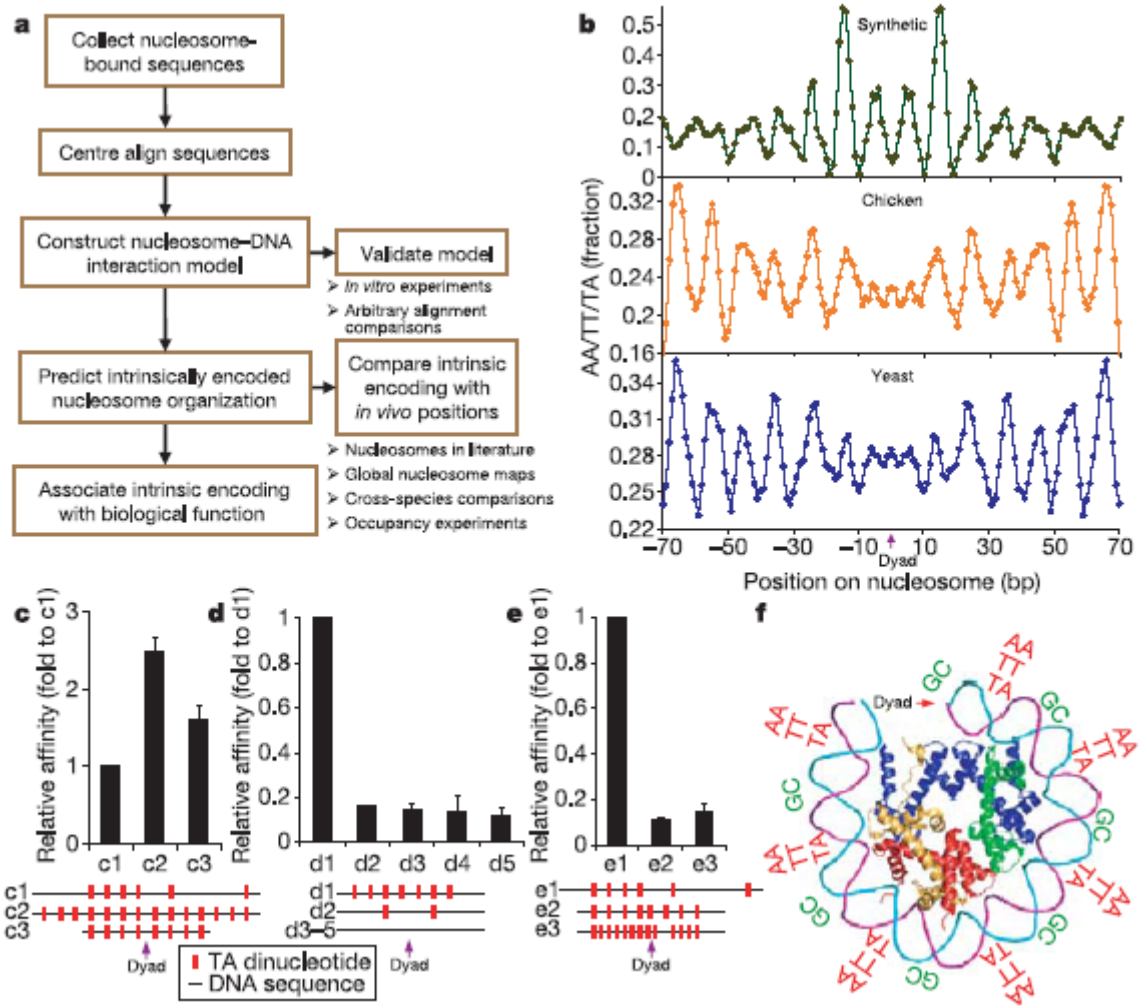
nature Vol 442|17 August 2006|doi:10.1038/nature04979

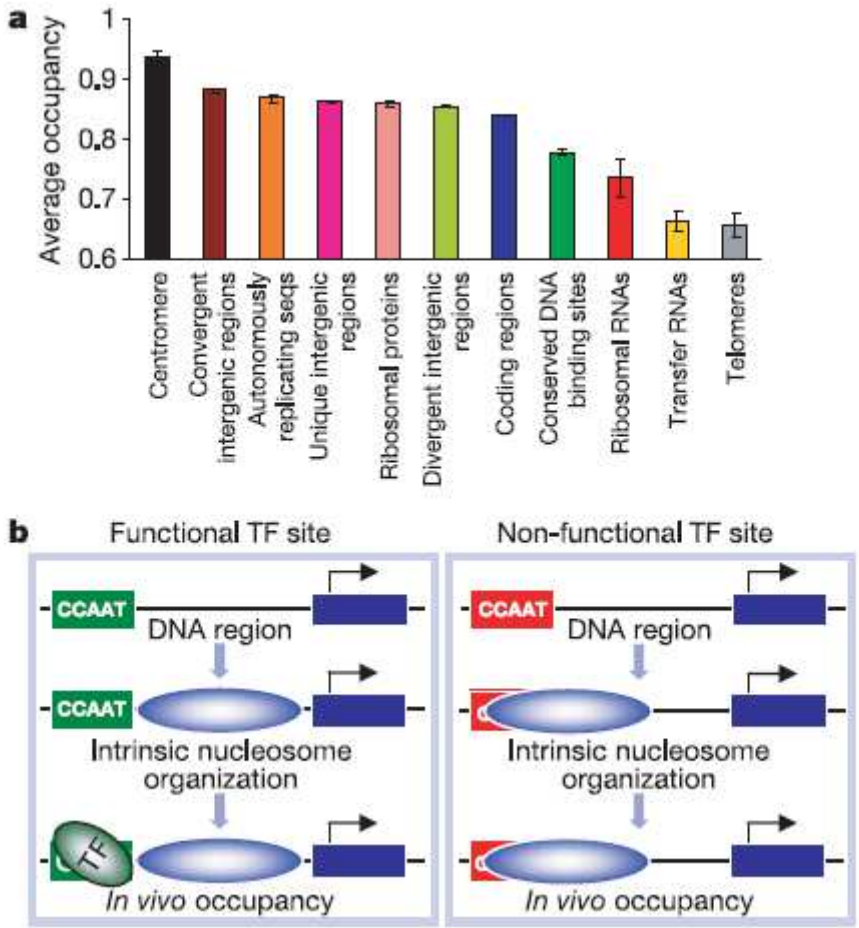
ARTICLES

A genomic code for nucleosome positioning

Eran Segal[1], Yvonne Fondufe-Mittendorf[2], Lingyi Chen[2], AnnChristine Thåström[2], Yair Field[1], Irene K. Moore[2], Ji-Ping Z. Wang[3] & Jonathan Widom[2]

Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. Nucleosomes have higher affinity for particular DNA sequences, reflecting the ability of the sequence to bend sharply, as required by the nucleosome structure. However, it is not known whether these sequence preferences have a significant influence on nucleosome position *in vivo*, and thus regulate the access of other proteins to DNA. Here we isolated nucleosome-bound sequences at high resolution from yeast and used these sequences in a new computational approach to construct and validate experimentally a nucleosome–DNA interaction model, and to predict the genome-wide organization of nucleosomes. Our results demonstrate that genomes encode an intrinsic nucleosome organization and that this intrinsic organization can explain ~50% of the *in vivo* nucleosome positions. This nucleosome positioning code may facilitate specific chromosome functions including transcription factor binding, transcription initiation, and even remodelling of the nucleosomes themselves.


a
Collect nucleosome-bound sequences
Centre align sequences
Construct nucleosome–DNA interaction model
Validate model
In vitro experiments
Arbitrary alignment comparisons
Predict intrinsically encoded nucleosome organization
Compare intrinsic encoding with in vivo positions
Nucleosomes in literature
Global nucleosome maps
Cross-species comparisons
Occupancy experiments
Associate intrinsic encoding with biological function
b
Synthetic
Chicken
Yeast
AA/TT/TA (fraction)
Position on nucleosome (bp)
Dyad
c
Relative affinity (fold to c1)
c1 c2 c3
Dyad
d
Relative affinity (fold to d1)
d1 d2 d3 d4 d5
d3–5
Dyad
e
Relative affinity (fold to e1)
e1 e2 e3
Dyad
TA dinucleotide
DNA sequence
f
Dyad


a
Average occupancy
1
0.9
0.8
0.7
0.6
Centromere
Convergent intergenic regions
Autonomously replicating seqs
Unique intergenic regions
Ribosomal proteins
Divergent intergenic regions
Coding regions
Conserved DNA binding sites
Ribosomal RNAs
Transfer RNAs
Telomeres
b
Functional TF site
Non-functional TF site
CCAAT
DNA region
Intrinsic nucleosome organization
TF
In vivo occupancy

OPEN ACCESS Freely available online PLoS COMPUTATIONAL BIOLOGY

Distinct Modes of Regulation by Chromatin Encoded through Nucleosome Positioning Signals

Yair Field[1☯], Noam Kaplan[1☯], Yvonne Fondufe-Mittendorf[2☯], Irene K. Moore[2], Eilon Sharon[1], Yaniv Lubling[1], Jonathan Widom[2*], Eran Segal[1,3*]

1 Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel, 2 Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois, United States of America, 3 Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Abstract

The detailed positions of nucleosomes profoundly impact gene regulation and are partly encoded by the genomic DNA sequence. However, less is known about the functional consequences of this encoding. Here, we address this question using a genome-wide map of ~380,000 yeast nucleosomes that we sequenced in their entirety. Utilizing the high resolution of our map, we refine our understanding of how nucleosome organizations are encoded by the DNA sequence and demonstrate that the genomic sequence is highly predictive of the in vivo nucleosome organization, even across new nucleosome-bound sequences that we isolated from fly and human. We find that Poly(dA:dT) tracts are an important component of these nucleosome positioning signals and that their nucleosome-disfavoring action results in large nucleosome depletion over them and over their flanking regions and enhances the accessibility of transcription factors to their cognate sites. Our results suggest that the yeast genome may utilize these nucleosome positioning signals to regulate gene expression with different transcriptional noise and activation kinetics and DNA replication with different origin efficiency. These distinct functions may be achieved by encoding both relatively closed (nucleosome-covered) chromatin organizations over some factor binding sites, where factors must compete with nucleosomes for DNA access, and relatively open (nucleosome-depleted) organizations over other factor sites, where factors bind without competition.

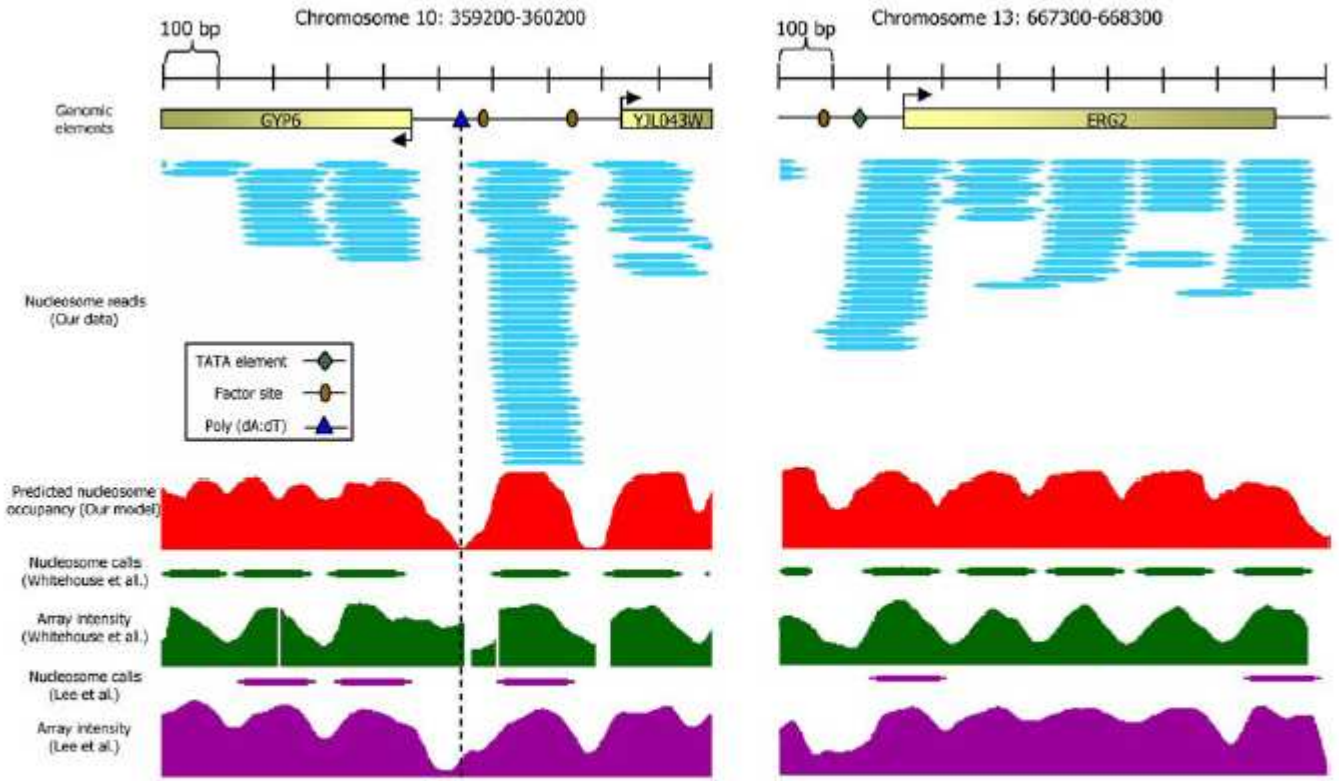
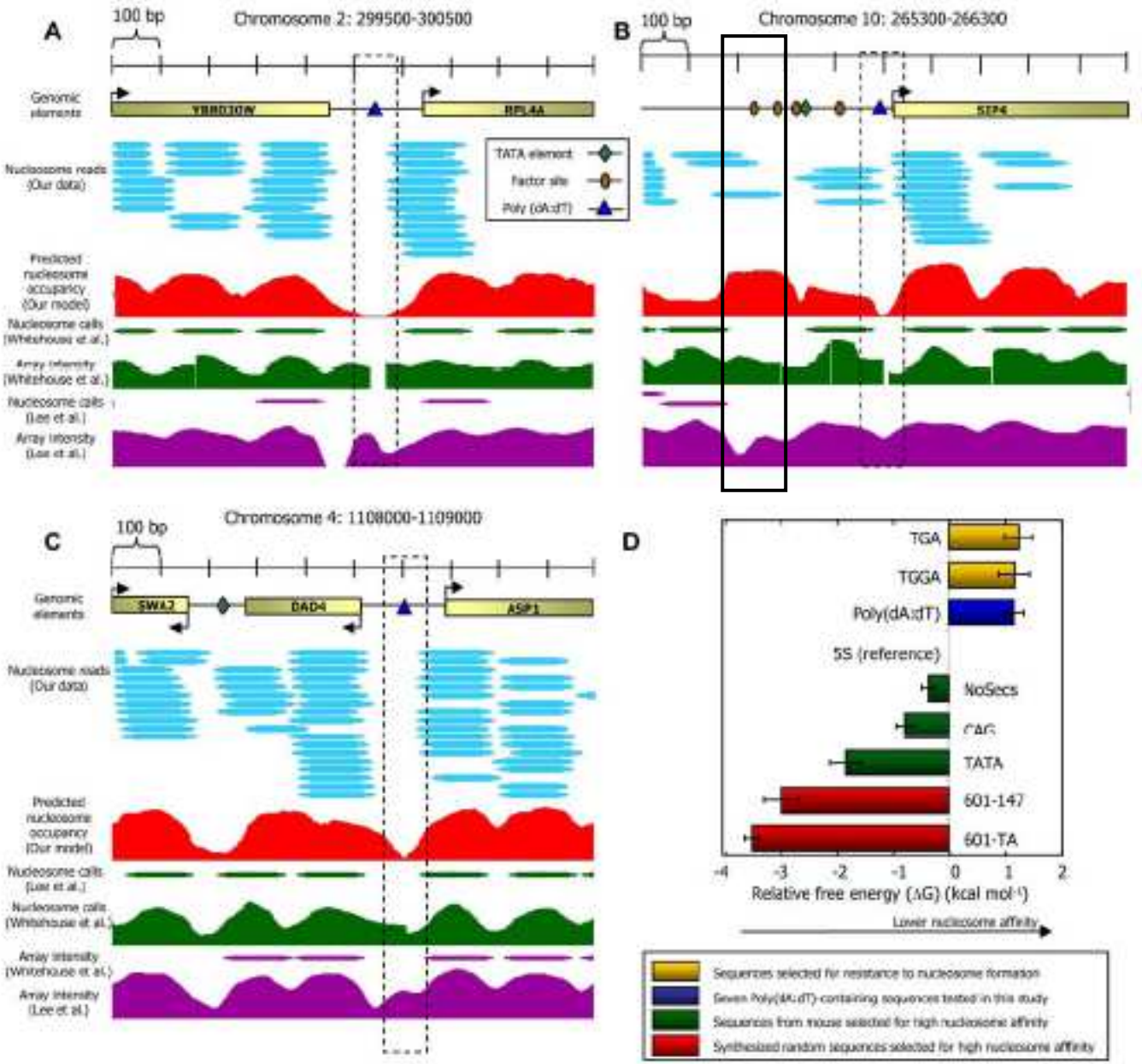
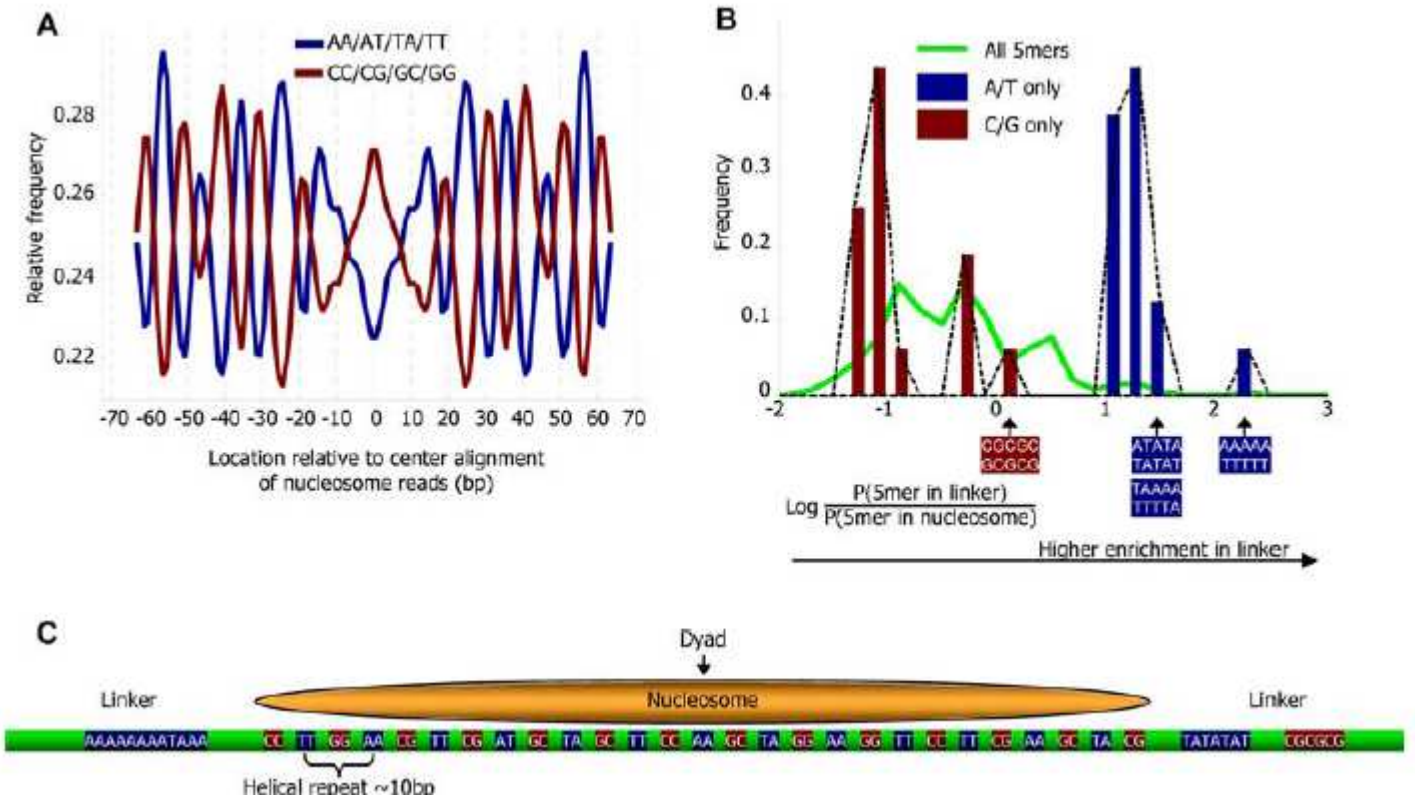
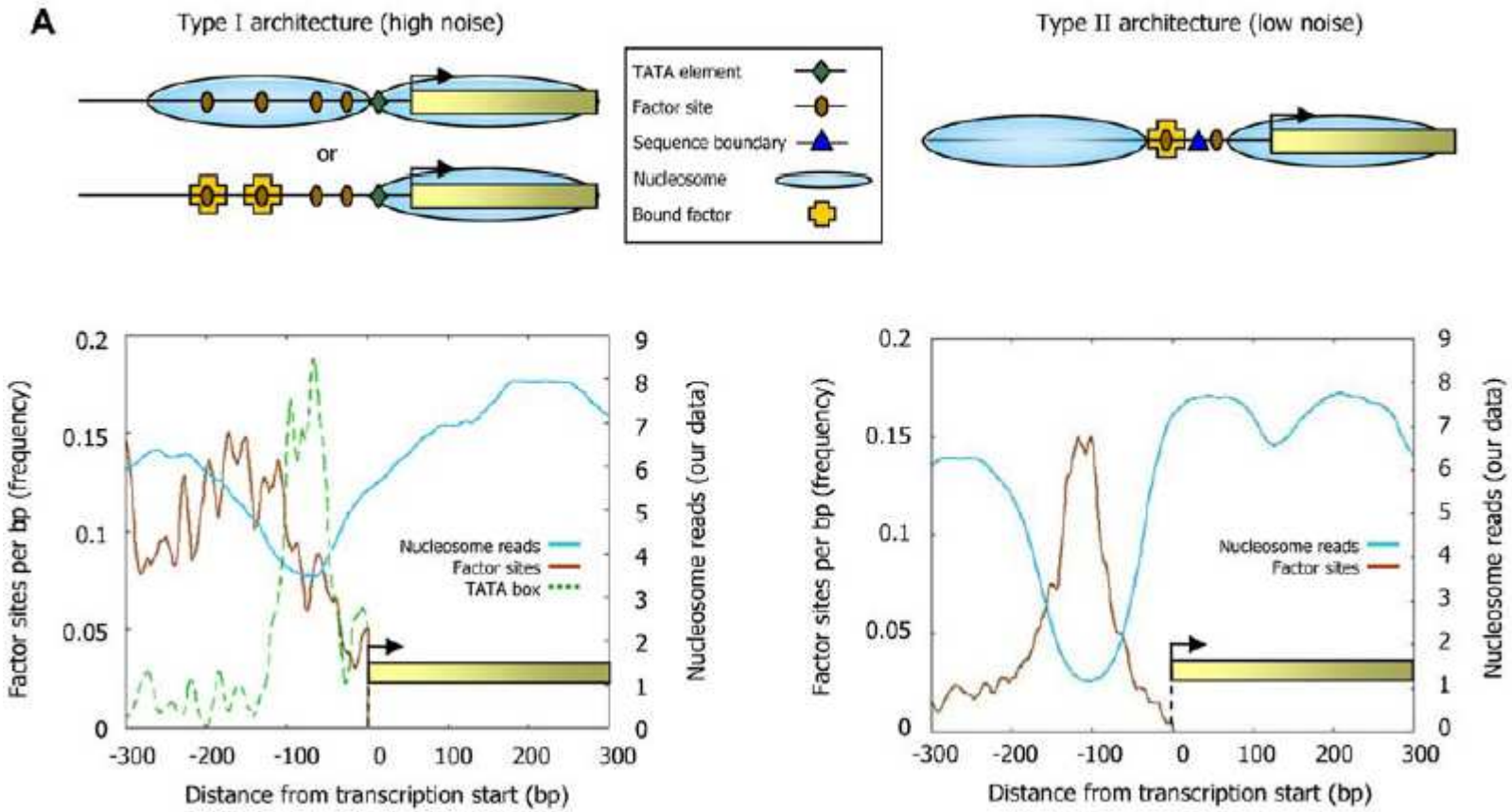
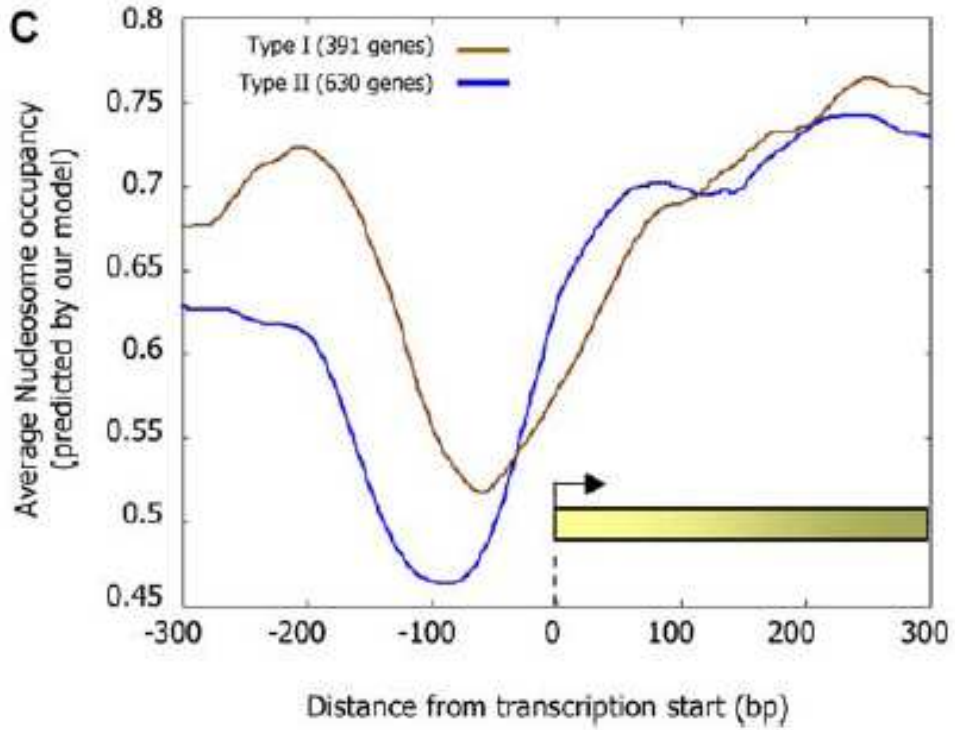


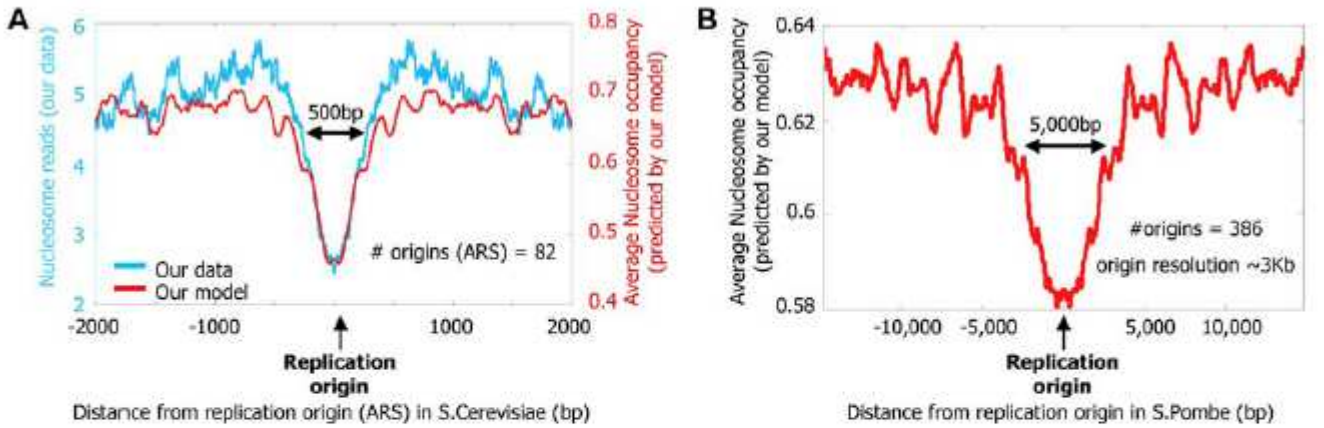
Figure 1. Nucleosome organization at two genomic regions. Shown are the raw data measured in this study at two 1000bp-long genomic regions. Every cyan oval represents the genomic location of one nucleosome that we sequenced in its entirety. Also shown is the average nucleosome occupancy per basepair predicted by the sequence-based nucleosome model that we developed here (red), the raw hybridization signal of two microarray-based nucleosome maps [5,10] (green and purple traces), and the locations of nucleosomes that were computationally inferred from these hybridization signals [5,10] (green and purple ovals). Note that although the nucleosome calls from the microarray maps are close to nucleosome locations from our map, the microarray map does not reveal the underlying variability in the detailed nucleosome read locations that we observe in our data. Annotated genes [63], transcription factor binding sites [47], TATA sequences [53], and Poly(dA:dT) elements in the region are

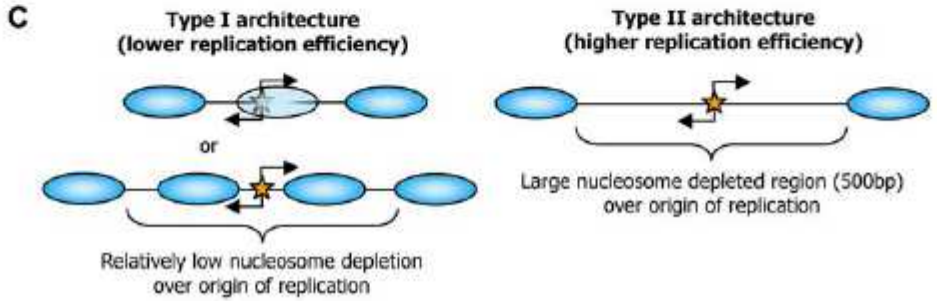

A
100 bp
Chromosome 2: 299500-300500
Genomic elements
YBR013W
RPL4A
Nucleosome reads (Our data)
TATA element
Factor site
Poly (dA:dT)
Predicted nucleosome occupancy (Our model)
Nucleosome calls (Whitehouse et al.)
Array intensity (Whitehouse et al.)
Nucleosome calls (Lee et al.)
Array intensity (Lee et al.)
B
100 bp
Chromosome 10: 265300-266300
STP4
C
100 bp
Chromosome 4: 1108000-1109000
Genomic elements
SWA2
DAD4
ASP1
Nucleosome reads (Our data)
Predicted nucleosome occupancy (Our model)
Nucleosome calls (Lee et al.)
Nucleosome calls (Whitehouse et al.)
Array intensity (Whitehouse et al.)
Array intensity (Lee et al.)
D
TGA
TGGA
Poly(dA:dT)
5S (reference)
NoSecs
TATA
601-147
601-TA
-4
-3
-2
-1
0
1
2
Relative free energy (ΔG) (kcal mol-1)
Lower nucleosome affinity
Sequences selected for resistance to nucleosome formation
Seven Poly(dA:dT)-containing sequences tested in this study
Sequences from mouse selected for high nucleosome affinity
Synthesized random sequences selected for high nucleosome affinity


A
AA/AT/TA/TT
CC/CG/GC/GG
Relative frequency
0.28
0.26
0.24
0.22
-70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
Location relative to center alignment
of nucleosome reads (bp)
B
All 5mers
A/T only
C/G only
Frequency
0.4
0.3
0.2
0.1
0
-2 -1 0 1 2 3
CGCGC
GCGCG
ATATA
TATAT
TAAAA
TTTTA
AAAAA
TTTTT
Log P(5mer in linker) / P(5mer in nucleosome)
Higher enrichment in linker
C
Dyad
Linker
Nucleosome
Linker
AAAAAAAATAAA
TATATAT
CGCGCG
Helical repeat ~10bp


A
Type I architecture (high noise)
or
TATA element
Factor site
Sequence boundary
Nucleosome
Bound factor
Type II architecture (low noise)
Factor sites per bp (frequency)
Nucleosome reads (our data)
Nucleosome reads
Factor sites
TATA box
Distance from transcription start (bp)
0.2
0.15
0.1
0.05
0
9
8
7
6
5
4
3
2
1
0
-300
-200
-100
0
100
200
300


C
Type I (391 genes)
Type II (630 genes)
Average Nucleosome occupancy
(predicted by our model)
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
-300
-200
-100
0
100
200
300
Distance from transcription start (bp)


A
Nucleosome reads (our data)
Average Nucleosome occupancy (predicted by our model)
500bp
origins (ARS) = 82
Our data
Our model
-2000
-1000
1000
2000
Replication origin
Distance from replication origin (ARS) in S.Cerevisiae (bp)
B
Average Nucleosome occupancy (predicted by our model)
5,000bp
#origins = 386
origin resolution ~3Kb
-10,000
-5,000
5,000
10,000
Replication origin
Distance from replication origin in S.Pombe (bp)


C
Type I architecture
(lower replication efficiency)
or
Relatively low nucleosome depletion
over origin of replication
Type II architecture
(higher replication efficiency)
Large nucleosome depleted region (500bp)
over origin of replication

Nucleosome mapping in the *Saccharomyces cerevisiae* genome shows that gene expression variability and chromatin remodelingcorrelate with nucleosome occupancy at promoters.

Nucleosome content of the genome

	Coverage (bp)	Fraction of total genome (total intergenic / total transcribed)	Number of nucleosomes
Array probe coverage	12,068,004	1 (1 / 1)	N/A
Well-positioned nucleosomes	4,970,908	0.41 (0.36 / 0.42)	40,095
Delocalized (fuzzy) nucleosomes	4,801,292	0.4 (0.17 / 0.45)	30,776
Total nucleosomal DNA	9,772,200	0.81 (0.53 / 0.87)	70,871
Non-nucleosomal ('linker') DNA	2,295,804	0.19 (0.47 / 0.13)	32.4 bp average length

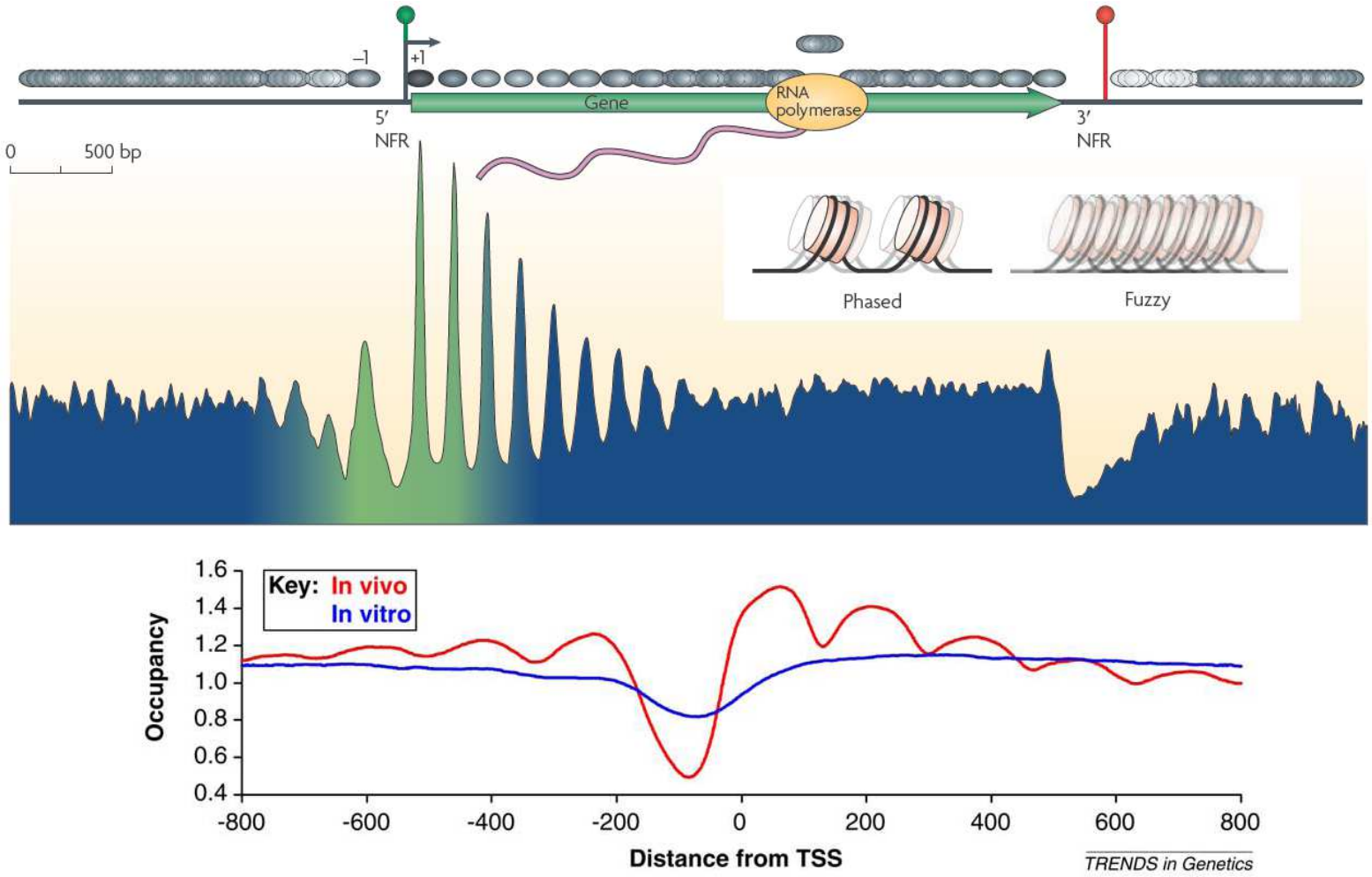
nature genetics

A high-resolution atlas of nucleosome occupancy in yeast

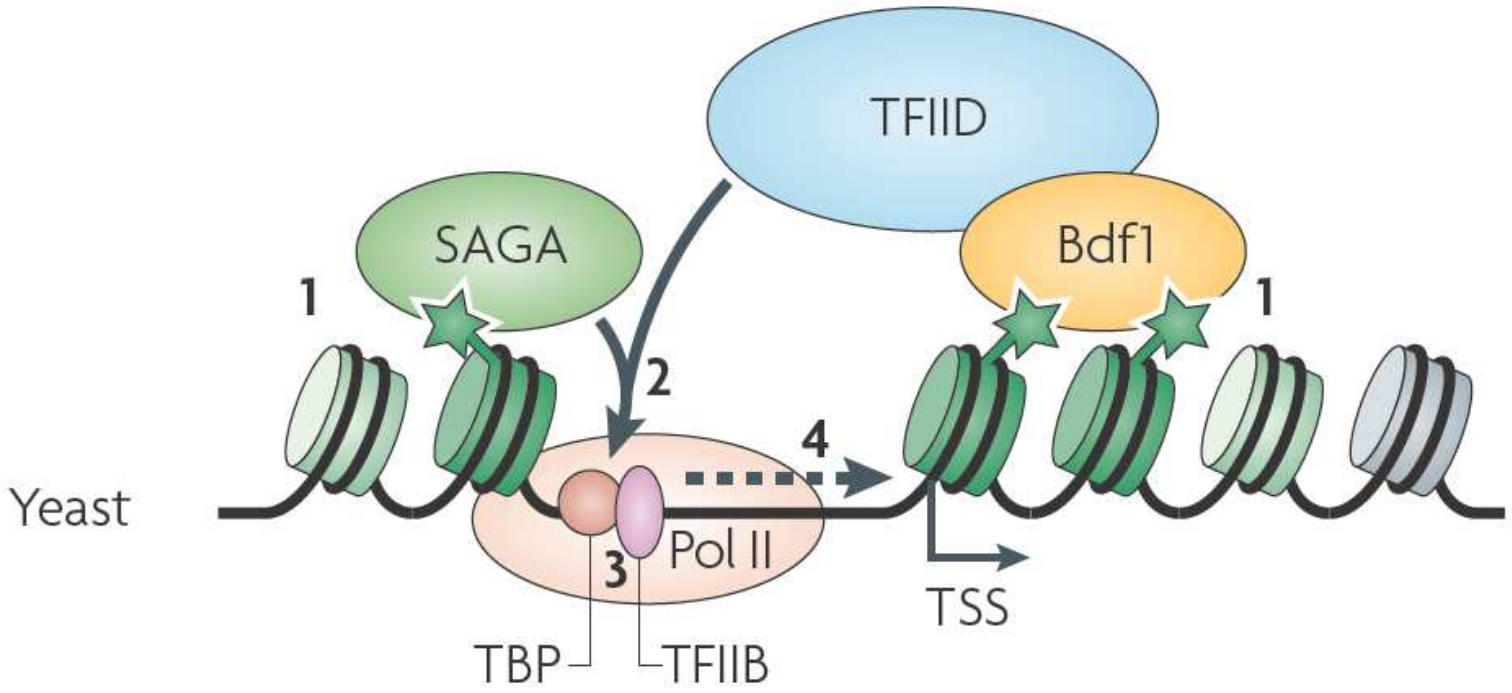
William Lee[1,2], Desiree Tillo[3], Nicolas Bray[3], Randall H Morse[4], Ronald W Davis[1,2], Timothy R Hughes[3,5,6] & Corey Nislow[3,5,6]

Tirosh et al., 2007

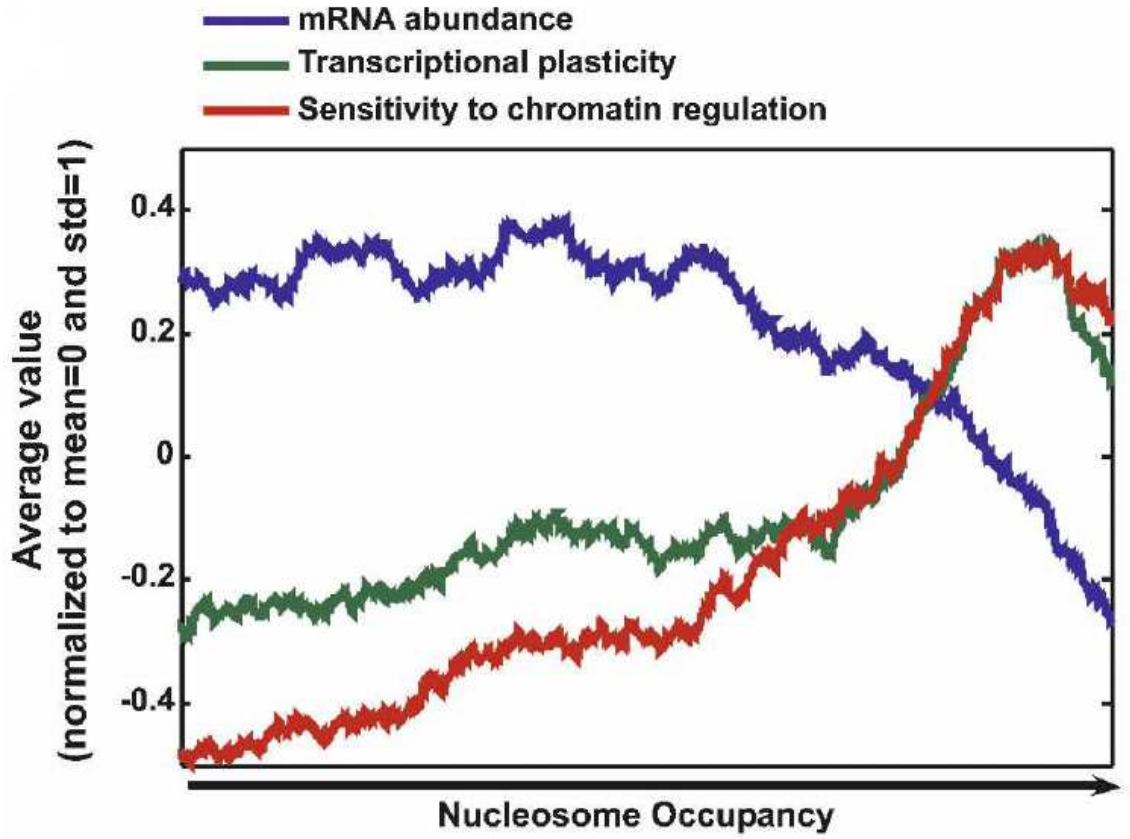
A common nucleosome organization is evident in active genes.



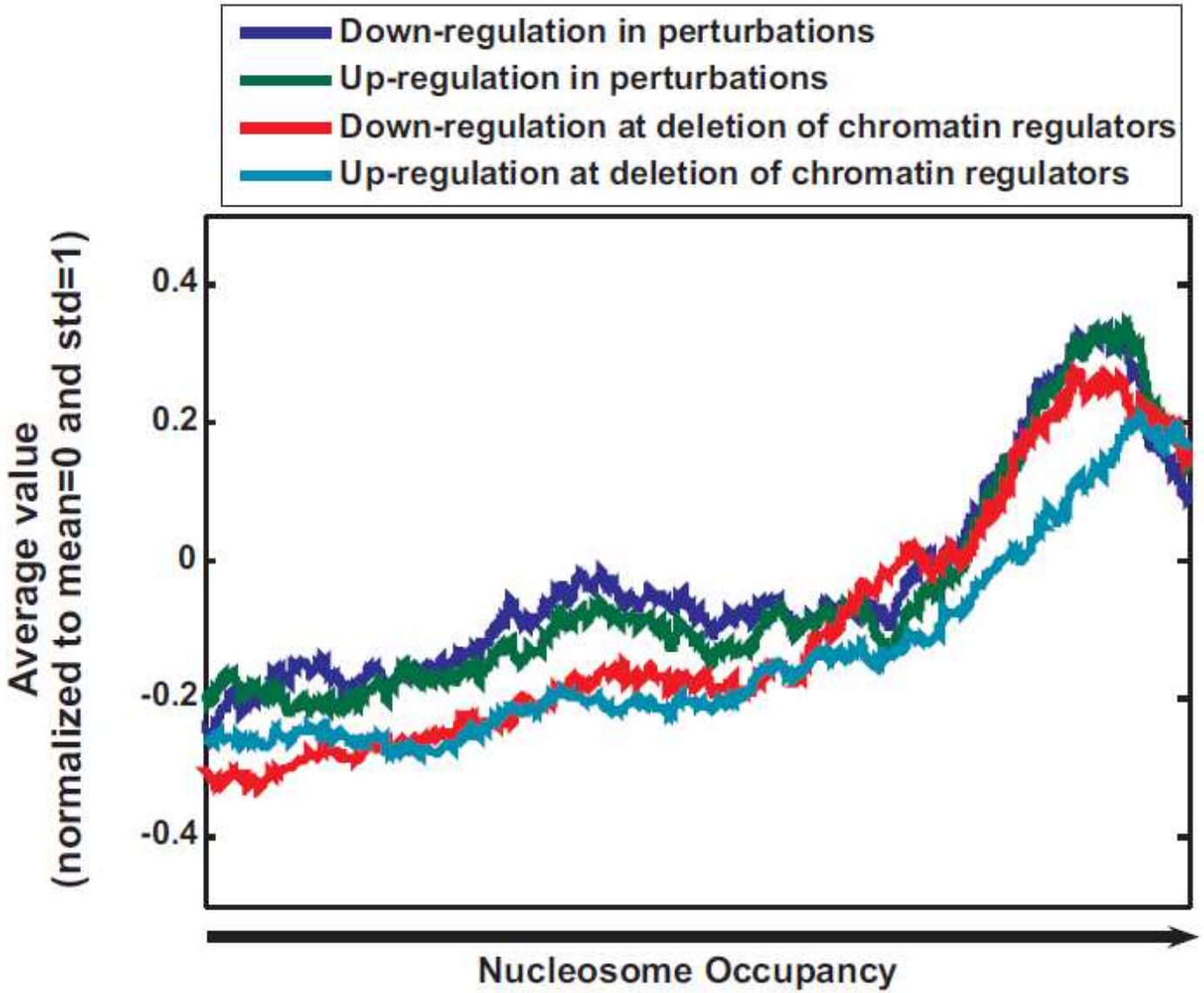
Transcription start site (TSS) is generally intrinsically devoid of nucleosomes.



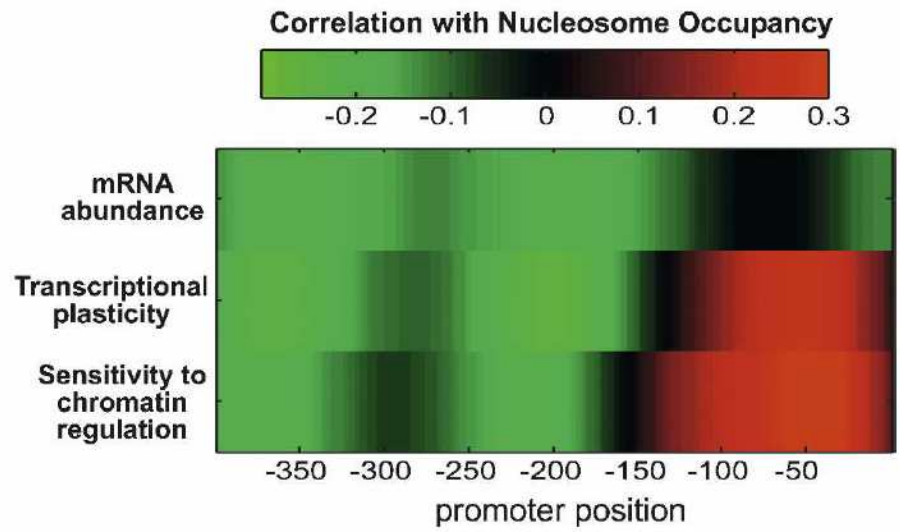
High nucleosome occupancy is associated to high transcriptional plasticity and sensitivity to chromatin remodeling.

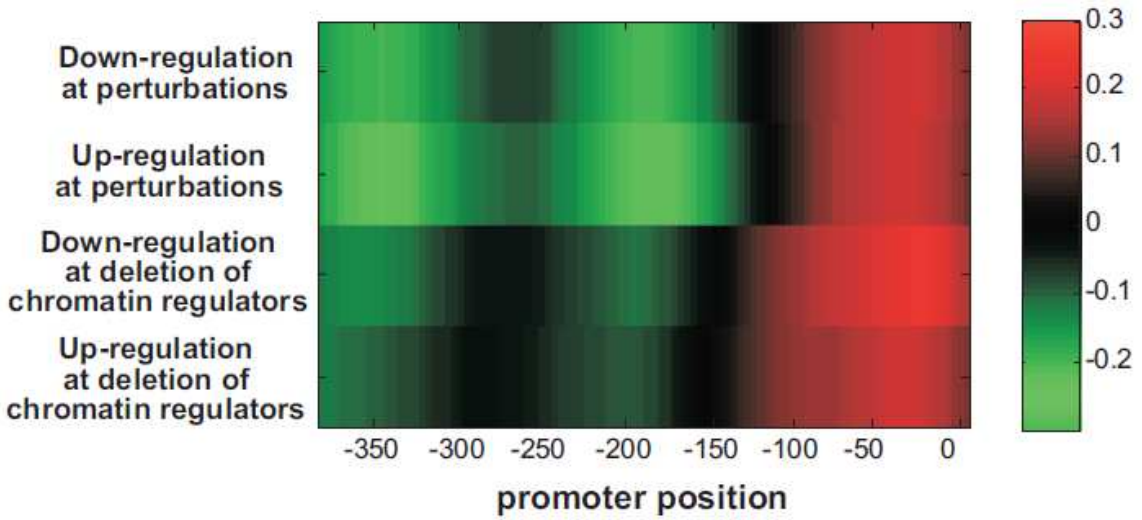


Tirosh and Barkai 2008

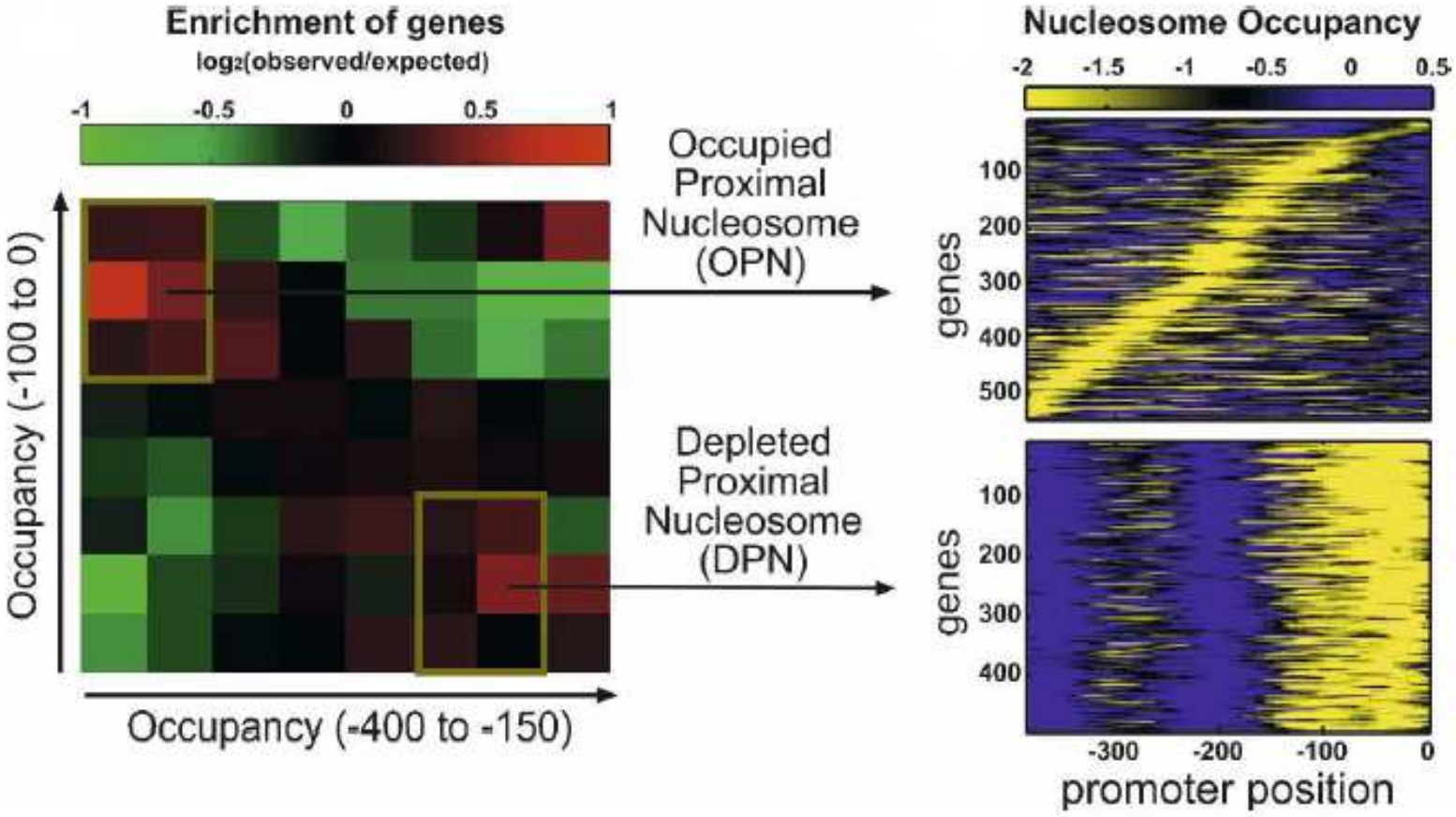

Down-regulation in perturbations
Up-regulation in perturbations
Down-regulation at deletion of chromatin regulators
Up-regulation at deletion of chromatin regulators
Average value
(normalized to mean=0 and std=1)
0.4
0.2
0
-0.2
-0.4
Nucleosome Occupancy

Regional positioning is important for transcription

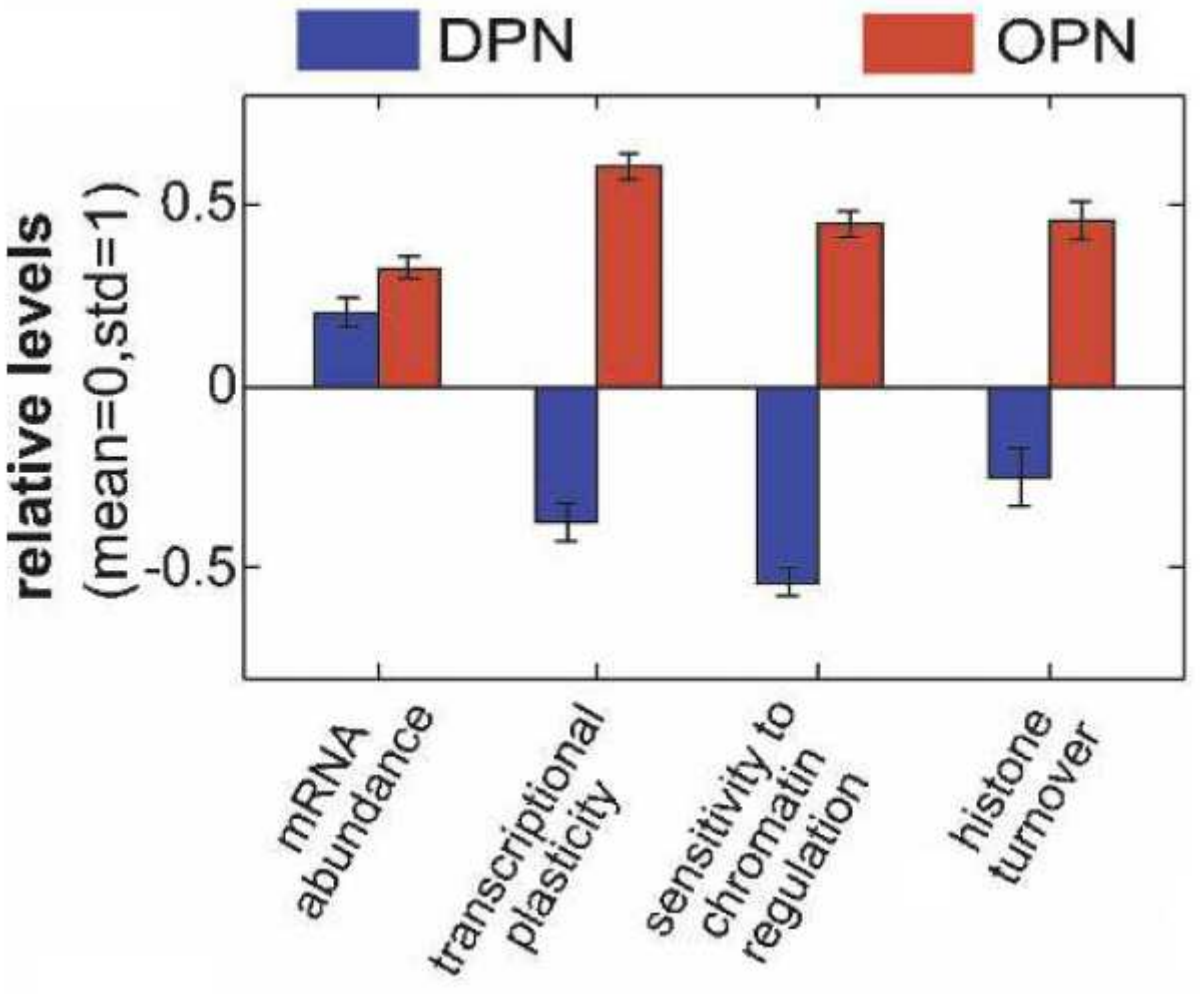




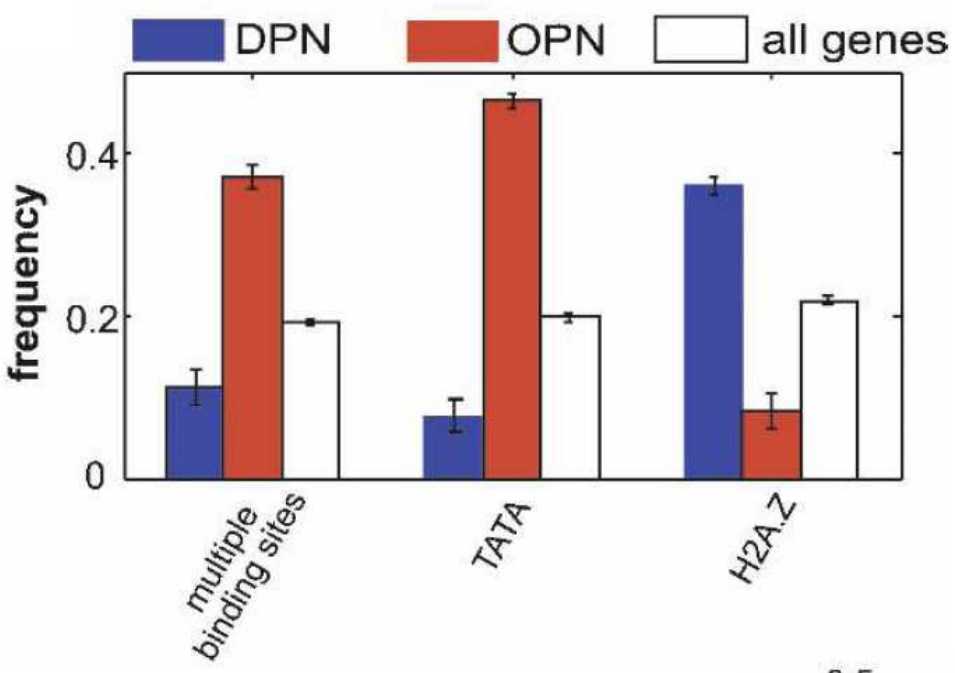
Two different patterns of nucleosome positioning in *Saccharomyces cerevisiae*.

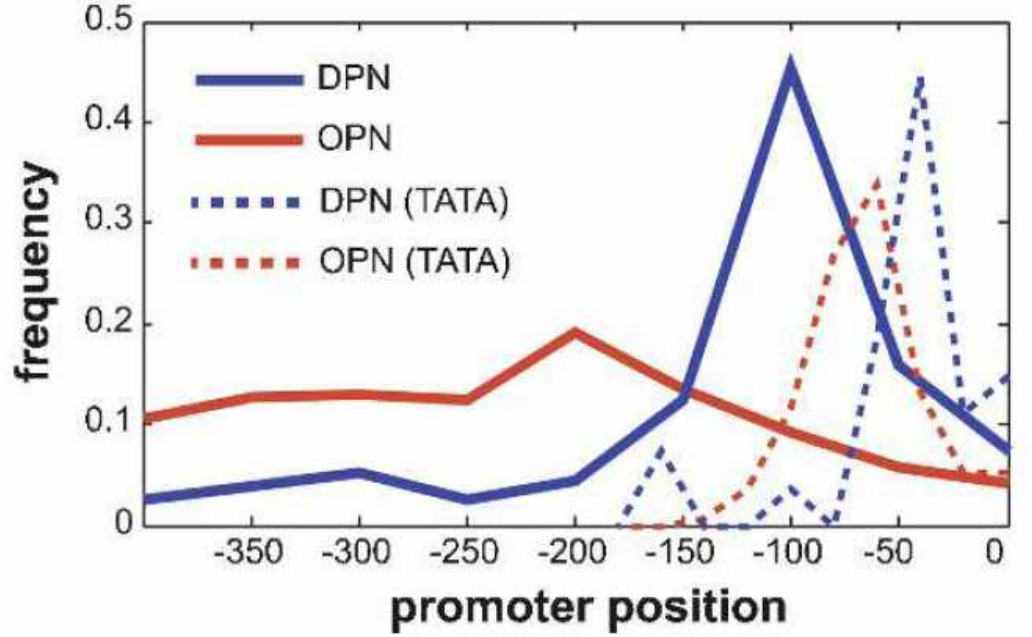


Tirosh and Barkai 2008

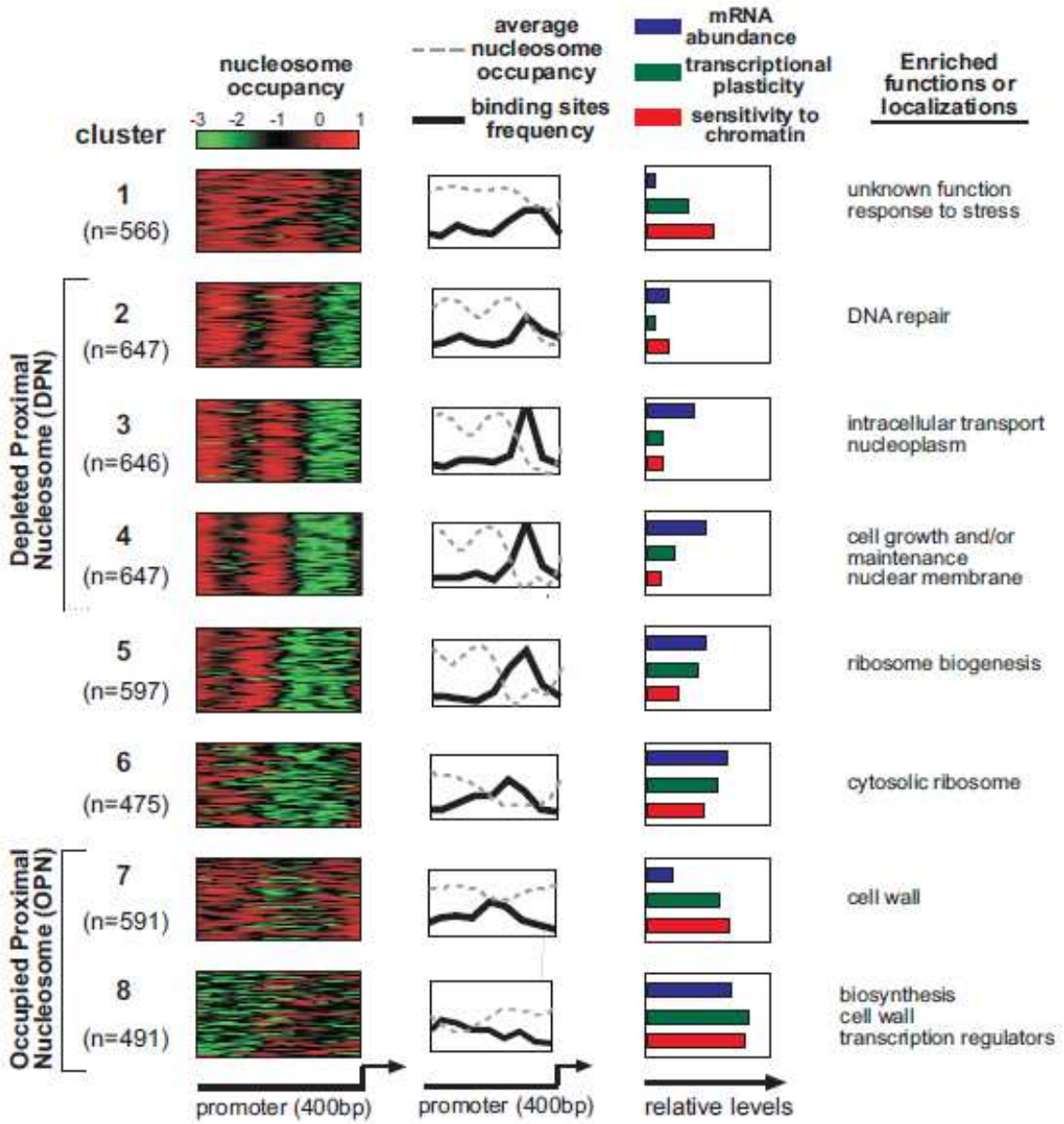


Tirosh and Barkai 2008

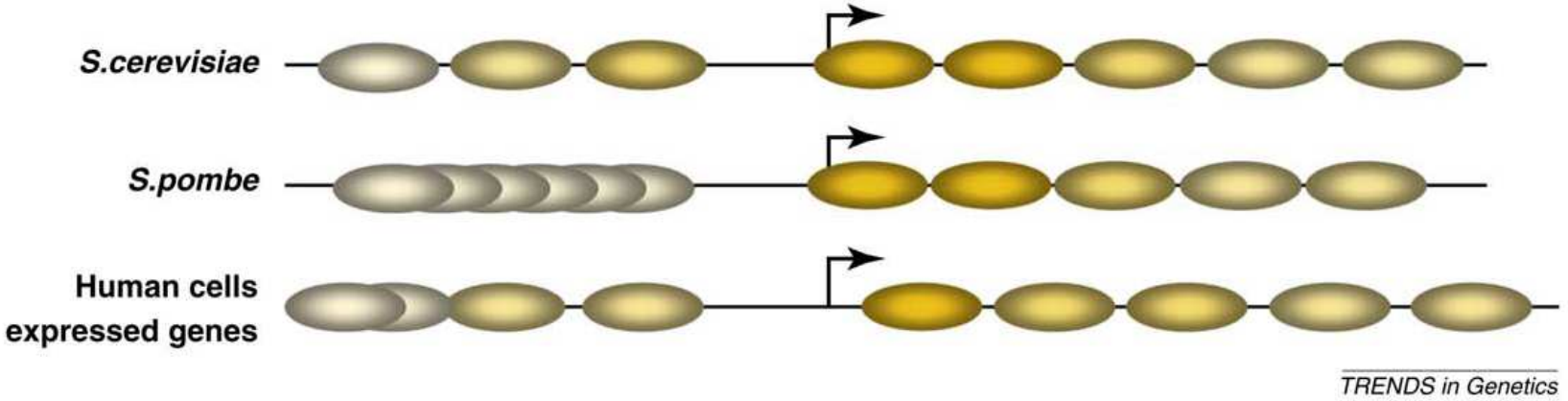




Tirosh and Barkai 2008


nucleosome occupancy
-3 -2 -1 0 1
average nucleosome occupancy
binding sites frequency
mRNA abundance
transcriptional plasticity
sensitivity to chromatin
Enriched functions or localizations
cluster
1 (n=566)
unknown function
response to stress
Depleted Proximal Nucleosome (DPN)
2 (n=647)
DNA repair
3 (n=646)
intracellular transport
nucleoplasm
4 (n=647)
cell growth and/or maintenance
nuclear membrane
5 (n=597)
ribosome biogenesis
6 (n=475)
cytosolic ribosome
Occupied Proximal Nucleosome (OPN)
7 (n=591)
cell wall
8 (n=491)
biosynthesis
cell wall
transcription regulators
promoter (400bp)
promoter (400bp)
relative levels

Nucleosome organizations at promoters is evolutionary conserved



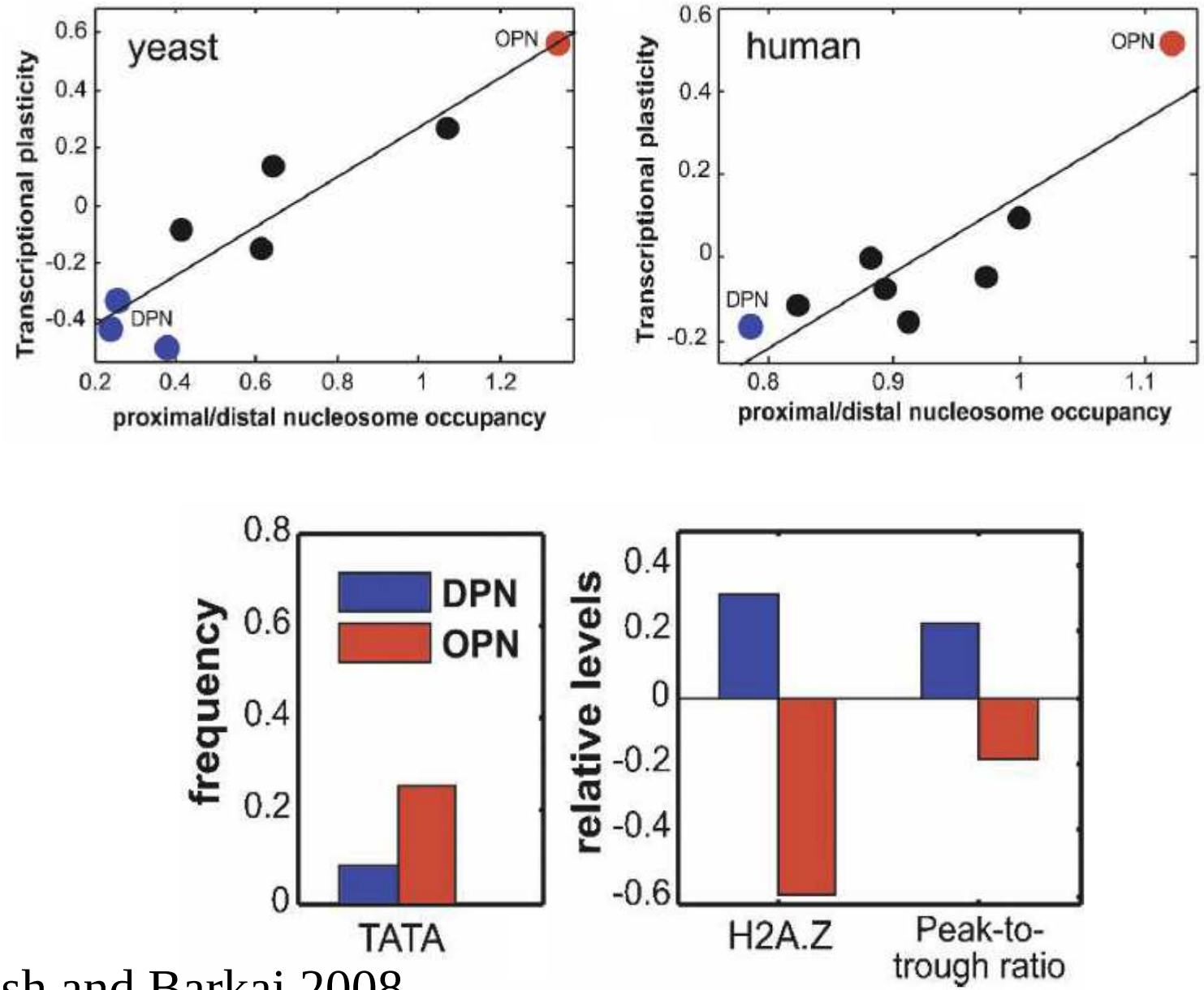
Review

Trends in Genetics Vol.26 No.11

Gene regulation by nucleosome positioning

Lu Bai[1] and Alexandre V. Morozov[2]

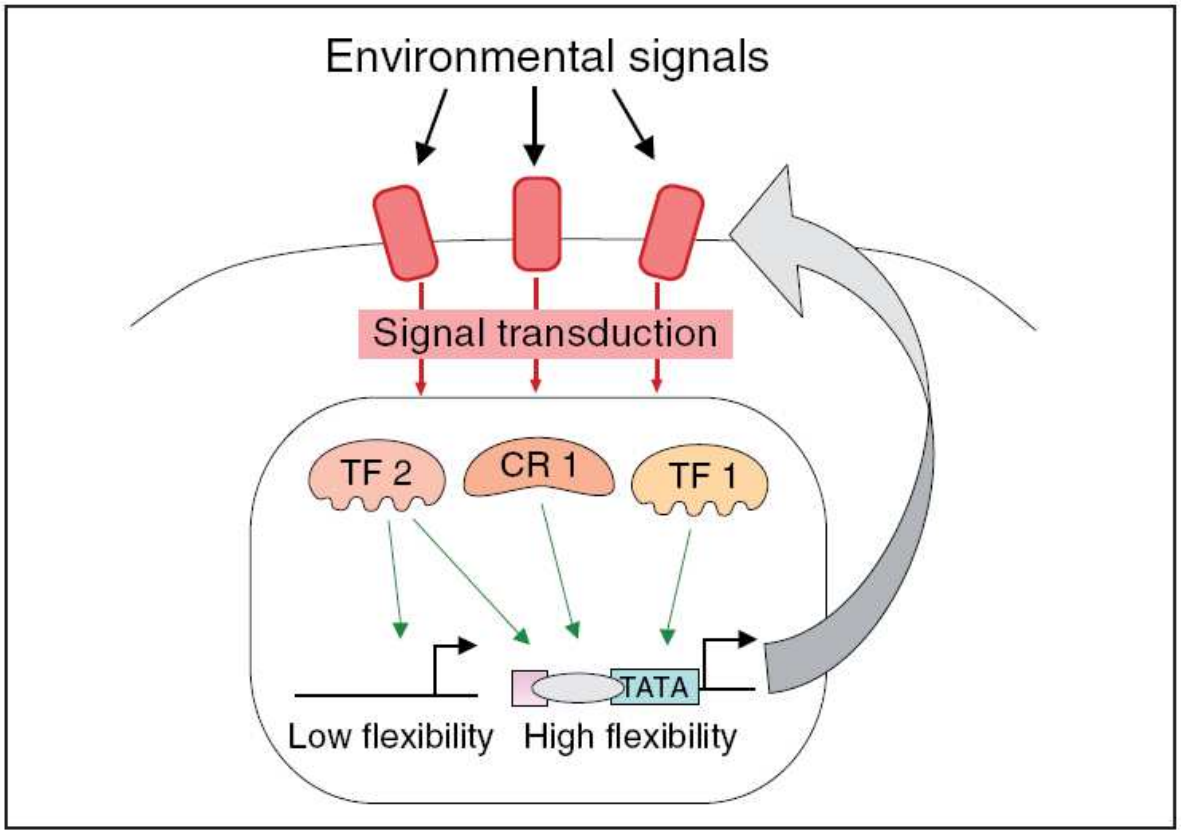
[1] The Rockefeller University, New York, NY, 10065, USA
[2] Department of Physics and Astronomy and BioMaPS Institute for Quantitative Biology, Rutgers University, Piscataway, NJ 08854, USA



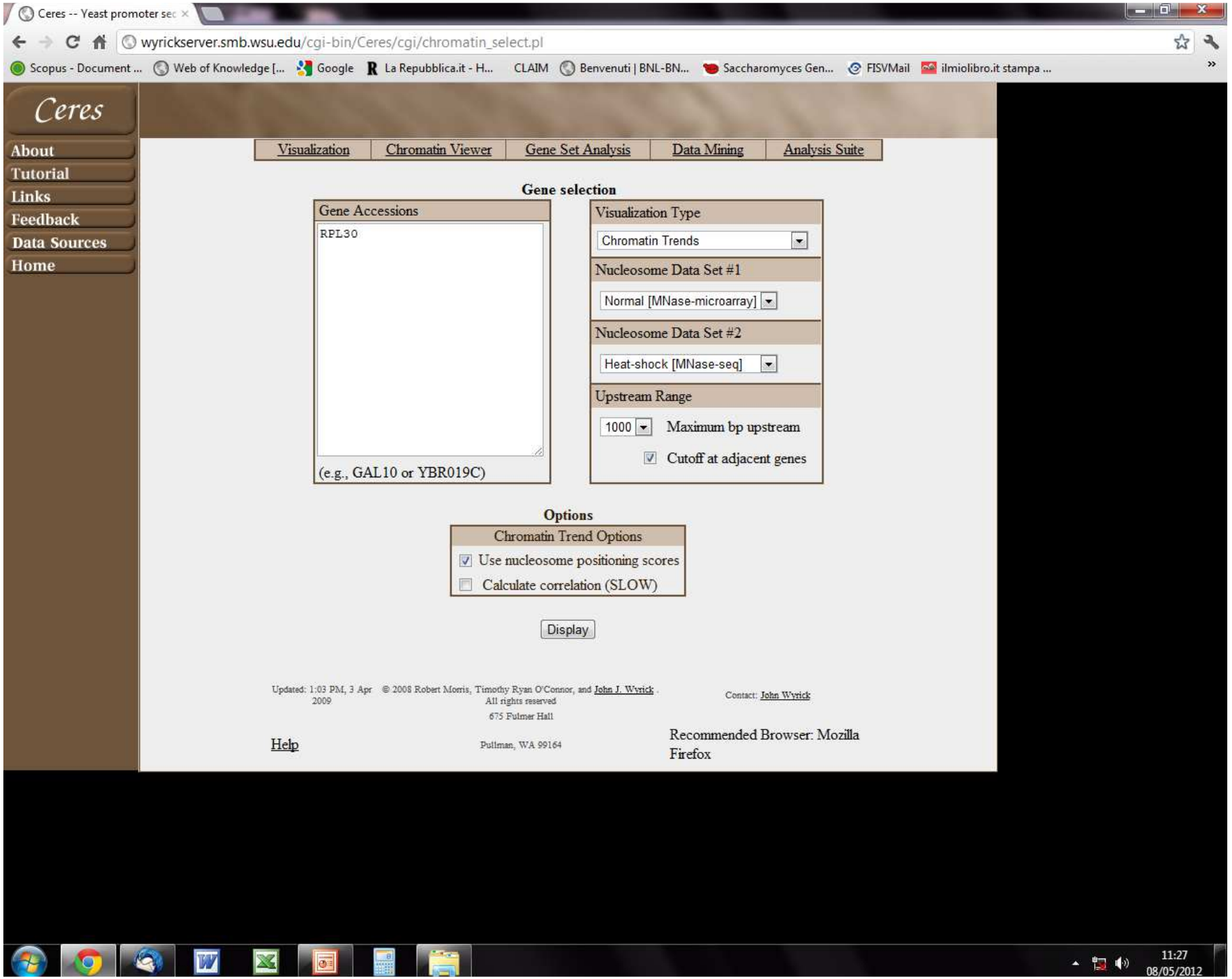
Tirosh and Barkai 2008

TATA box containing promoters, nucleosome occupancy proximal to TSS and multiple TF binding sitesare extensively regulated and exhibit high plasticity.

They have high capacity of signal integration. Transcriptional plasticity is often coupled to evolutionary plasticity.



Tirosh et al.2009


Ceres -- Yeast promoter sec
wyrickserver.smb.wsu.edu/cgi-bin/Ceres/cgi/chromatin_select.pl
Scopus - Document ...
Web of Knowledge [...
Google
La Repubblica.it - H...
CLAIM
Benvenuti | BNL-BN...
Saccharomyces Gen...
FISVMail
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Gene Set Analysis
Data Mining
Analysis Suite
Gene selection
Gene Accessions
RPL30
(e.g., GAL10 or YBR019C)
Visualization Type
Chromatin Trends
Nucleosome Data Set #1
Normal [MNase-microarray]
Nucleosome Data Set #2
Heat-shock [MNase-seq]
Upstream Range
1000
Maximum bp upstream
Cutoff at adjacent genes
Options
Chromatin Trend Options
Use nucleosome positioning scores
Calculate correlation (SLOW)
Display
Updated: 1:03 PM, 3 Apr 2009
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Pullman, WA 99164
Contact: John Wyrick
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Recommended Browser: Mozilla Firefox
11:27
08/05/2012

Ceres -- Analysis tools displ

wyrickserver.smb.wsu.edu/cgi-bin/Ceres/cgi/chromatin.pl

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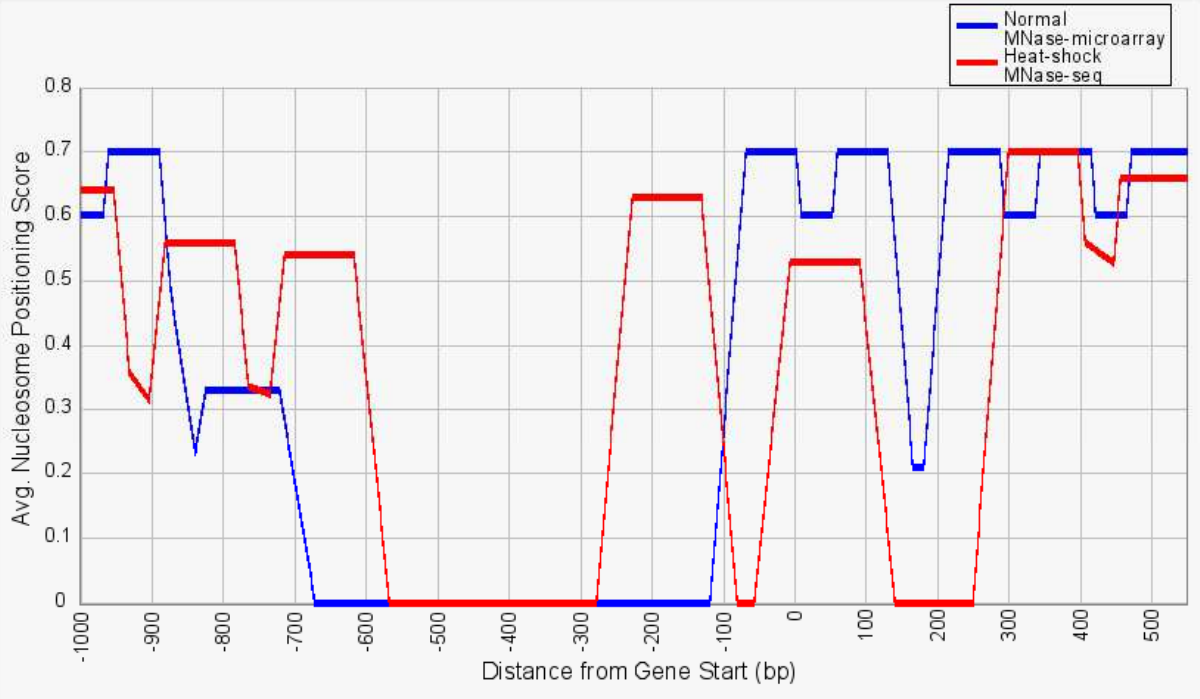


Updated: 1:03 PM, 3 Apr 2009

675 Fulmer Hall

Pullman, WA 99164

Contact: John Wyrick

Help

Recommended Browser: Mozilla Firefox

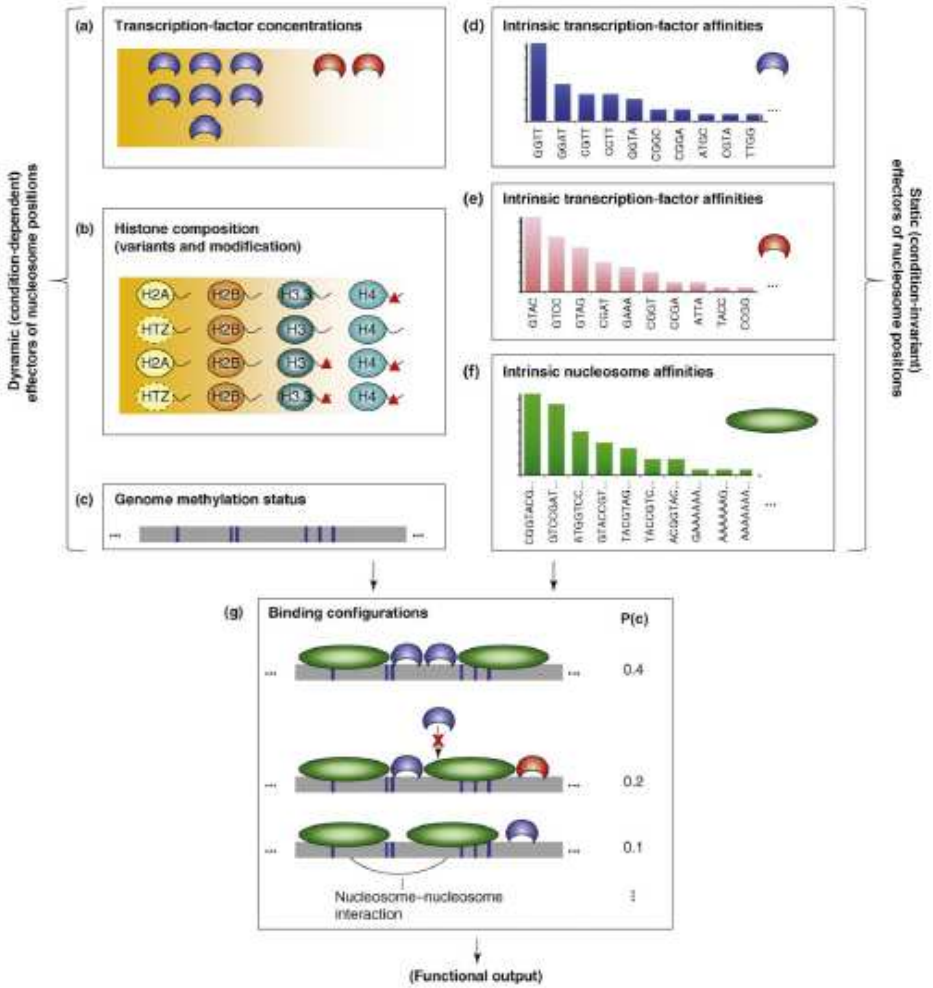


Figure 1.

A unified framework for nucleosome positioning. Here, we present an illustration of our unified view, in which nucleosome positions are explained by the combined effect of both static, condition-invariant inputs such as nucleosome and TF sequence preferences (**d–f**), and dynamic, condition-dependent inputs such as the concentrations of histones (not shown) and TFs, the composition of histones, and the methylation status of the genome (**a–c**). Two different TFs are colored blue and red; different histones are colored yellow (H2A), orange (H2B) or turquoise (H3, H4); histone variants are outlined with a dashed line; PTMs are shown as red triangles; resulting nucleosomes are colored green; methylated CpGs are shown as blue lines through the grey DNA; and an occluded TF-binding site is indicated by a red X. Each input

Histone variants

-Structural variants

-They exist in all species

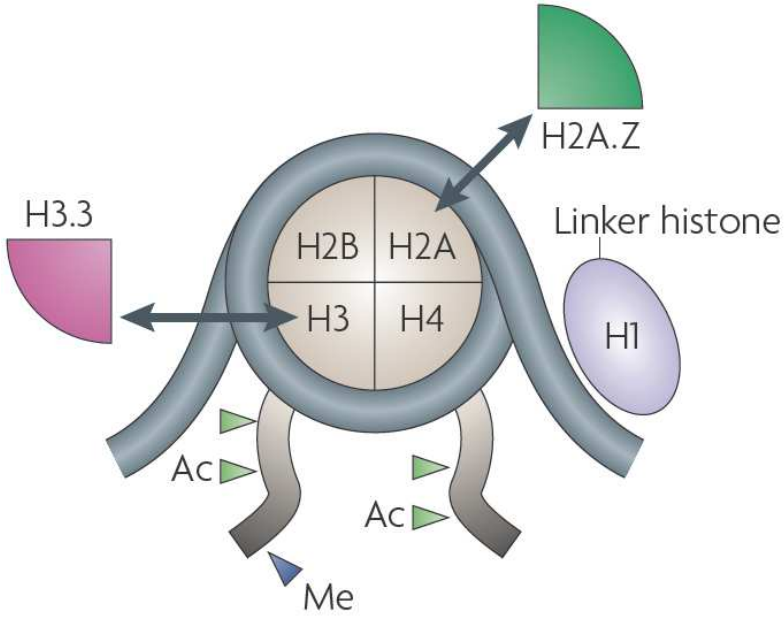
-Can be cell type-specific

-Regulated during development

-More common in H2A and H2B than in H3 and H4

-Some form are highly specialized (CENP A and Macro H2A)

Histone variants



Histone Variants

H2A → H2A.Z (Htz1)

H2A → H2A.X (10-15%)

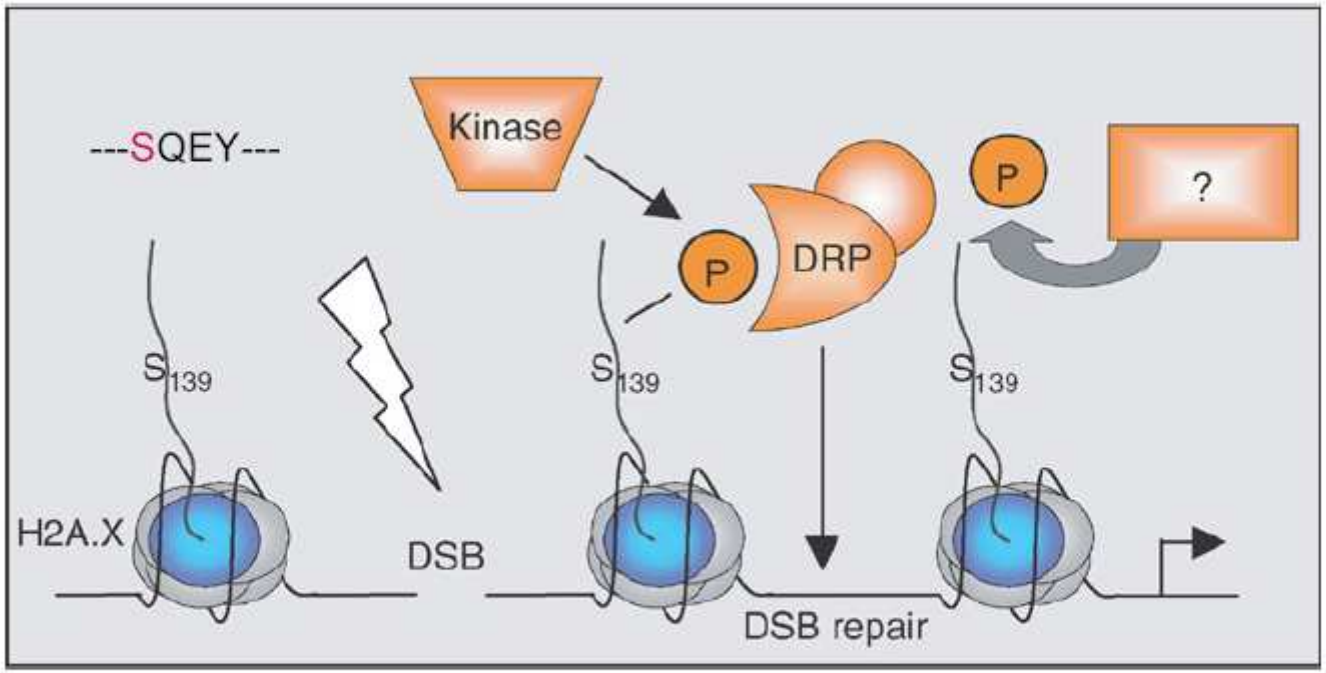
H2A → macroH2A

H3 → H3.3

H3 → CENP-A, Cid

Histone variants

Phosphorylation of H2A.X Signals DNA Damage

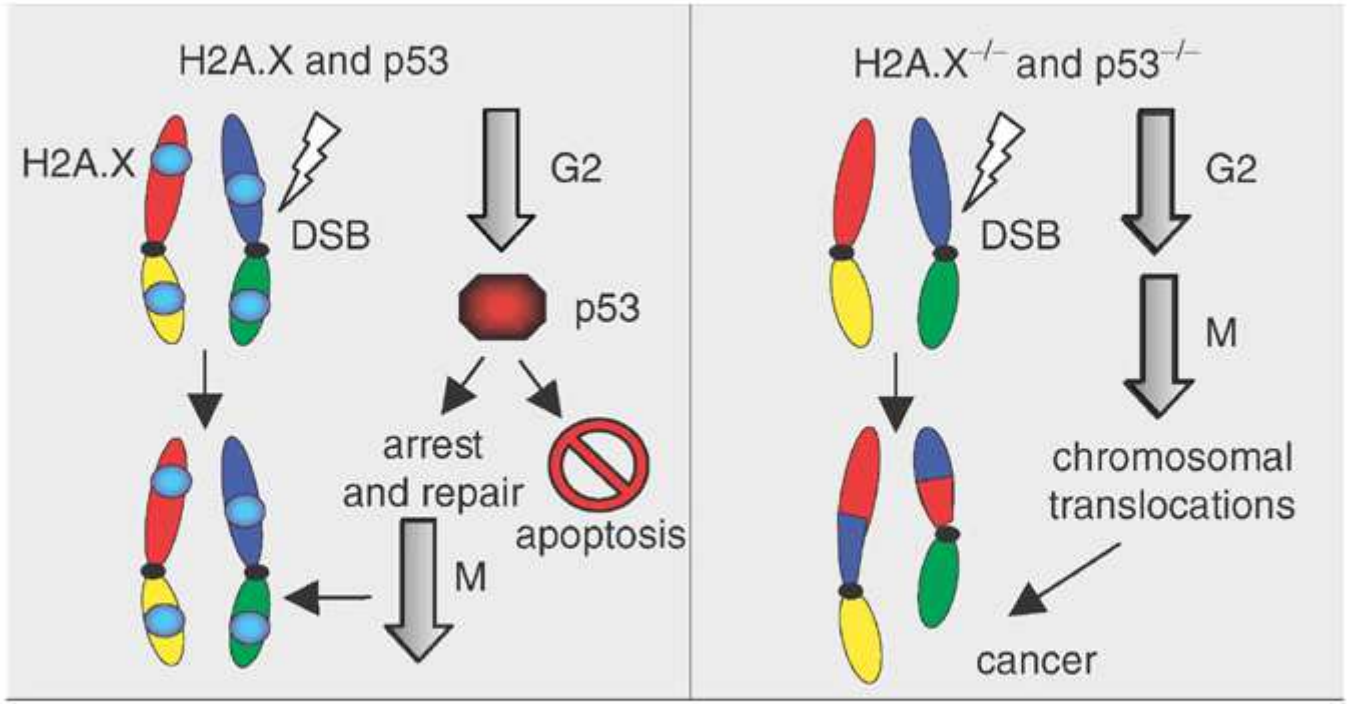


H2AX ~10-15% total. Long C-terminal tail

Hake, Xiao and Allis 2004 Br J. Cancer 90: 761

Histone variants

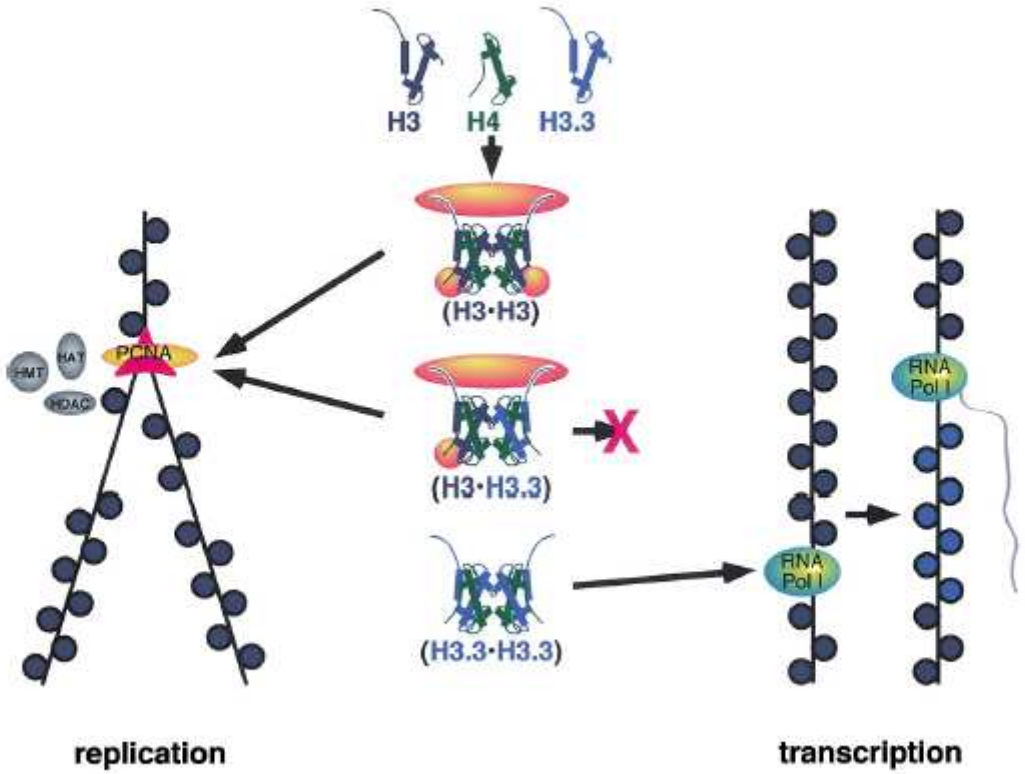
Loss of H2A.X Leads to Genomic Instability



Hake, Xiao and Allis 2004 Br J. Cancer 90: 761

Histone variants

H3 and H3.3 are loaded by different mechanisms



Ahmad & Henikoff 2002

Histone variants

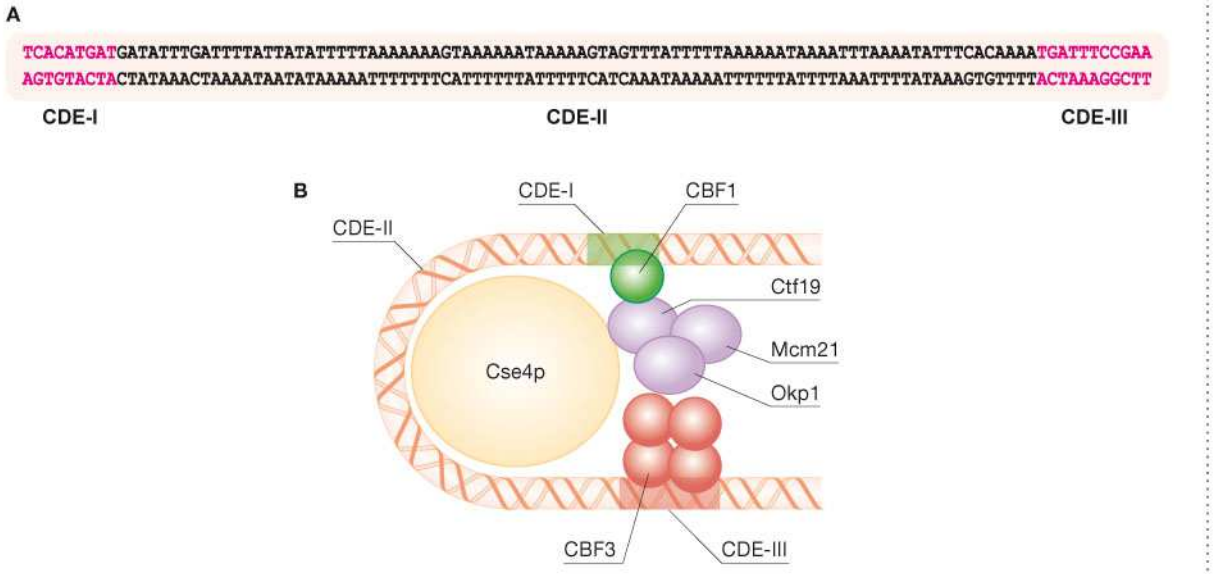


Figura 4.10 **Regione CEN del centromero di lievito.**
Tale regione è costituita dai domini CDE-I, CDE-II e CDE-III (**A**) ed è avvolta intorno a un complesso proteico (**B**).

Histone variants

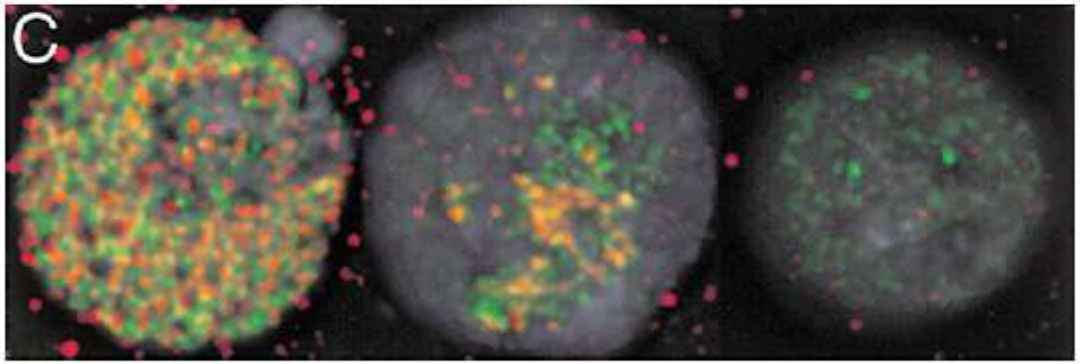
Differ by
3 amino acids

H3 'Major histone'

H3.3

*C*id

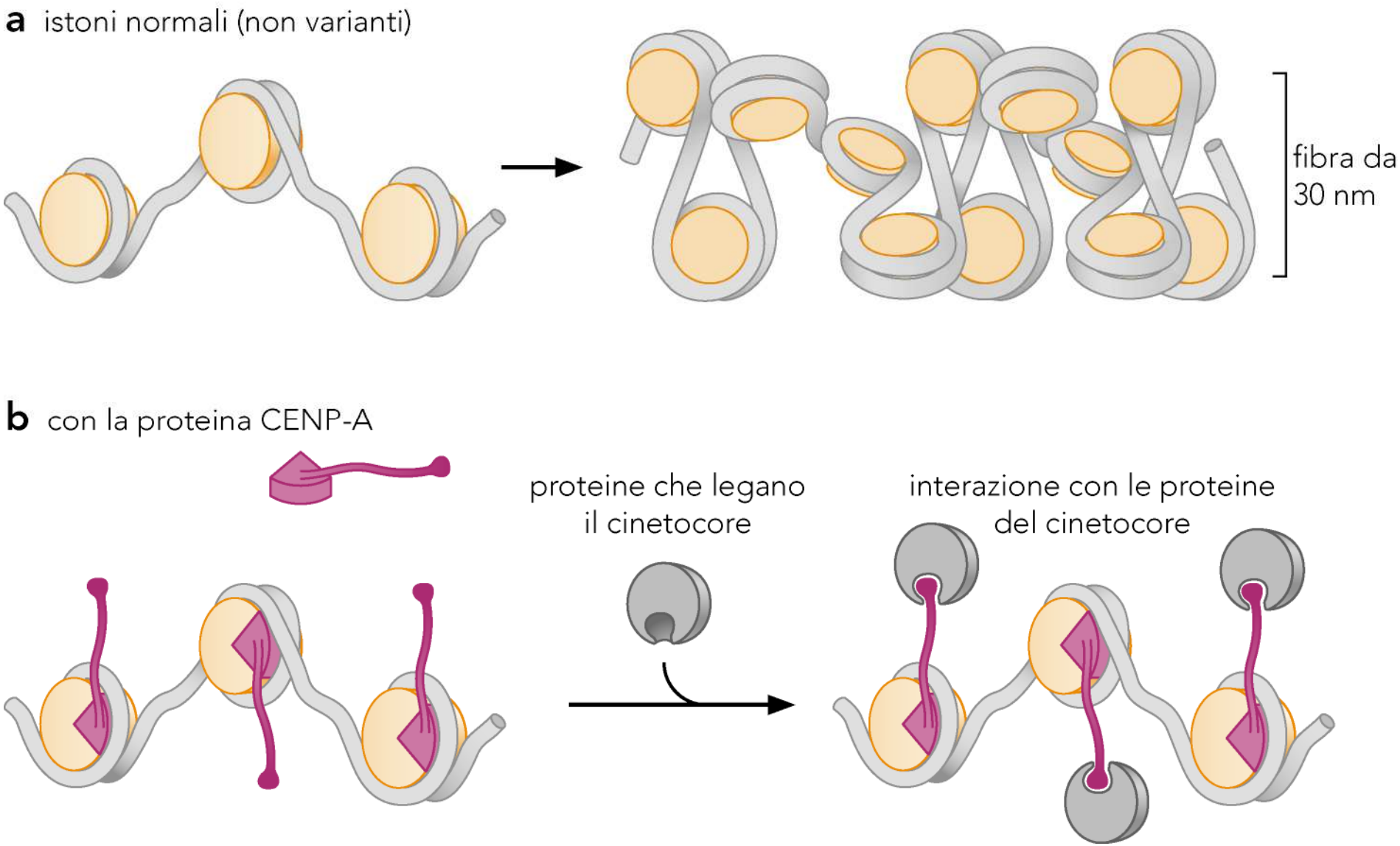
'Replacement histones'



Mid-S early-S G1 or G2

PCNA GFP-H3.3 merge

Ahmad & Henikoff 2002


a istoni normali (non varianti)
fibra da
30 nm
b con la proteina CENP-A
proteine che legano
il cinetocore
interazione con le proteine
del cinetocore

Histone variants

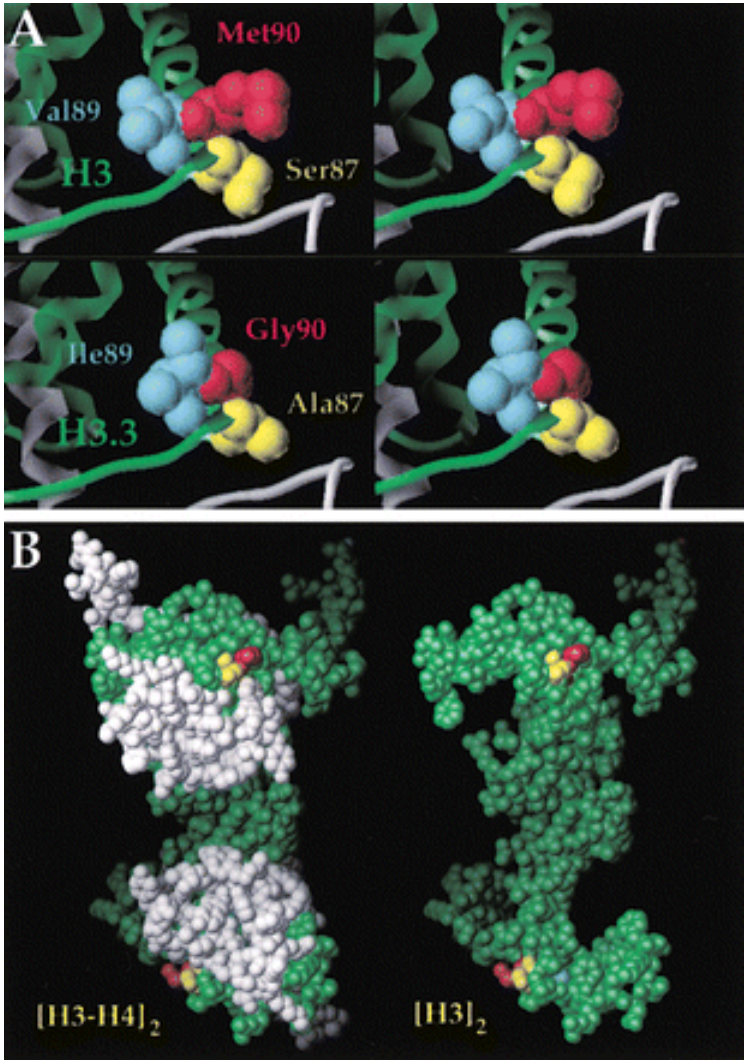
Histone H3.3:

Only 4 aminoacids modified in comparison with H3

Costitutive expression

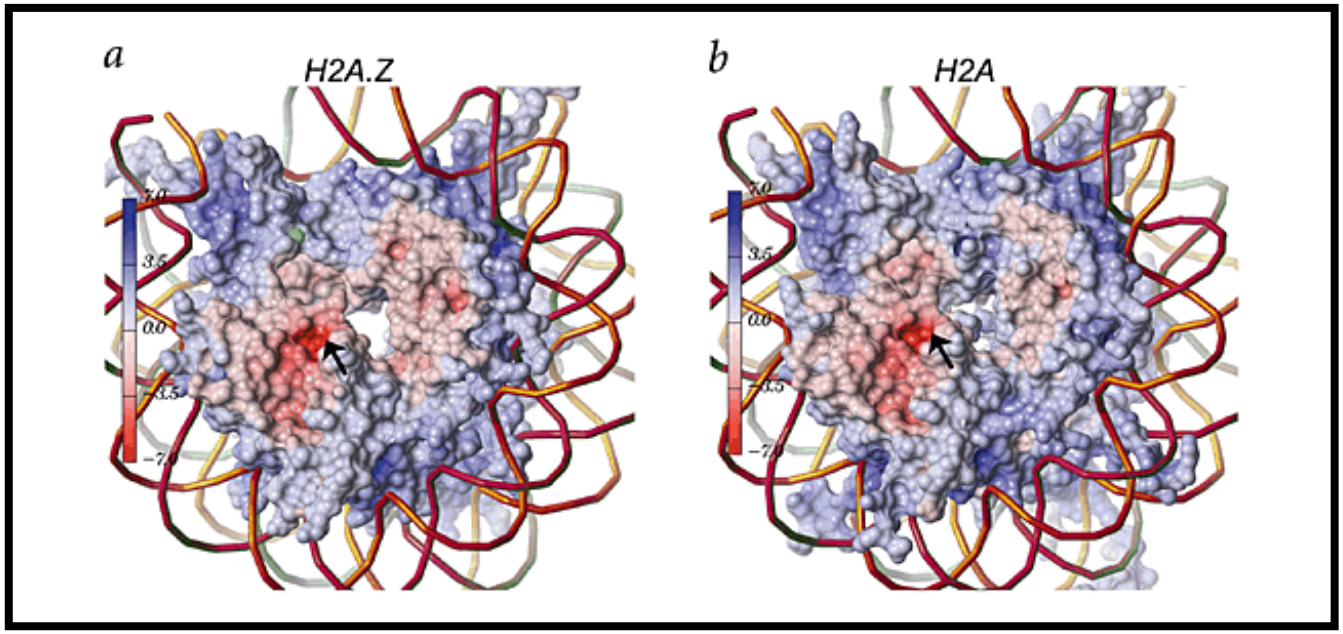
Can be positioned in replication-independent mode

Substitution oh H3 with H3.3 can lead to transcription and H3 methylation reprogramming



From: Smith, MM Mol Cell 9, 1158 (2002)

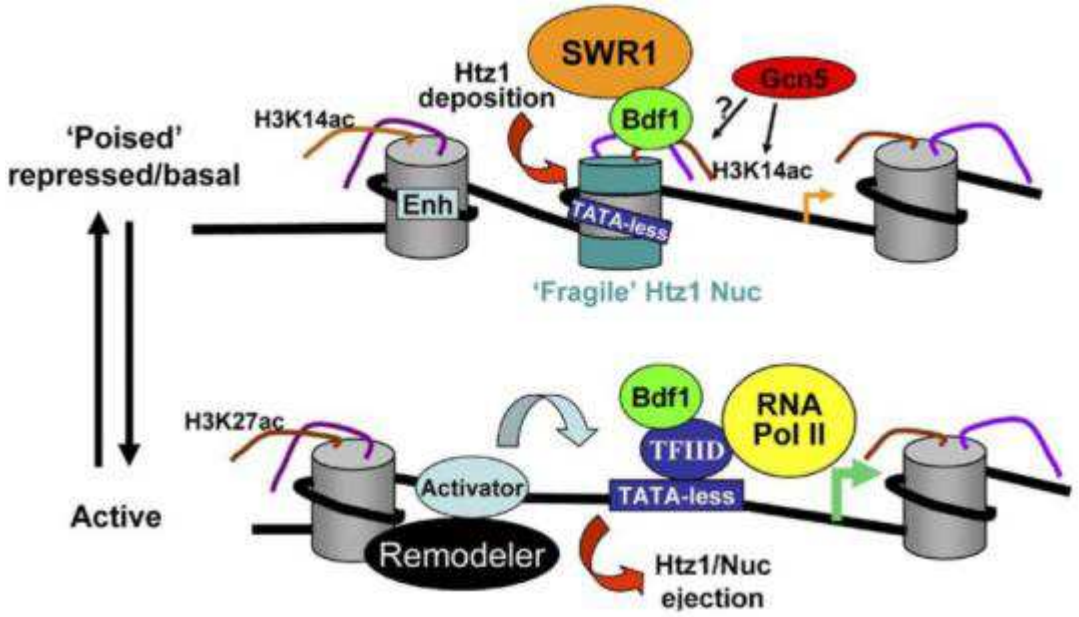
Comparison between H2A and H2A.Z structure

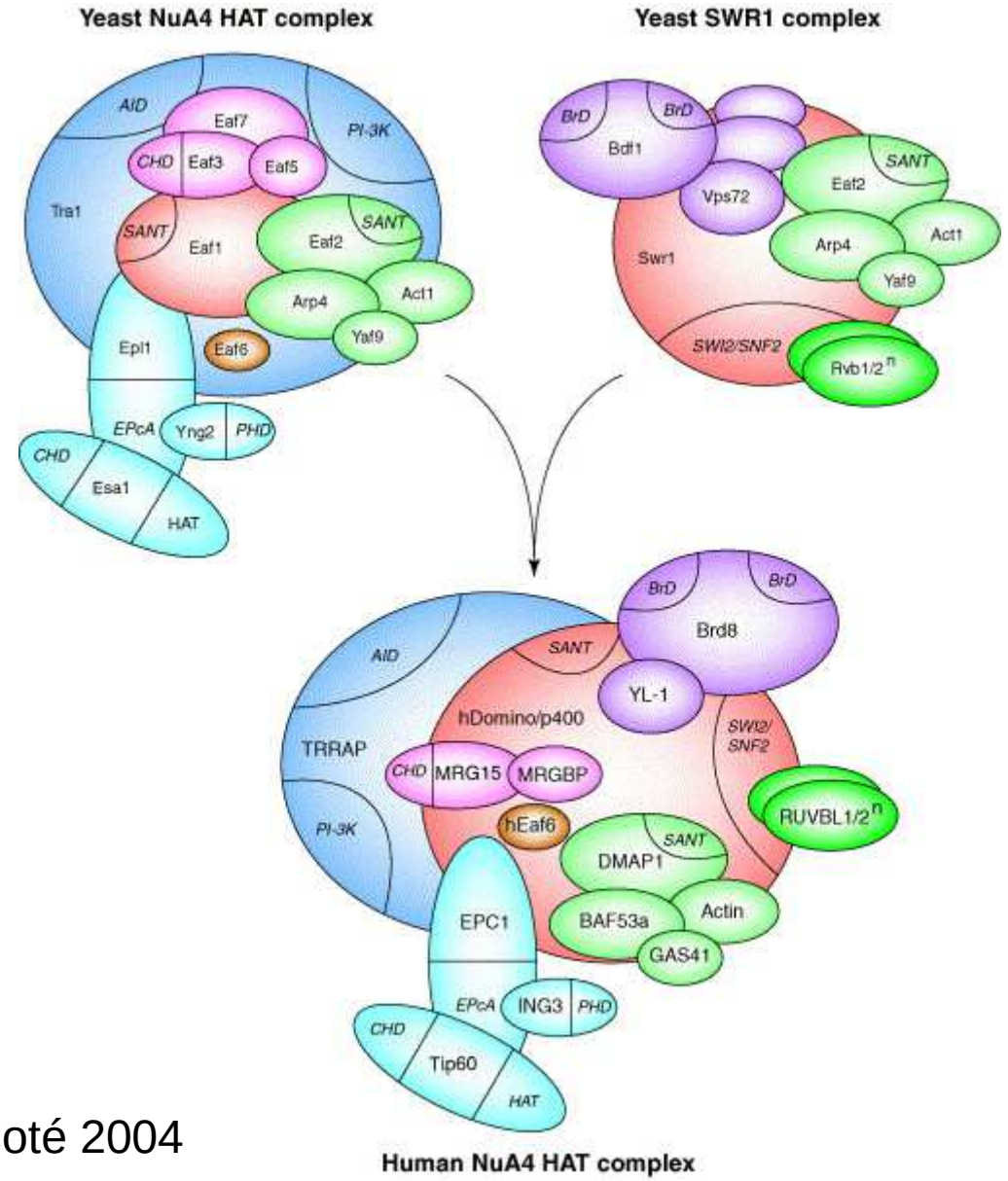


Cell. 2005 Oct 21;123(2):219-31.

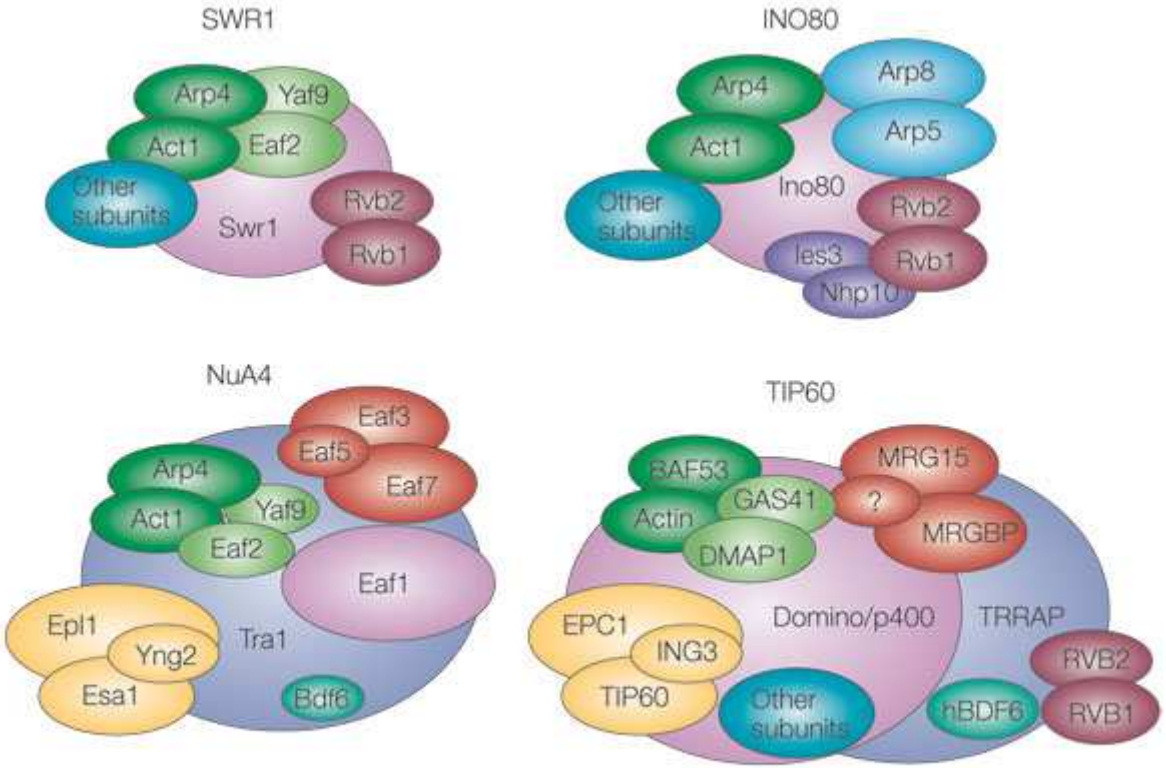
Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss.

Zhang H, Roberts DN, Cairns BR.



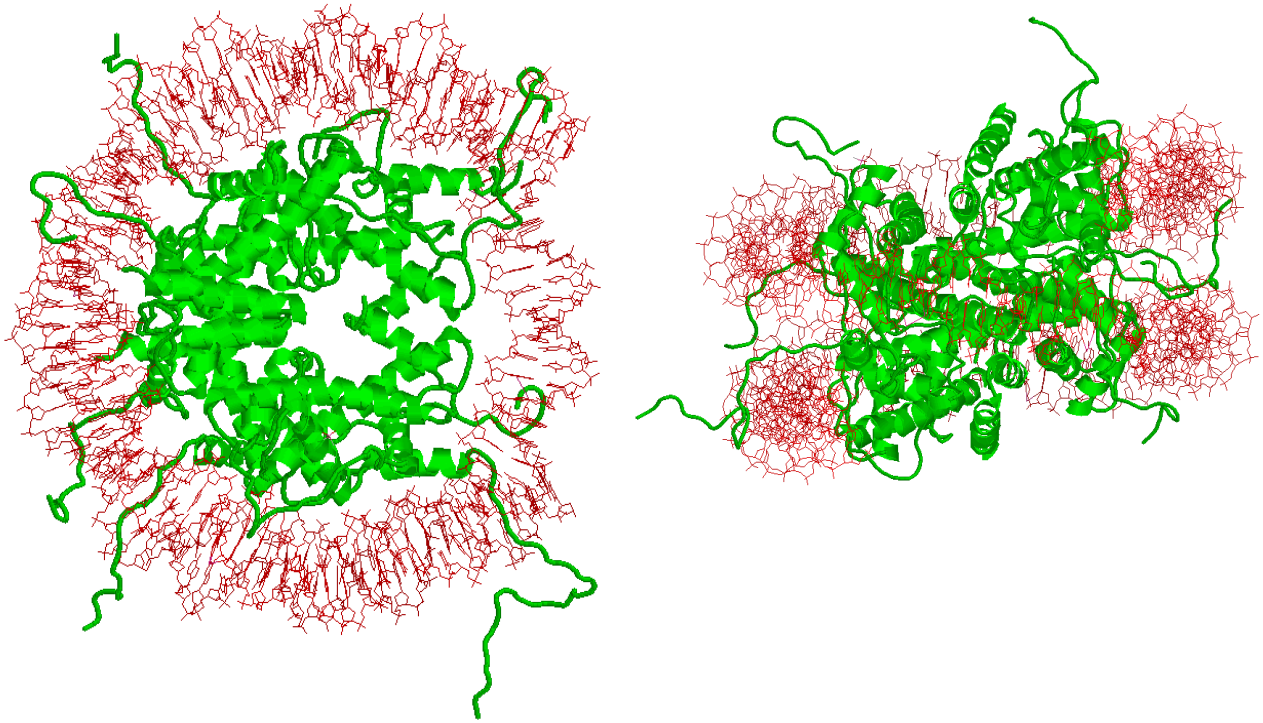


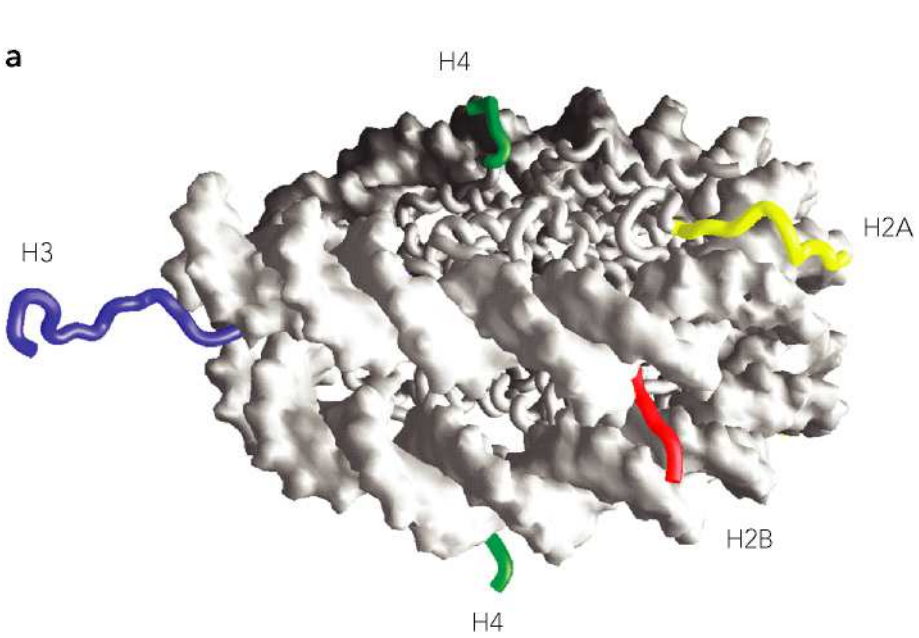
Doyon and Coté 2004

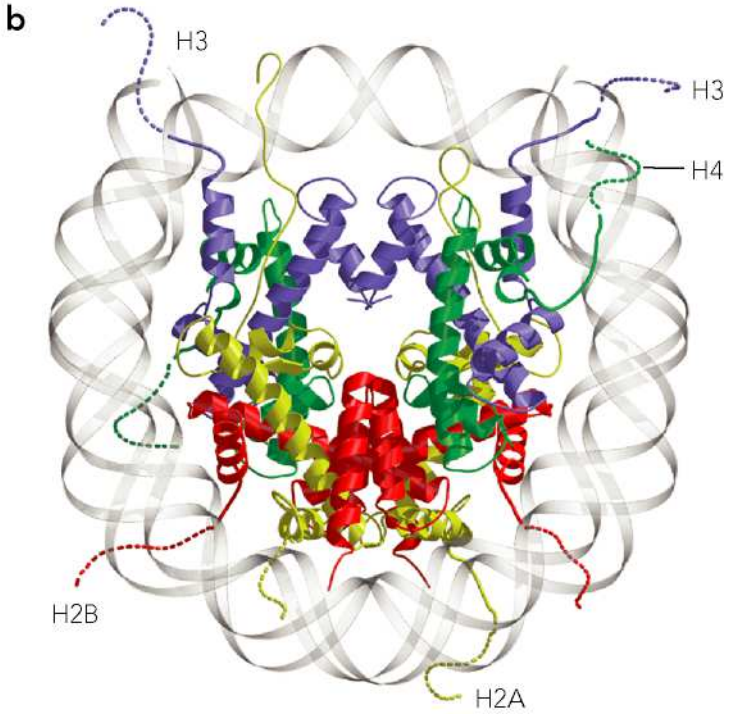


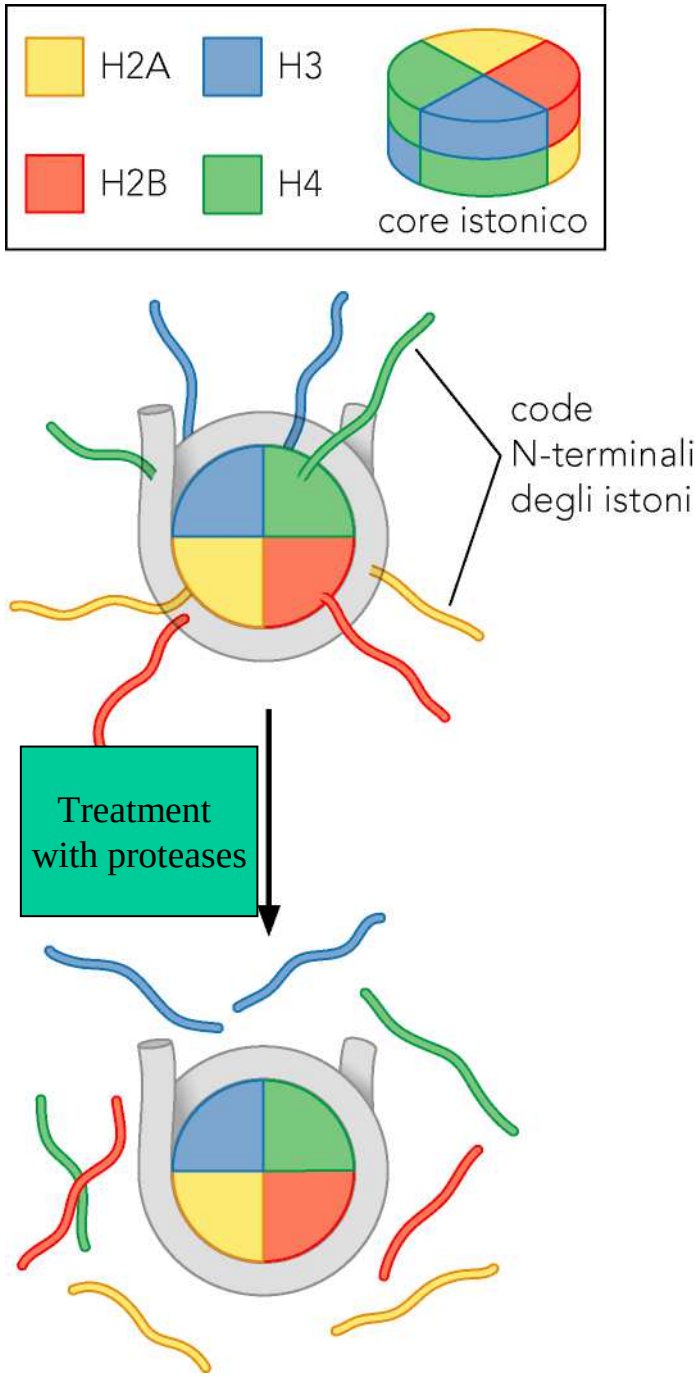
Nature Reviews | Molecular Cell Biology

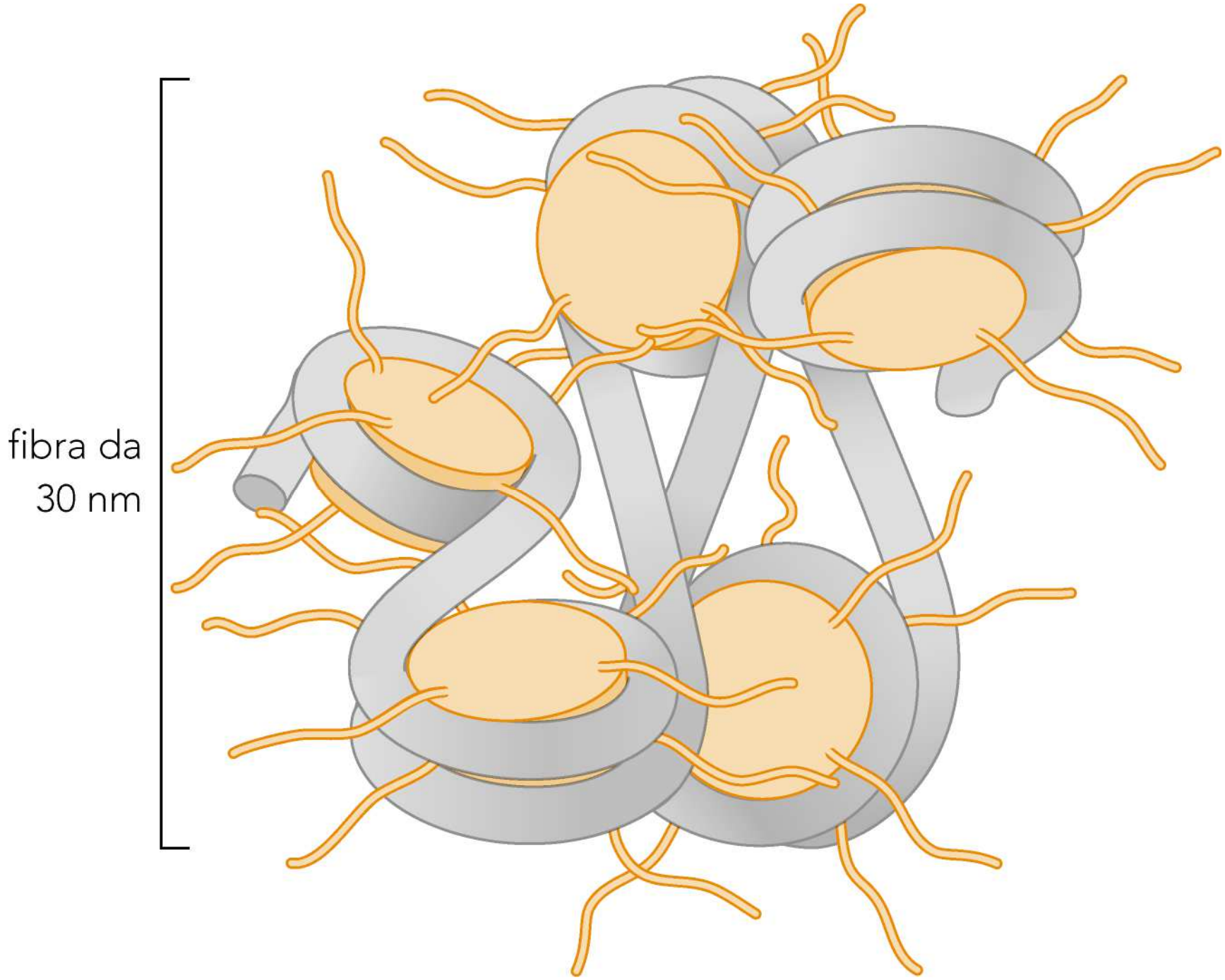
Histone tails




a
H4
H3
H2A
H2B
H4


b
H3
H3
H4
H2B
H2A


H2A
H3
H2B
H4
core istonico
code
N-terminali
degli istoni
Treatment
with proteases


fibra da
30 nm

Histone tails

Known and well carachterized
Histone tail modifications:

methylation

acethylation

ADP ribosilation

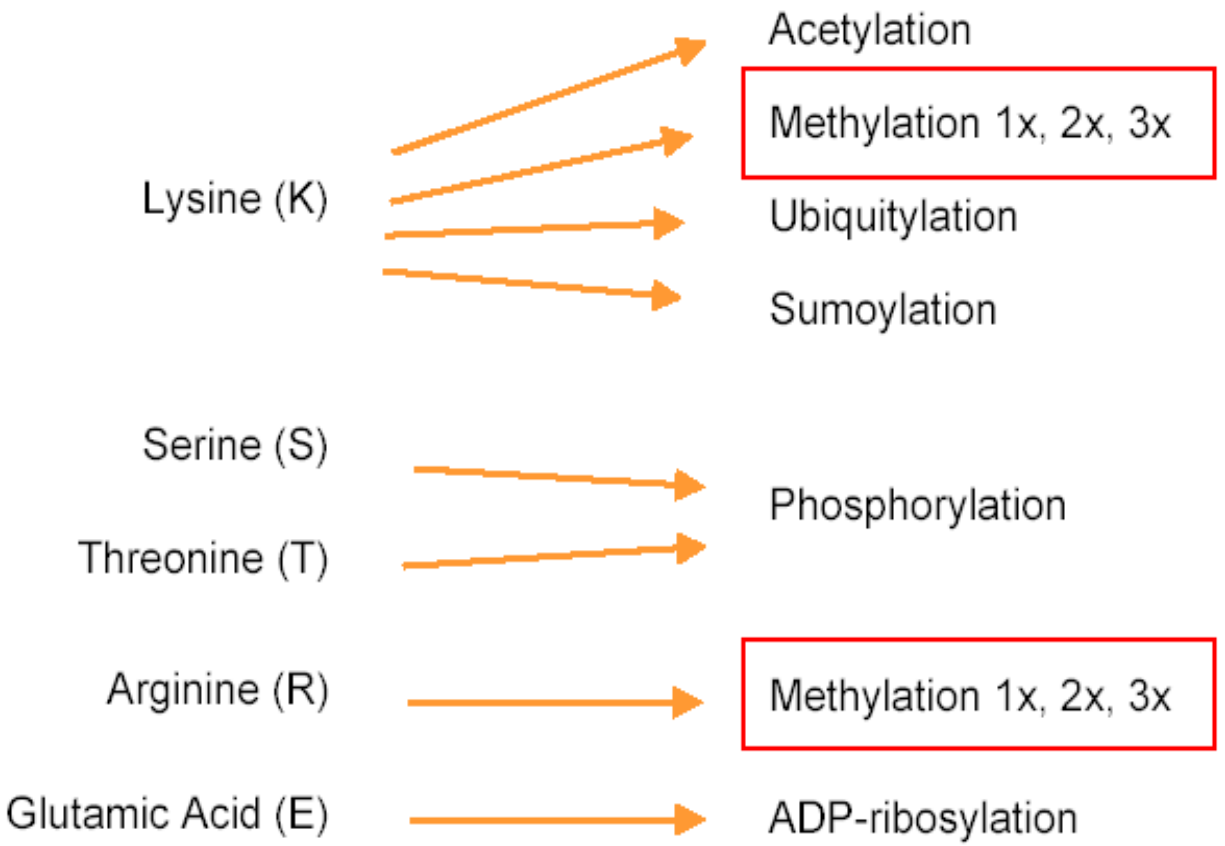
ubiquitination

sumolilation

Phosphorylation

The contemporary occurrence and the possible mutual influence of more than one modification at a time should be considered

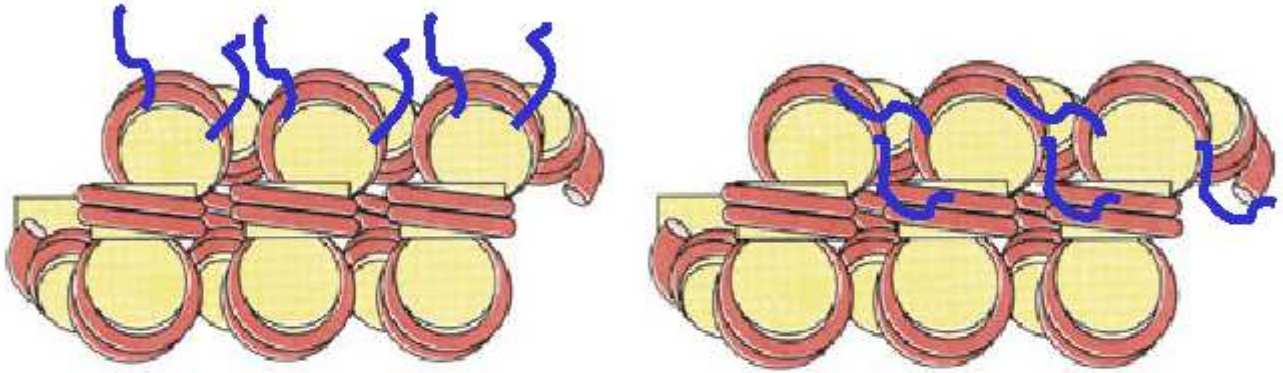
Histone modifications



David Allis et al.

Acetilation

Histone Acetylation Contributes to the Control of Gene Expression



- **Deacetylase Inhibitors (e.g. sodium butyrate and Trichostatin A)**
- **Non-random distribution of hyper- and hypo-acetylated chromatin**
- **Mutation acetylation sites in yeast H4**

Vincent Allfrey 1960's
Michael Grunstein 1980-90's

Acetilation

Why So Many HATs?

	Histone preference	*Non-histone target*
GCN5	H3 (H4,H2B)	?
PCAF	H3 (H4)	E2F, p53 etc
CBP/p300	All four	p53, TFIIF etc
SRC/p160	H3 &H4	?
$TAF_{II}250$	H3 (H4)	TFIIE
MYST family	H4 (H3)	?
TFIIIC	H3	?

Many HATs are also FATs

Acetilation

Acetylation is reversible!

Anti-cancer drugs

- Trichostatin A (TSA), Trapoxin, Sodium Butyrate

- Inhibit histone deacetylation
- Alter gene expression (promote expression)
- Cause G1 and G2 cell cycle arrests (checkpoint activation?).
- Promote cellular differentiation

Acetilation

Plenty of Deacetylases As Well!

	Mammals	Yeast	
Class 1			
	HDAC1	(yRPD3)	Deacetilasi attiva a livello globale su tutte le code istoniche
	HDAC2	(yRPD3)	
	HDAC3		
Class 2			
	HDAC4	(yHDA1)	Deacetilasi attiva a livello globale ma solo sulle code degli istoni H3 e H2B
	HDAC5		
	HDAC6		
	HDAC7		
Class 3			
	hSir2	(ySir2)	Deacetilasi NADH dipendente
Class 4	Hos Family		Intervengono sui promotori o ORF di geni attivamente trascritti

Acetilation

Creates a new code of gene activation/inactivation

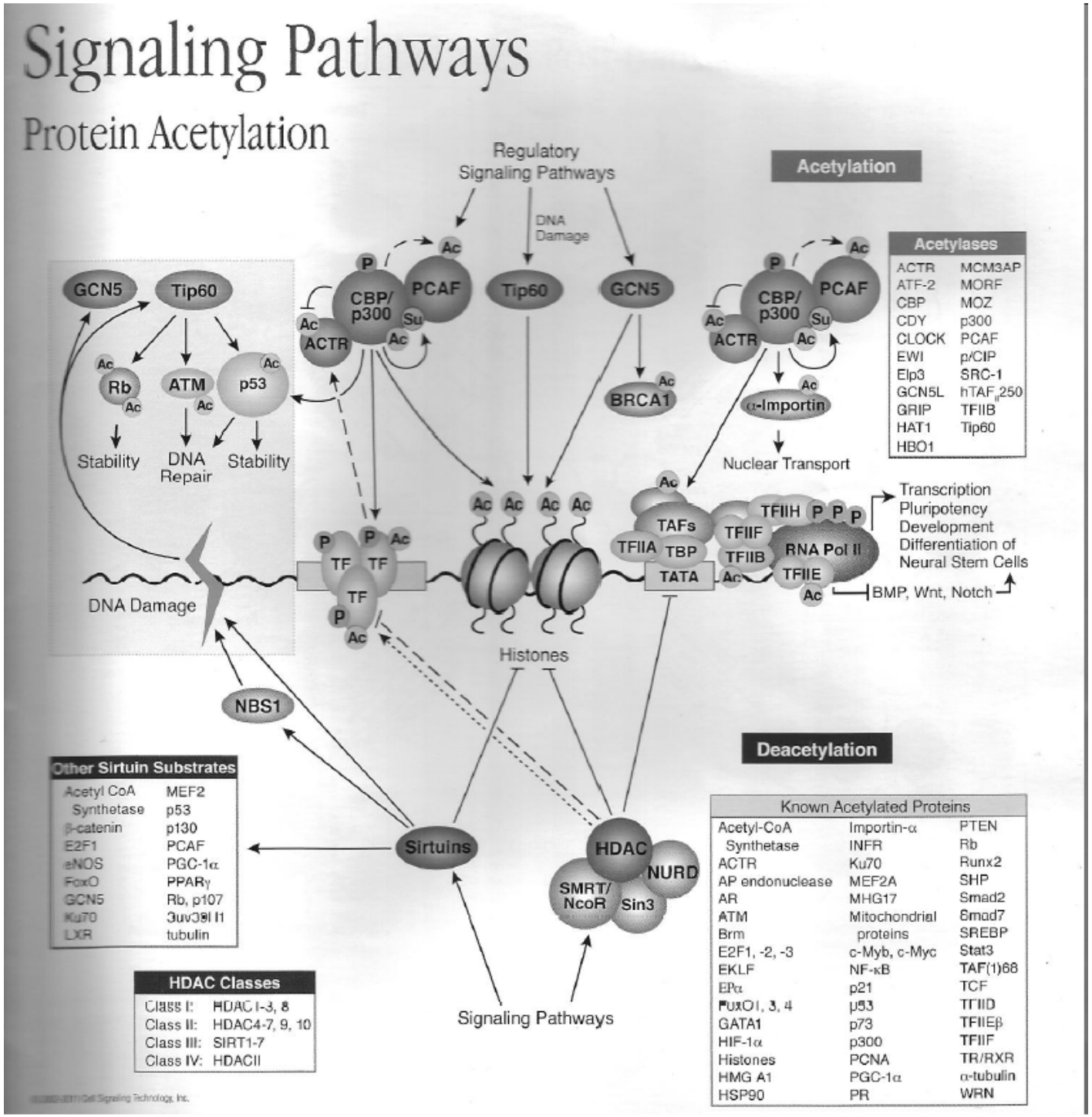
Produces new domains for protein binding

Is crucial in DNA repair process

Regulates chromatin structure

Signaling Pathways

Protein Acetylation

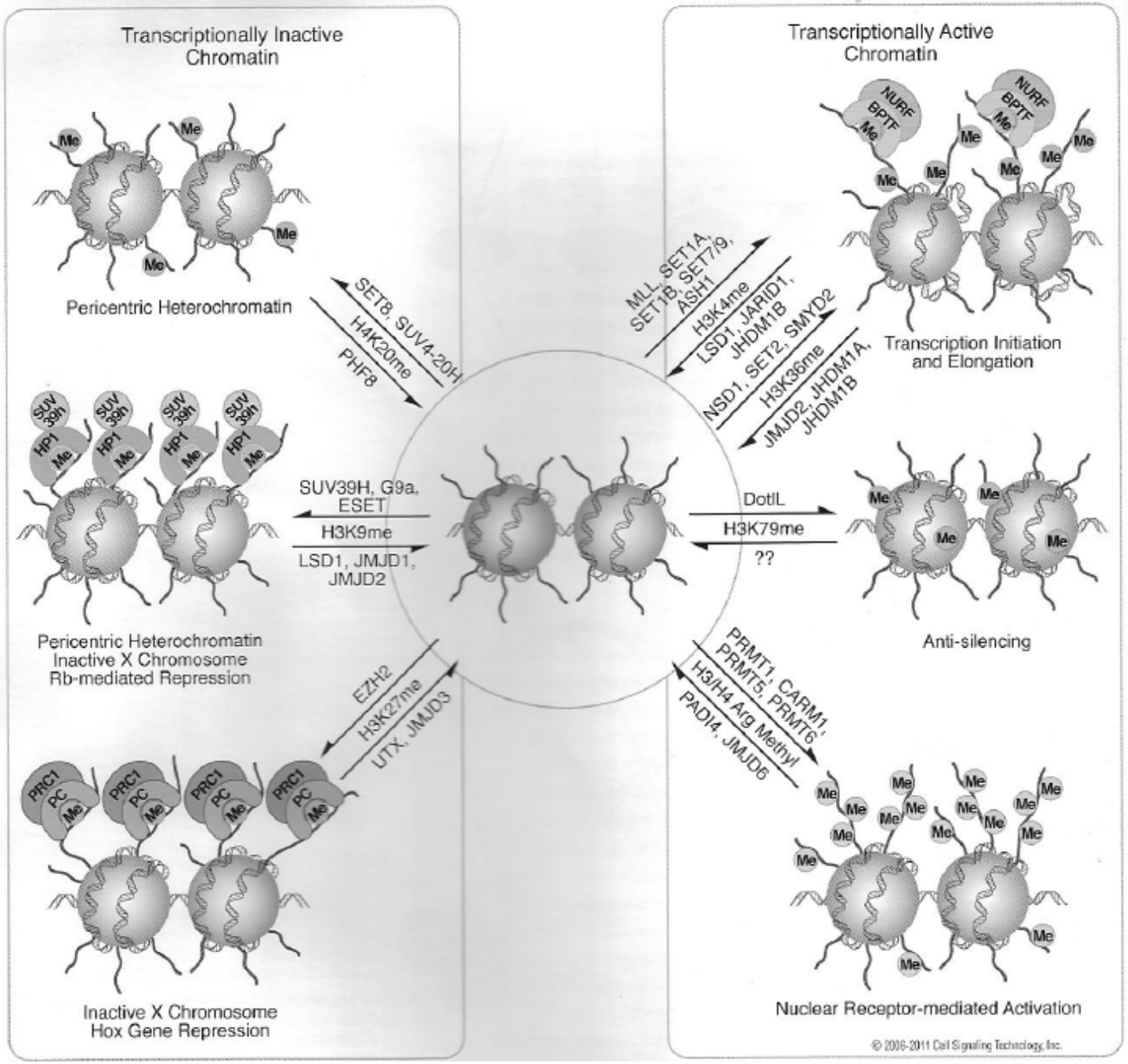


Acetylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys4 (S. cerevisiae)	Esa1	transcriptional activation	(1)
	Lys5 (mammals)	Tip60, p300/CBP	transcriptional activation	(2,3)
	Lys7 (S. cerevisiae)	Hat1	unknown	(4)
		Esa1	transcriptional activation	(1)
H2B	Lys5	p300, ATF2	transcriptional activation	(3,5)
	Lys11 (S. cerevisiae)	Gcn5	transcriptional activation	(6)
	Lys12 (mammals)	p300/CBP, ATF2	transcriptional activation	(3,5)
	Lys15 (mammals)	p300/CBP, ATF2	transcriptional activation	(3,5)
	Lys16 (S. cerevisiae)	Gcn5, Esa1	transcriptional activation	(6)
	Lys20	p300	transcriptional activation	(3)
H3	Lys4 (S. cerevisiae)	Esa1	transcriptional activation	(1)
		Hpa2	unknown	(7)
	Lys9	unknown	histone deposition	(8)
		Gcn5, SRC-1	transcriptional activation	(9,10)
	Lys14	unknown	histone deposition	(8)
		Gcn5, PCAF	transcriptional activation	(3,11)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		SRC-1	transcriptional activation	(10)
		Elp3	transcriptional activation (elongation)	(13)
		Hpa2	unknown	(7)
		hTFIIIC90	RNA polymerase III transcription	(14)
		TAF1	RNA polymerase II transcription	(15)
		Sas2	euchromatin	(16)
		Sas3	transcriptional activation (elongation)	(17)
		p300	transcriptional activation	(3)
	Lys18	Gcn5	transcriptional activation, DNA repair	(9)
		p300/CBP	DNA replication, transcriptional activation	(3,18)
	Lys23	unknown	histone deposition	(8)
		Gcn5	transcriptional activation, DNA repair	(9)
		Sas3	transcriptional activation (elongation)	(17)
		p300/CBP	transcriptional activation	(3,18)
	Lys27	Gcn5	transcriptional activation	(6)
	Lys56 (S. cerevisiae)	Spt10	transcriptional activation	(19)
			DNA repair	(20)

H4	Lys5	Hat1	histone deposition	(21)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Hpa2	unknown	(7)
		p300	transcriptional activation	(3)
	Lys8	Gcn5, PCAF	transcriptional activation	(3,22)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Elp3	transcriptional activation (elongation)	(13)
		p300	transcriptional activation	(3)
	Lys12	Hat1	histone deposition	(21)
			telomeric silencing	(23)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		Hpa2	unknown	(7)
		p300	transcriptional activation	(3)
	Lys16	Gcn5	transcriptional activation	(22)
		MOF (D. melanogaster)	transcriptional activation	(24)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Sas2	euchromatin	(2,6)
	Lys91 (S. cerevisiae)	Hat1/Hat2	chromatin assembly	(25)

Histone Methylation



Metylation

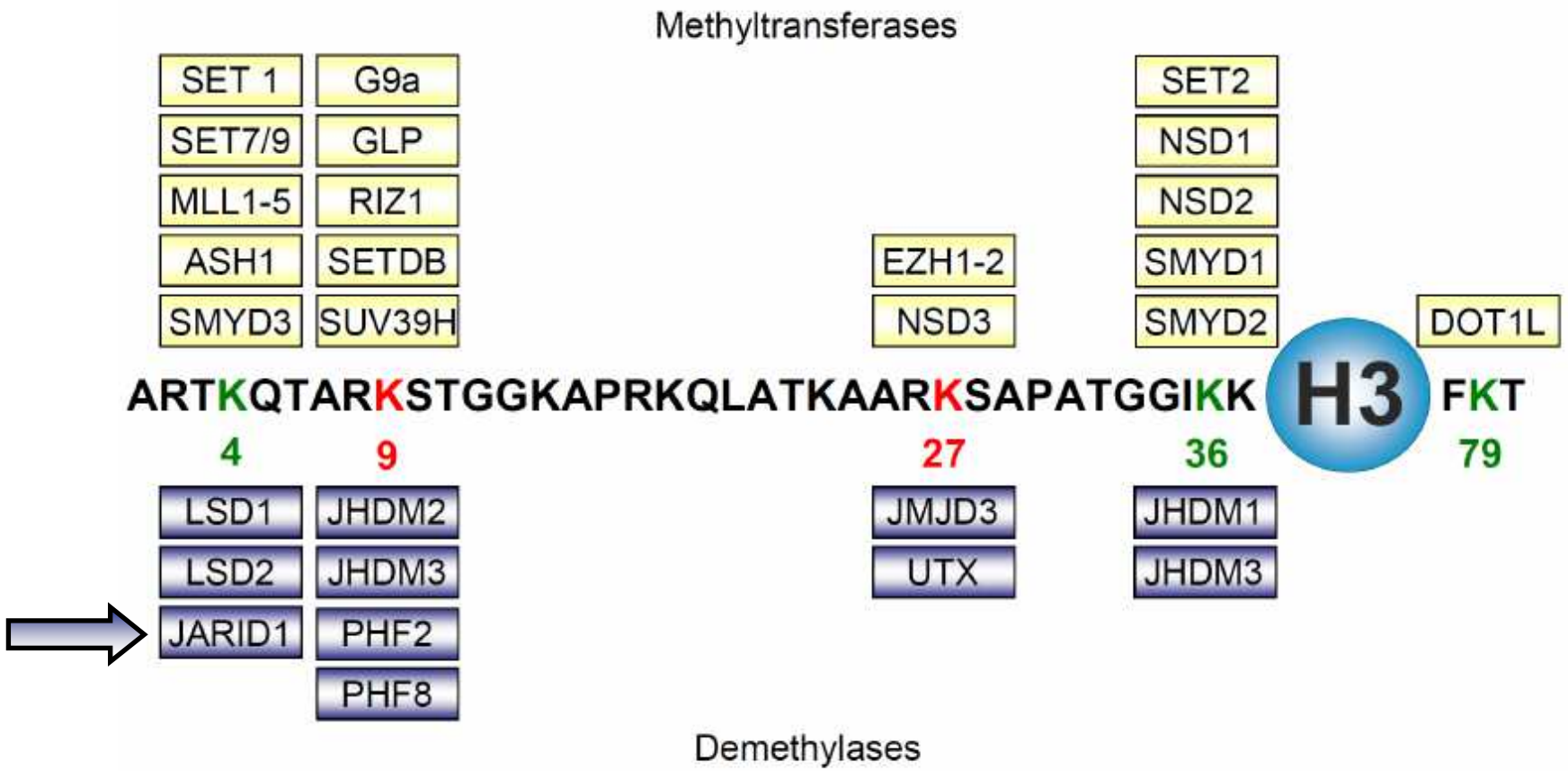
Histone Methyltransferases

HMT	Histone	Sites	SET domain	Organisms	Chromatin
Set1	H3	K4	+	S. cerevisiae	
Set2	H3	K36	+	S. cerevisiae	
Clr4	H3	K9	+	S. pombe	
G9a	**H3**	**K9, 27**	+	Human	Eu
Suv39h1,h2	**H3**	**K9**	+	Murine	He
Set9	H3	K4	+	Human	Eu
Dot1	H3	K79	-	S. cerevisiae	Eu
PR-Set7	H4	K20	+	Human	He
Ezh2	H3	K27	+	Drosophila	He

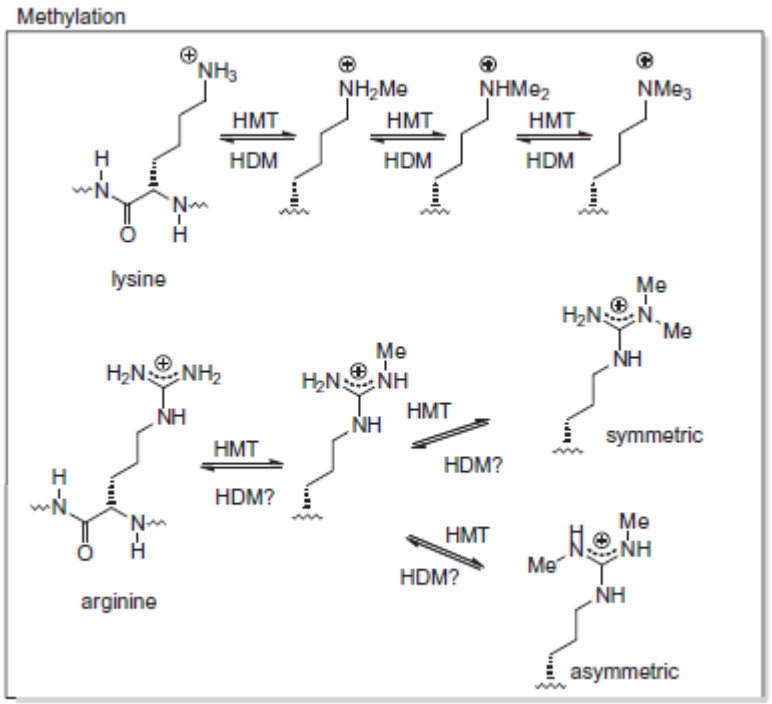
References:

Cell, Vol 9, 1201-1213, June 2002

Science. 2003 Apr 4;300(5616):131-5



The first 37 amino acid of the histone H3 tail and lysine residue 79 plus neighbouring amino acids are shown. Lysine residues that undergo methylation are highlighted in green to indicate a function in active transcription, or in red to indicate involvement in transcriptional repression.



Histone methylation is reversible !

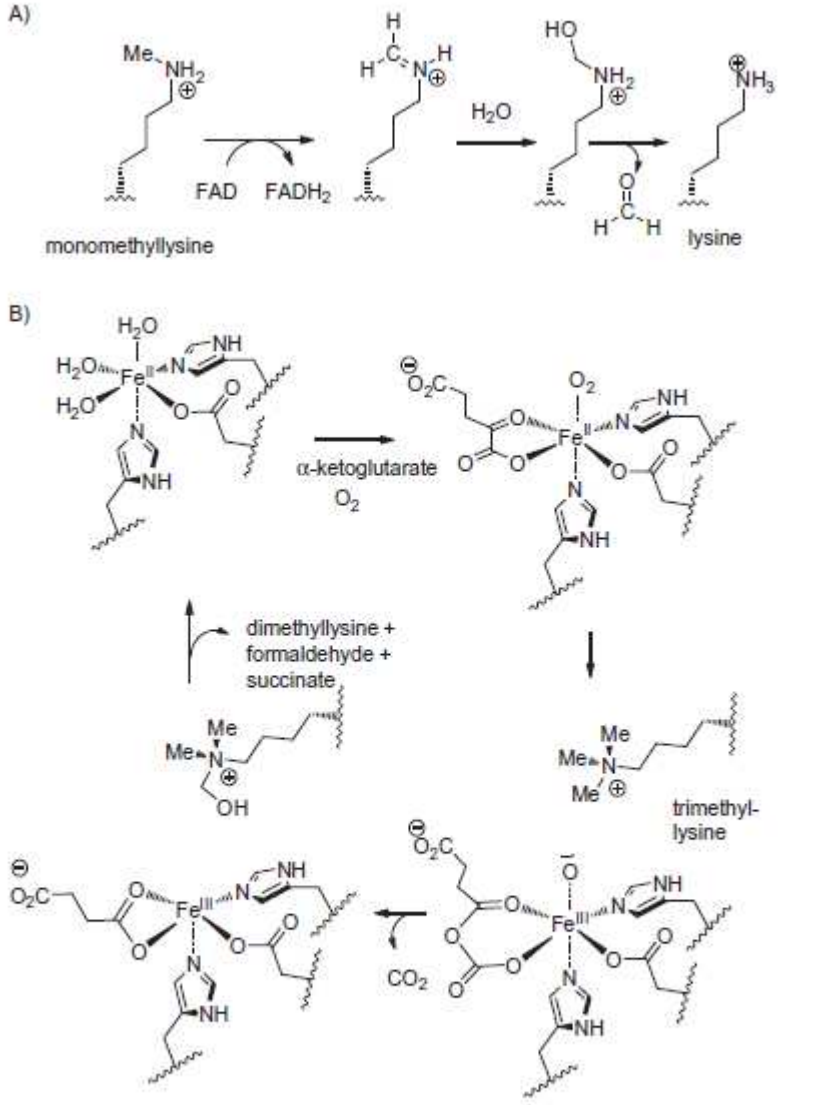


Figure 2. Reaction mechanisms for the demethylation of lysines at histones. (A) FAD dependent LSD demethylases and (B) Fe(II) and α-KG dependent JmjC demethylases.

Two major classes of HDMs in mammalians

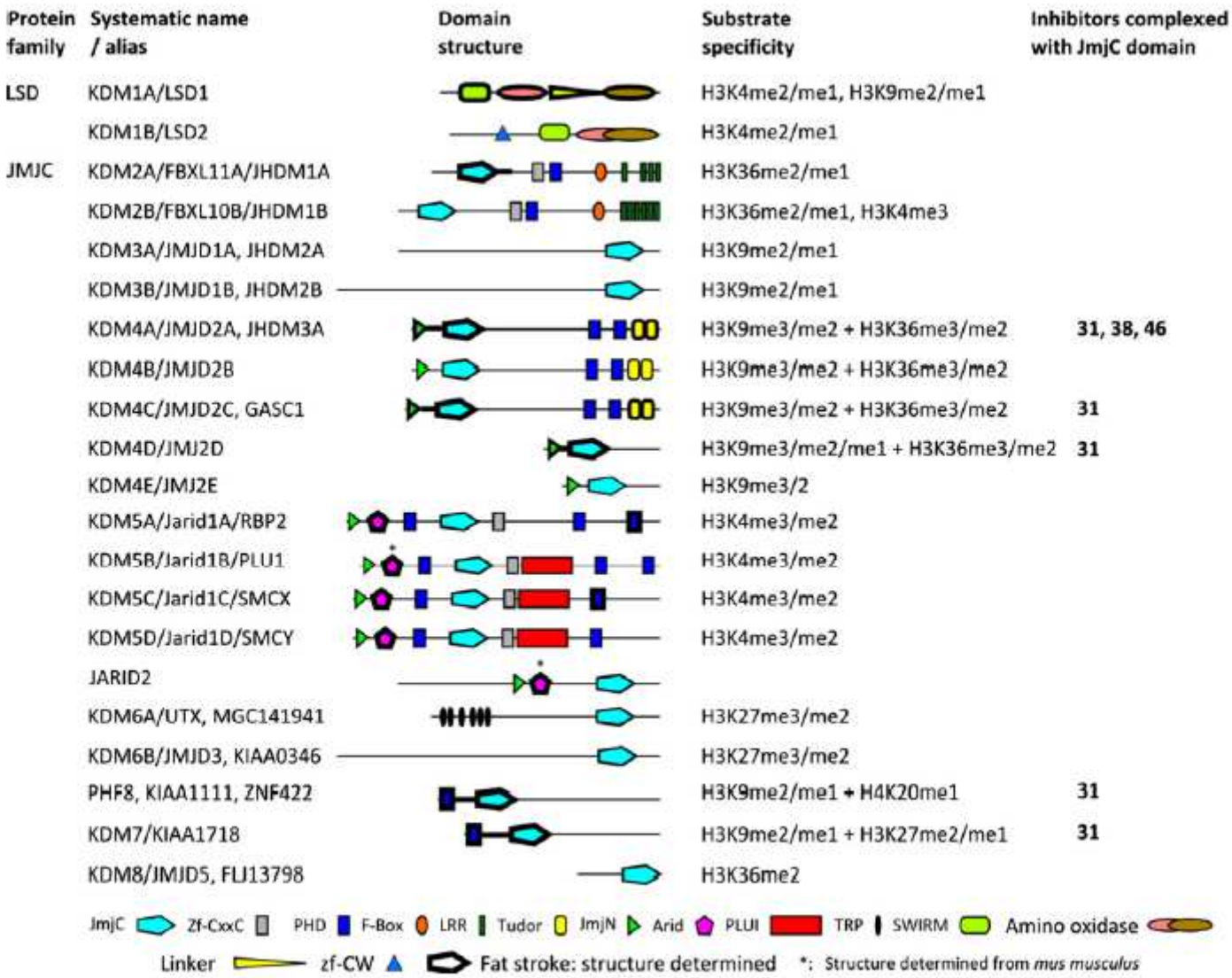
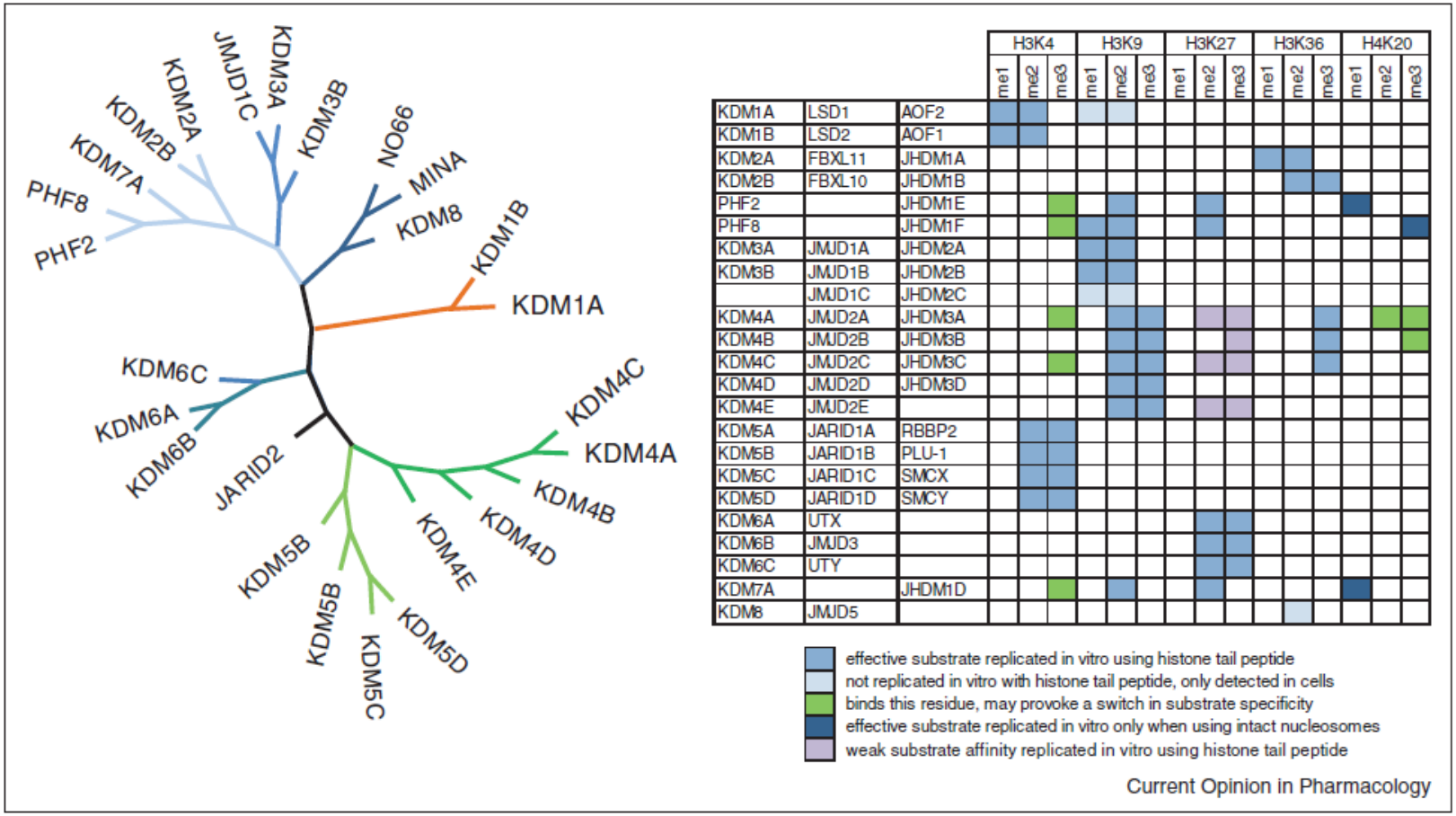


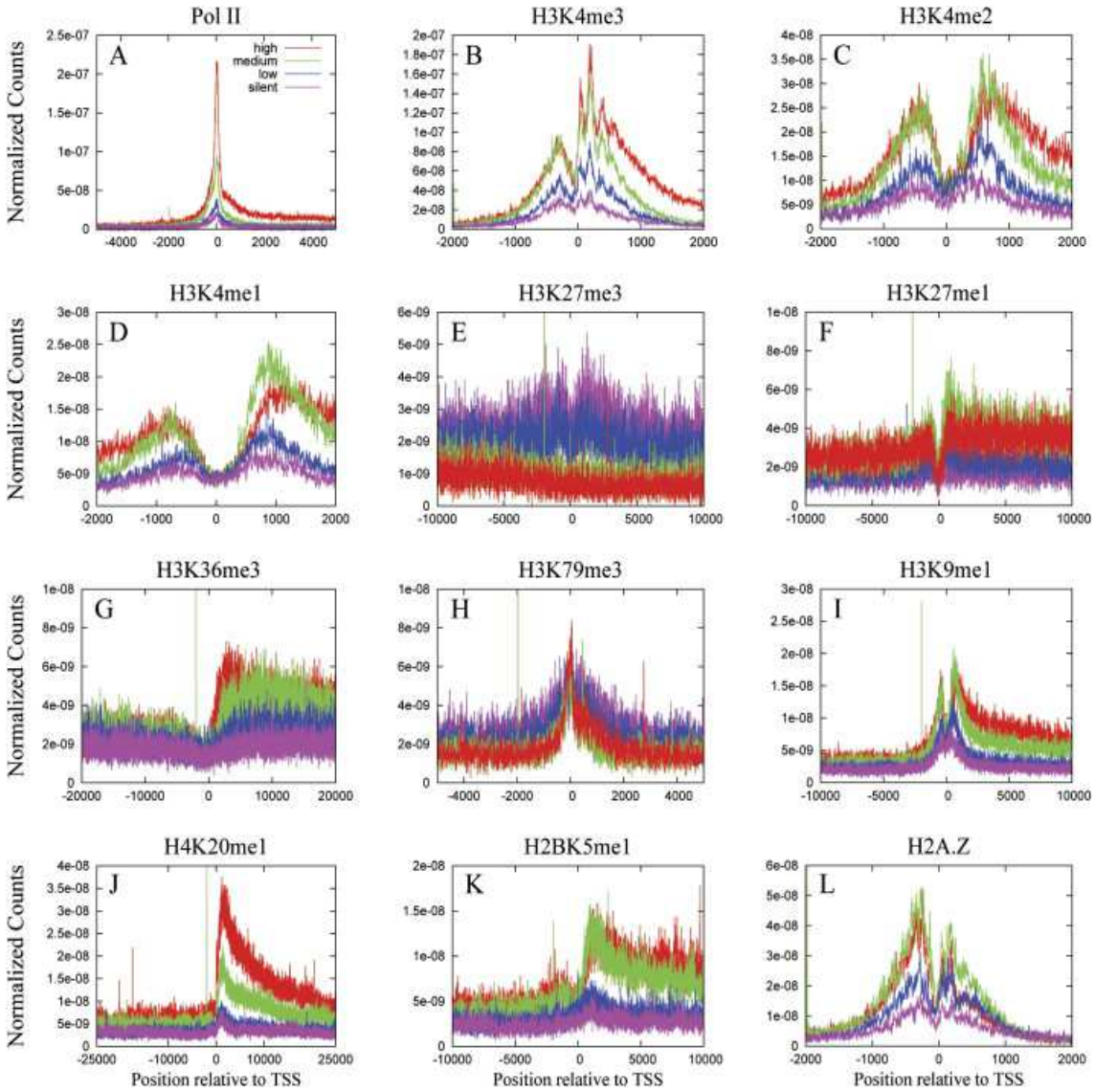
Figure 9. Domain architecture within the human KDM family. Domains for which structural data are available are marked with thick borders. Proteins are named according to the Protein Knowledgebase (UniProtKB).

Please cite this article in press as: Lohse, B.; et al. *Bioorg. Med. Chem.* (2011), doi:10.1016/j.bmc.2011.01.046

Figure 3



Substrate recognition and demethylation by Histone Lysine Demethylases. Left: Phylogenetic tree of histone lysine demethylases. Right: table reflecting binding and demethylation specificities reported for the different enzymes.



Histone PTMs around TSS region in Human genome according to transcriptional activity (red > green > blue > purple). ChIP-Seq reads highlight the diverse distribution of different PTMs according to both distance from TSS and transcriptional activity: H3K4 methylation level of active genes peaks in the immediate surroundings of the TSS, fading out in a regular fashion (me3 than me2 and finally me1) in both directions; H3K79me3 is only present in correspondence to TSSs; H3K36me3 and H4K20me1 are specifically enriched in the first region of gene bodies of transcriptionally active genes; H3K27me3 anti-correlates with transcriptional activity and its distribution spans over the whole region (up- and downstream of the TSS). From Barski et al., 2007

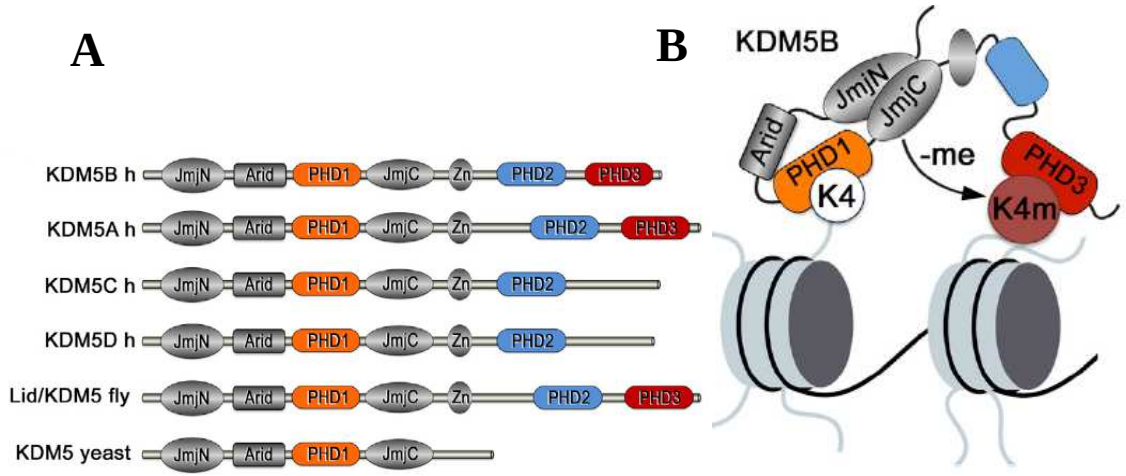


Figure The family of JARID1/KDM5 proteins in human and its orthologues in drosophila and yeast (left). PHD1 recognises unmethylated Lysine 4 while the trymethylated form is recognised by PHD3 (right). From Klein et al., 2014.

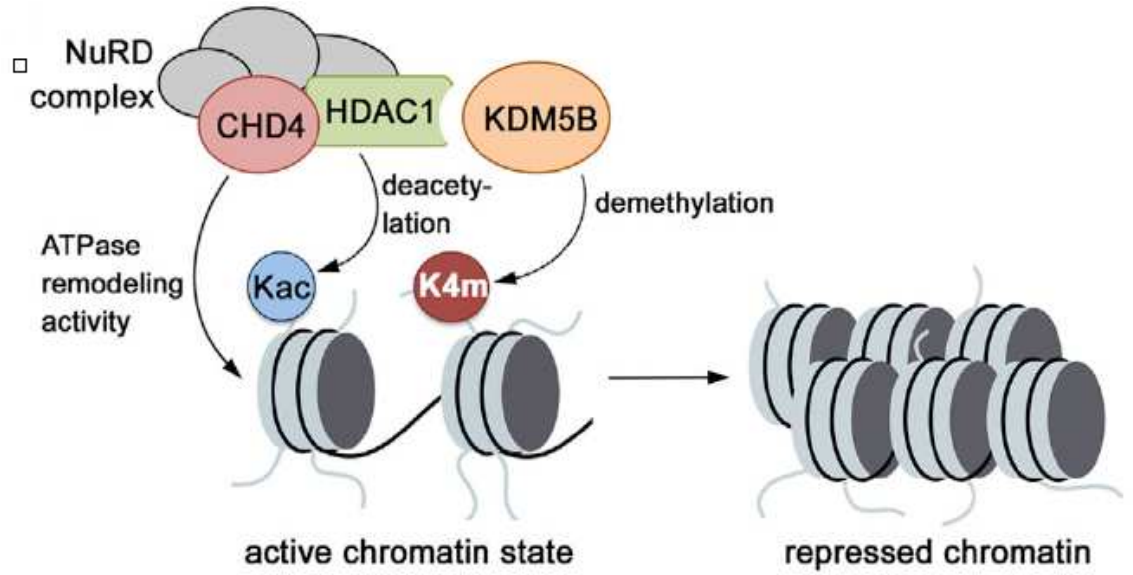


Figure JARID1B/KDM5B cooperates with NuRD complex to remove activating marks (H3K4me3) and remodel chromatin to produce a completely repressed state at the level of promoters (adapted from Klein et al., 2014)

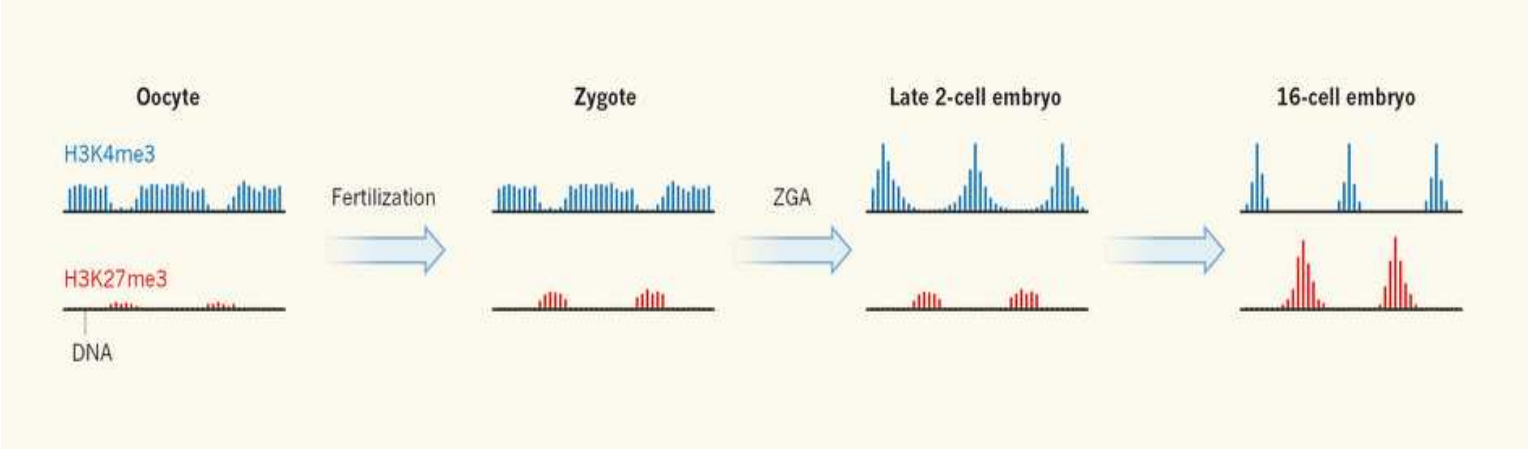


Figure Dynamics of H3K4me3 domains during development: oocyte genome contains large regions marked with low levels of H3K4me3, that are progressively defined and shaped into focused peaks corresponding to the promoters of actively transcribed genes. This process is brought on in parallel with the deposition of methyl groups on H3K27 in complementary (inactive) regions. From Vaquerizas and Torres-Padilla, 2016

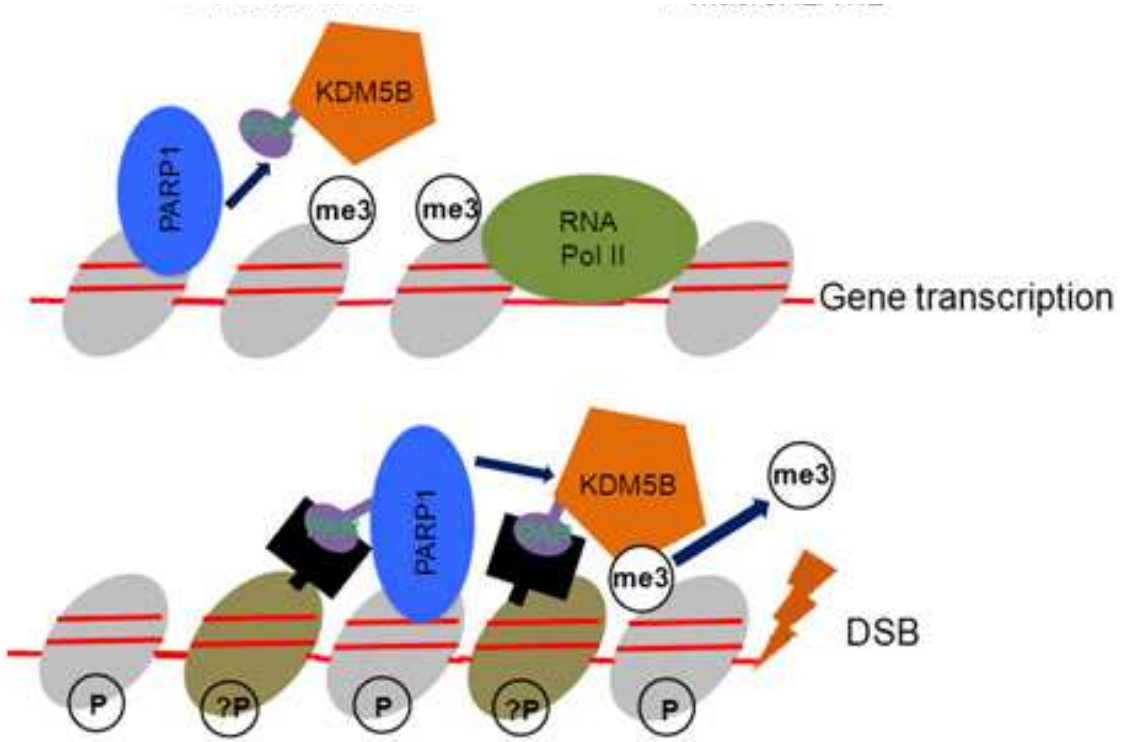


Figure The model proposed by Li et al., 2014 (supplemental information): JARID1B/KDM5B is recruited to the site of damage through its parrylation by PARP1 and recognition by macro domain of macroH2A1.1. JARID1B demethylates H3K4me3 facilitating DDR proteins recruitment.

Vicky W. Zhou et al., 2011

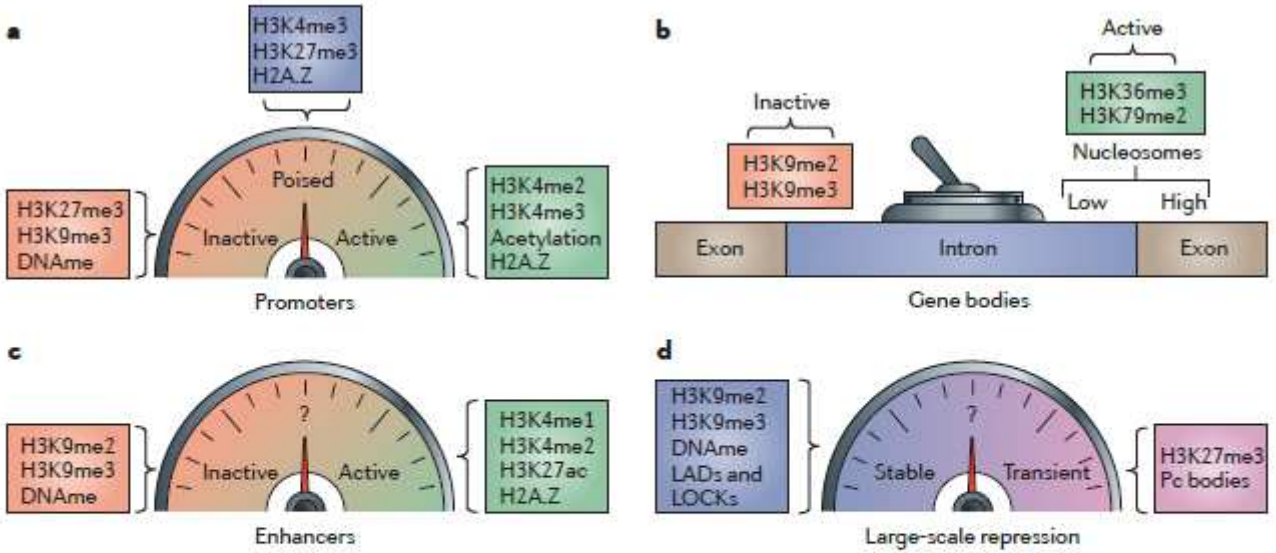
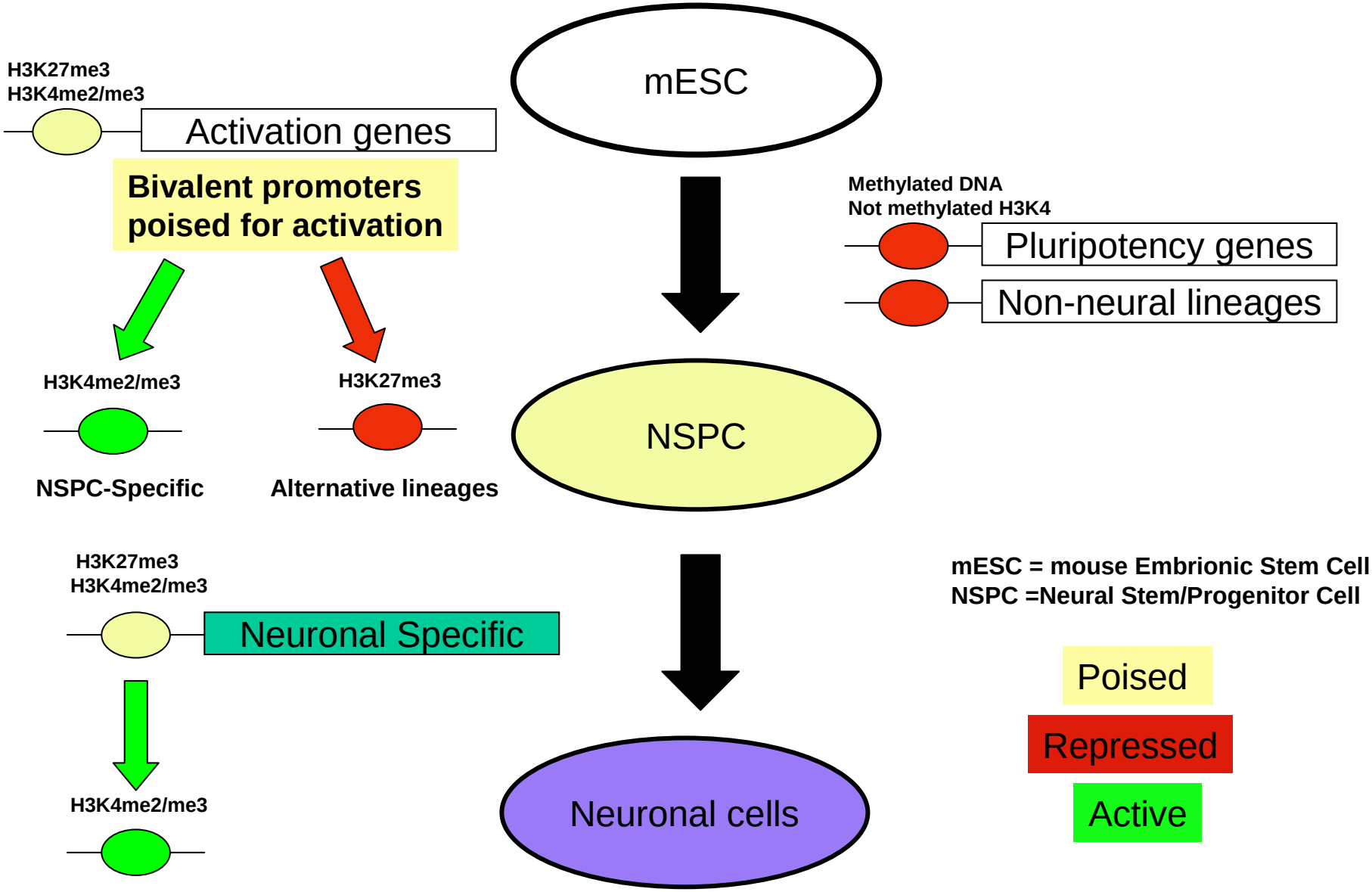


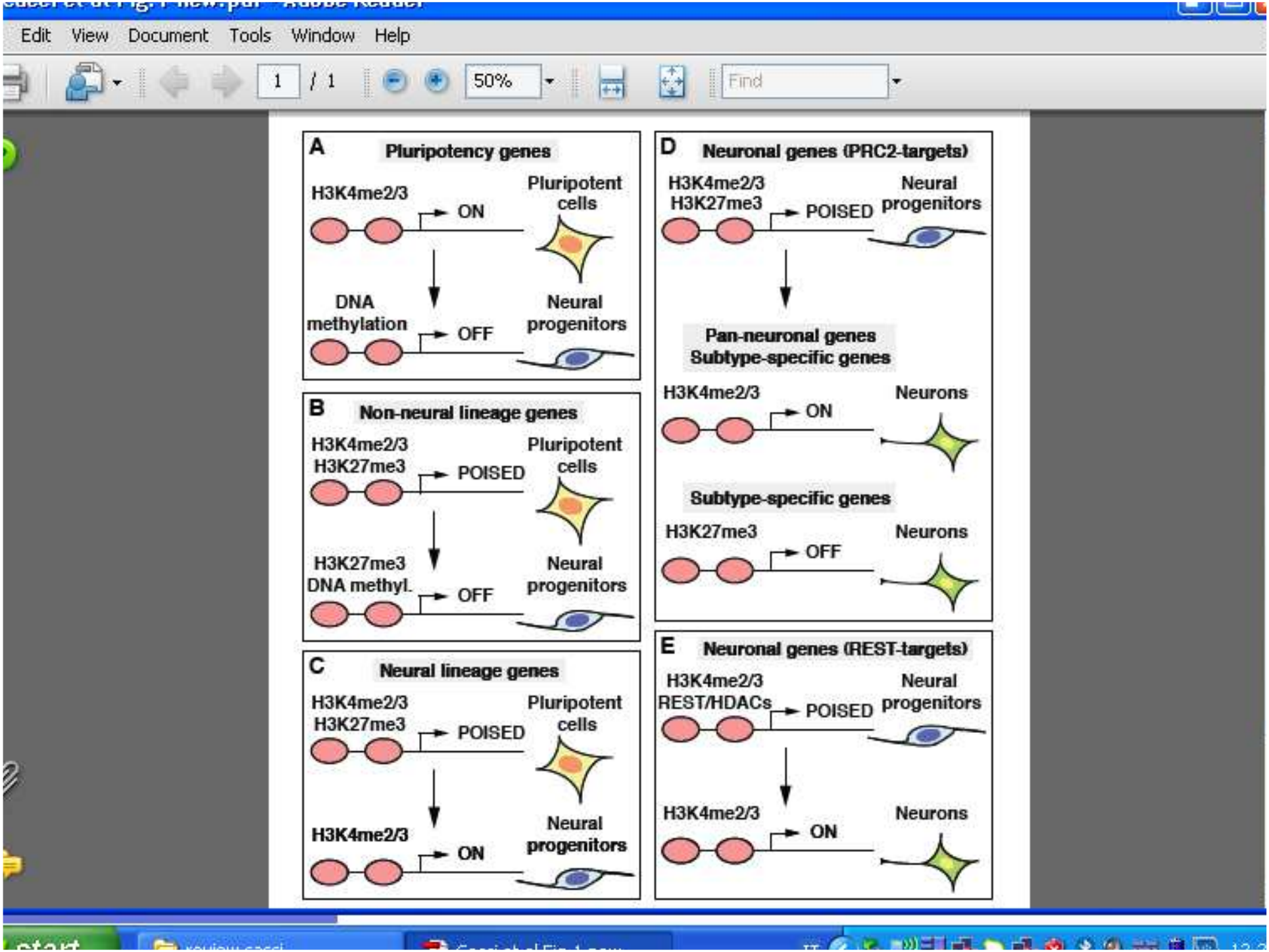
Figure 4 | **'Dashboard' of histone modifications for fine-tuning genomic elements.** In addition to enabling annotation, histone modifications may serve as 'dials' or 'switches' for cell type specificity. **a** | At promoters, they can contribute to fine-tuning of expression levels — from active to poised to inactive — and perhaps even intermediate levels. **b** | At gene bodies, they discriminate between active and inactive conformations. In addition, exons in active genes have higher nucleosome occupancy and thus more histone H3 lysine 36 trimethylation (H3K36me3) and H3K79me2-modified histones than introns. **c** | At distal sites, histone marks correlate with levels of enhancer activity. **d** | On a global scale, they may confer repression of varying stabilities and be associated with different genomic features. For example, lamina-associated domains (LADs) in the case of stable repression and Polycomb (Pc) bodies in the case of context-specific repression. DNAme, DNA methylation; LOCK, large organized chromatin K modification.

Epigenetic control of neural stem/progenitor cell self-renewal and differentiation

E. Cacci, R. Negri, S. Biagioni, G. Lupo

Current Topics in Medicinal Chemistry (CTMC) 2015 *under revision*

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Neural progenitors
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Non-neural lineage genes
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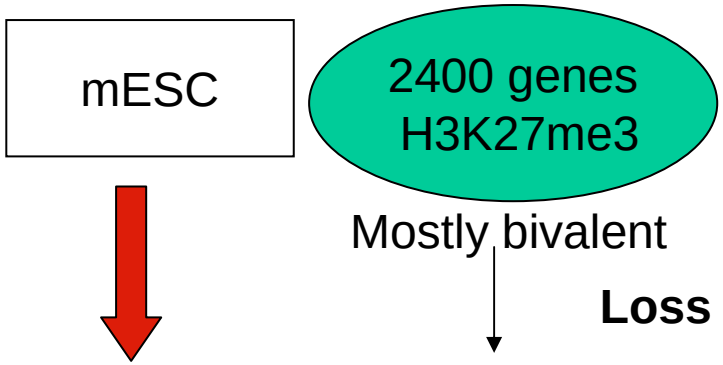
Neurons

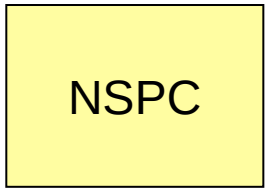
Mohn et al. 2008 Mol Cell 30: 755-766

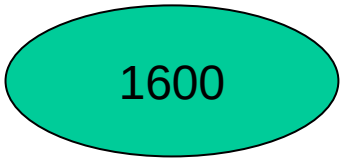


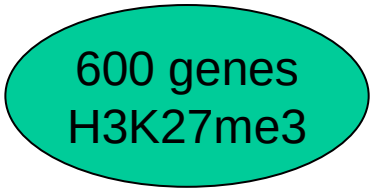
Mostly bivalent

Loss

Gain



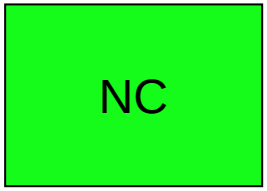


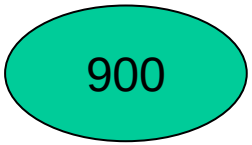


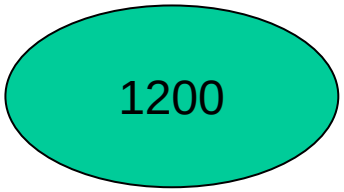
Mostly bivalent

Loss

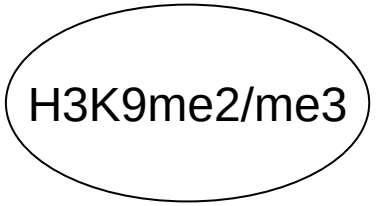
Gain







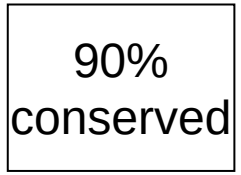
Lienert et al.2011



Total

2200

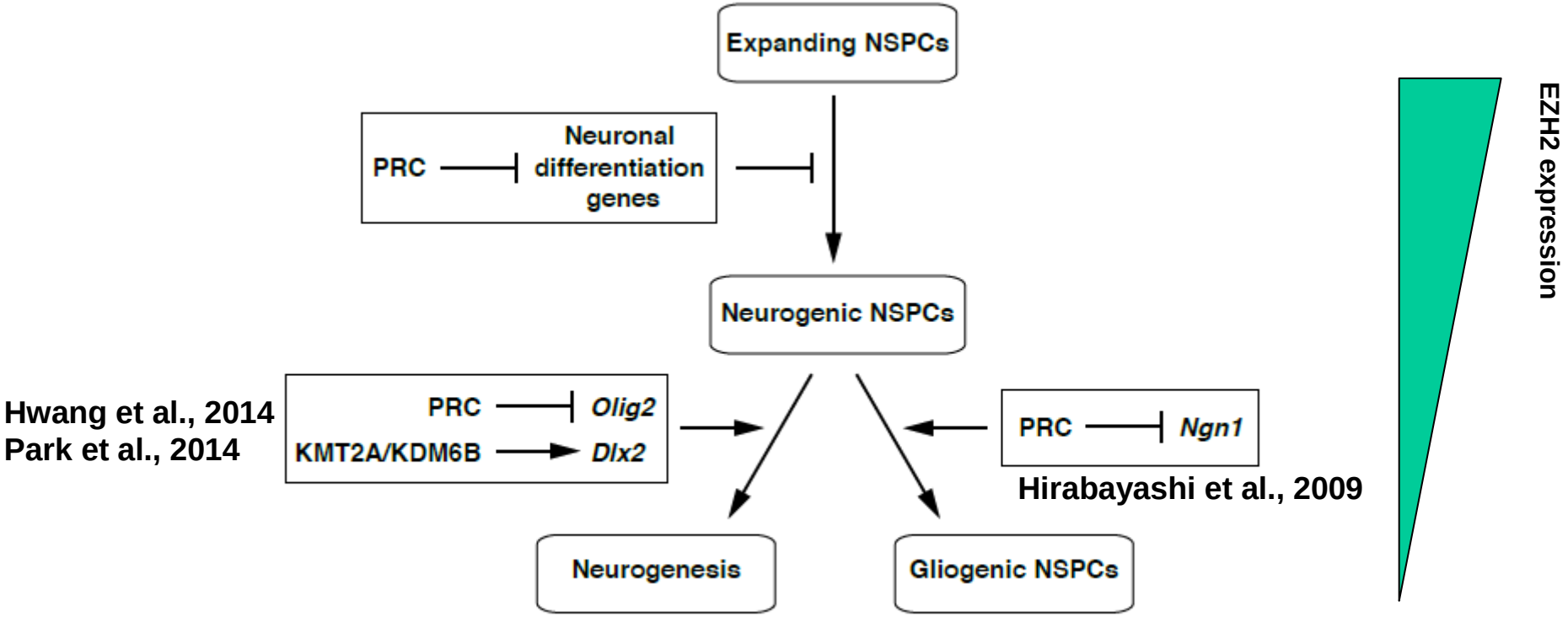
2100



PRC: polycomb repressive complex
EZH1/2 HMT

→ H3K27me3 Deposition ⊣ KDM6B

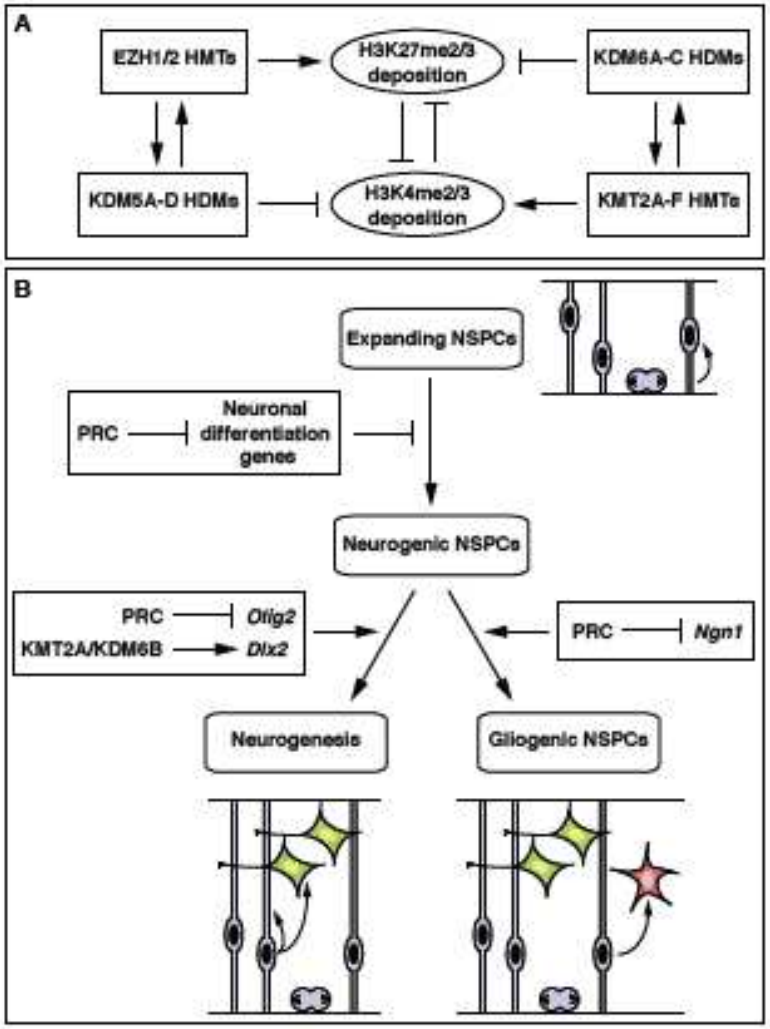
KMT2A → H3K4me2/3 ⊣ KDM5A-D



Hwang et al., 2014
Park et al., 2014

Hirabayashi et al., 2009

Figure 2



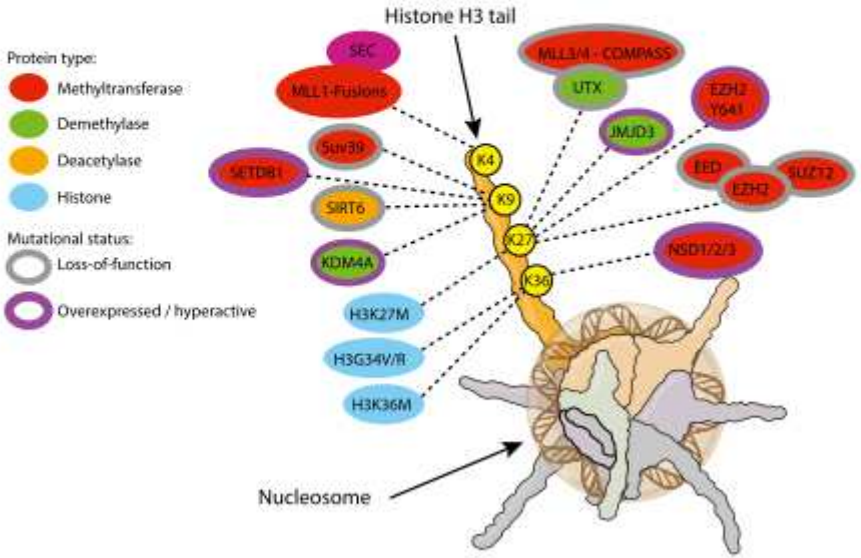
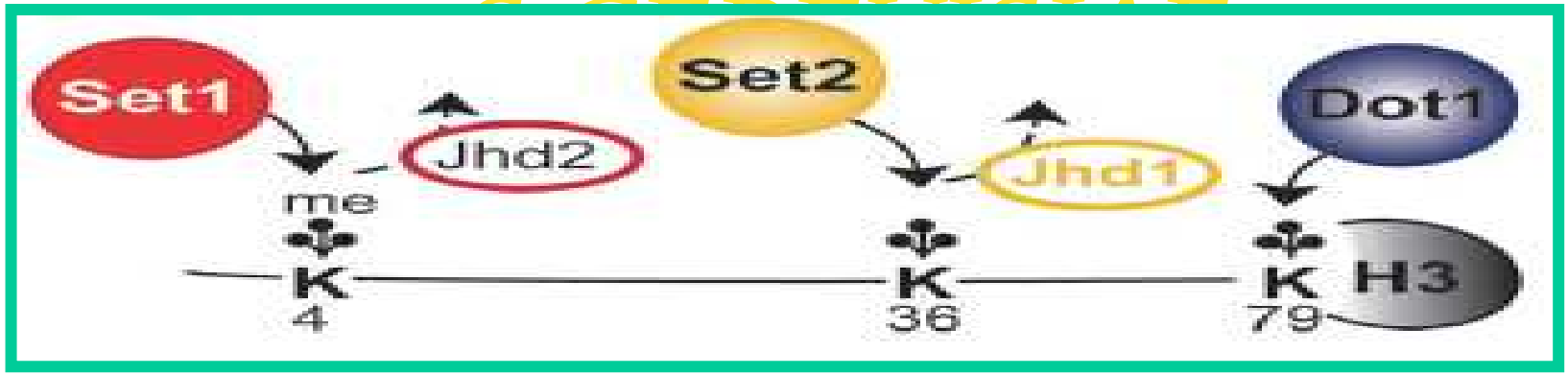


Figure 1. Chromatin proteins mutated in cancer. A summary of cancer mutations that affect post-translational modifications of the histone H3 N-terminal tail. Protein classes are indicated by the fill color for the ovals ([red] methyltransferase; [green] demethylase; [orange] deacetylase; [blue] histone), whereas mutational status is indicated by the outline color ([gray] loss of function; [purple] overexpressed/hyperactive). Dashed lines indicate the residue of histone H3 that is expected to be modified due to the indicated cancer mutations.

Chromatin signatures of cancer Marc A. Morgan and Ali Shilatifard, 2015

H3 LYSINE METHYLATION IN



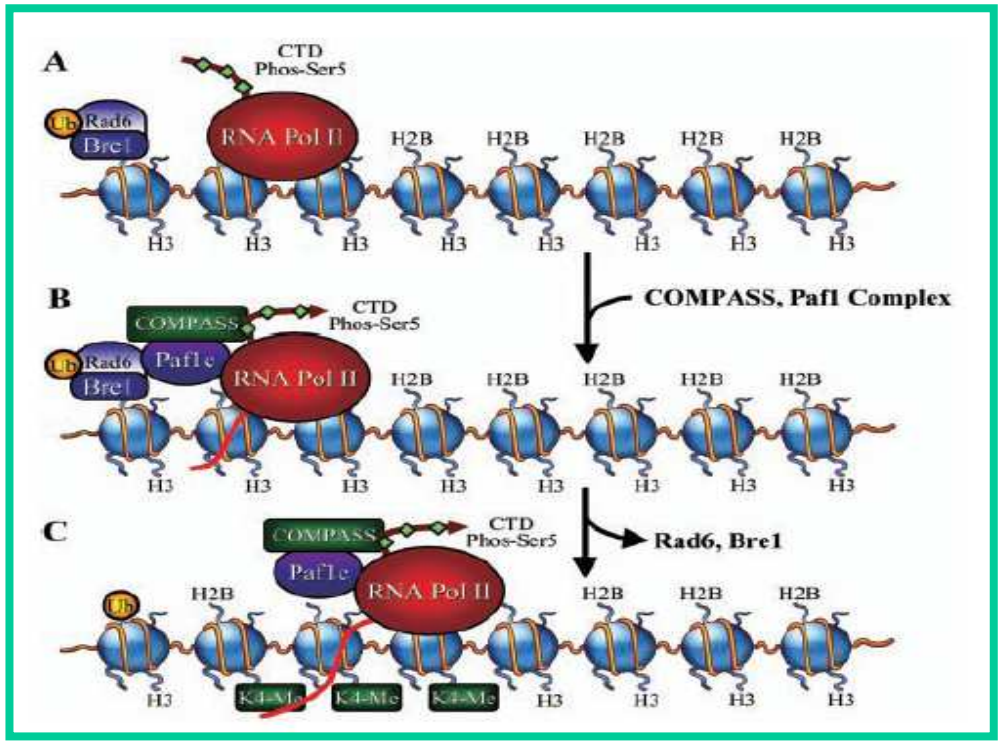
- Transcriptional memory
- Telomeric silencing
- rDNA silencing
- DNA repair

- Contrasting cryptic initiation
- RNA Pol II elongation

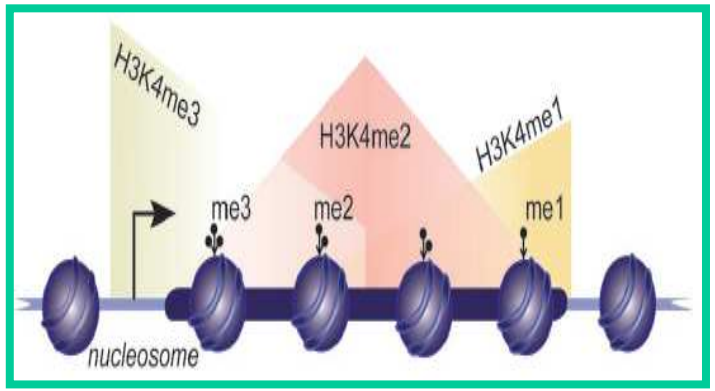
- Telomeric silencing
- DNA damage checkpoint
- DNA repair

H3K4 TRIMETHYLATION

H3K4 IS TRIMETHYLATED BY COMPASS DURING TRANSCRIPTIONAL INITIALIZATION, IN CONCERT WITH PHOSPHORYLATION ON SER5 OF RPB1 CTD



IT PEAKS ON 5’ OF TRANSCRIBED GENES, PRESENT ON THE FIRST TWO OR THREE NUCLEOSOMES

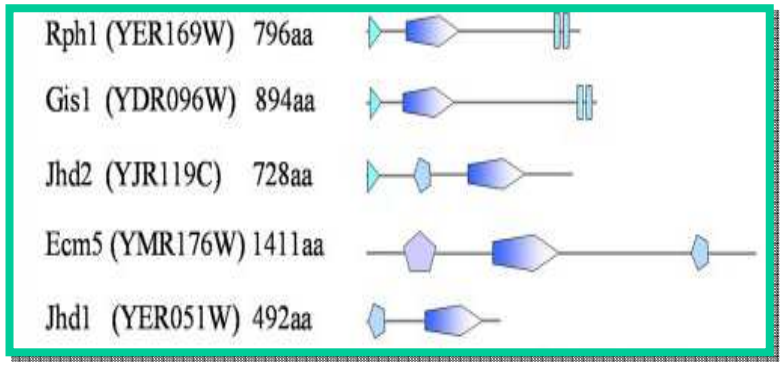


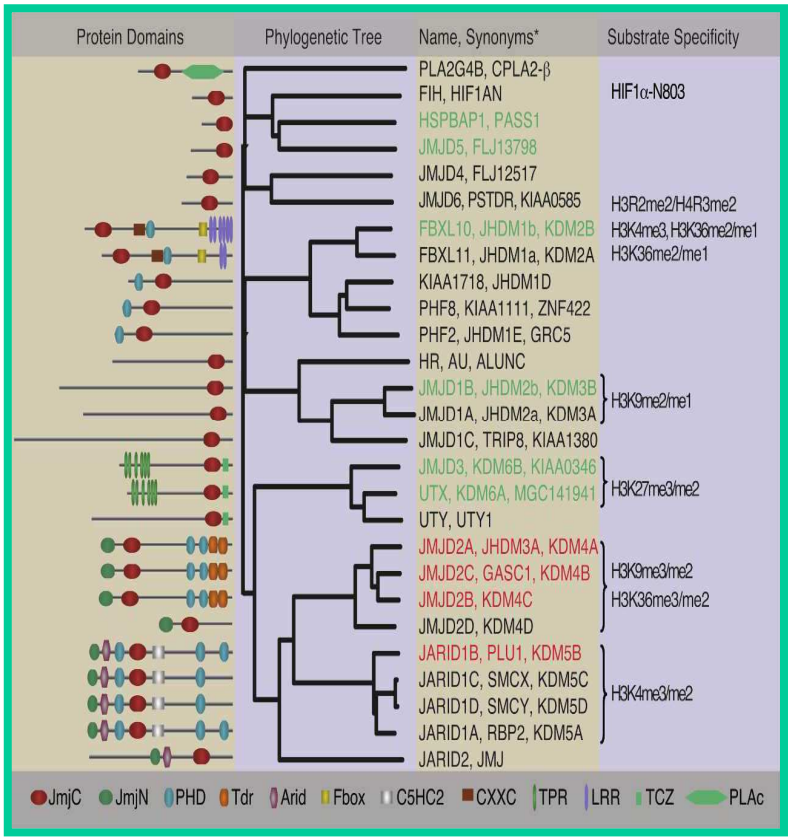
HISTONE LYSINE DEMETHYLATION

1. LSD1 FAMILY (KDM1)

2. JMJC FAMILY (KDM2-6):

- 5 MEMBERS IN *S.CEREVISIAE*
- 27 MEMBERS IN *H.SAPIENS*





JHD2

Identification of Histone Demethylases in *Saccharomyces cerevisiae*

Received for publication, October 23, 2006, and in revised form, February 15, 2007 Published, JBC Papers in Press, March 16, 2007, DOI 10.1074/jbc.M609900200

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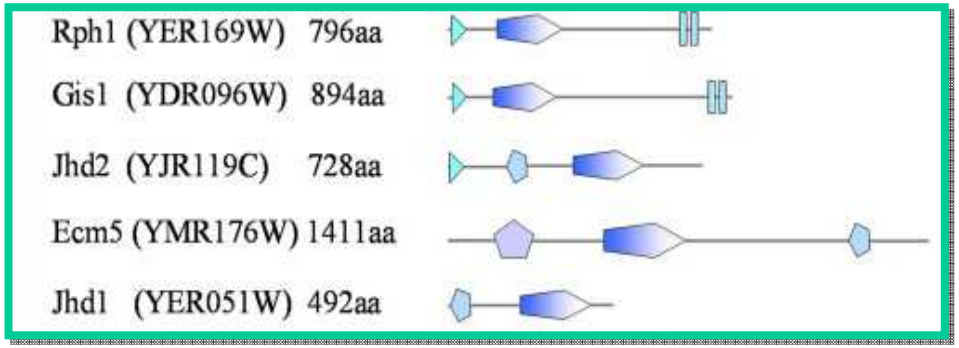
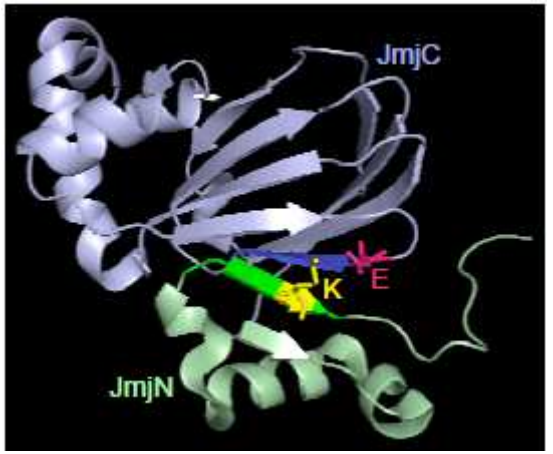
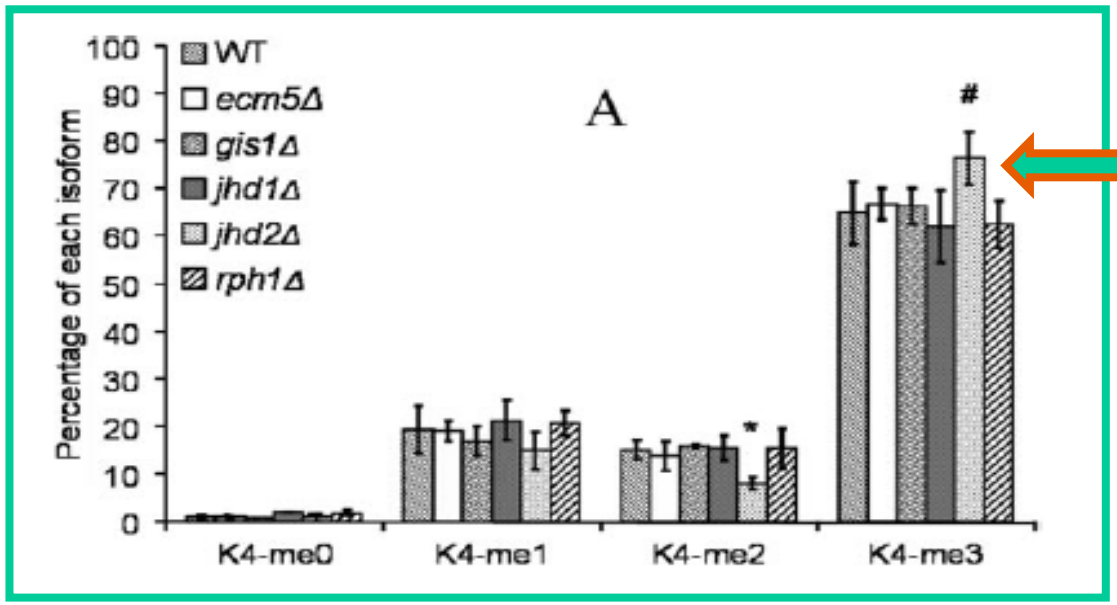




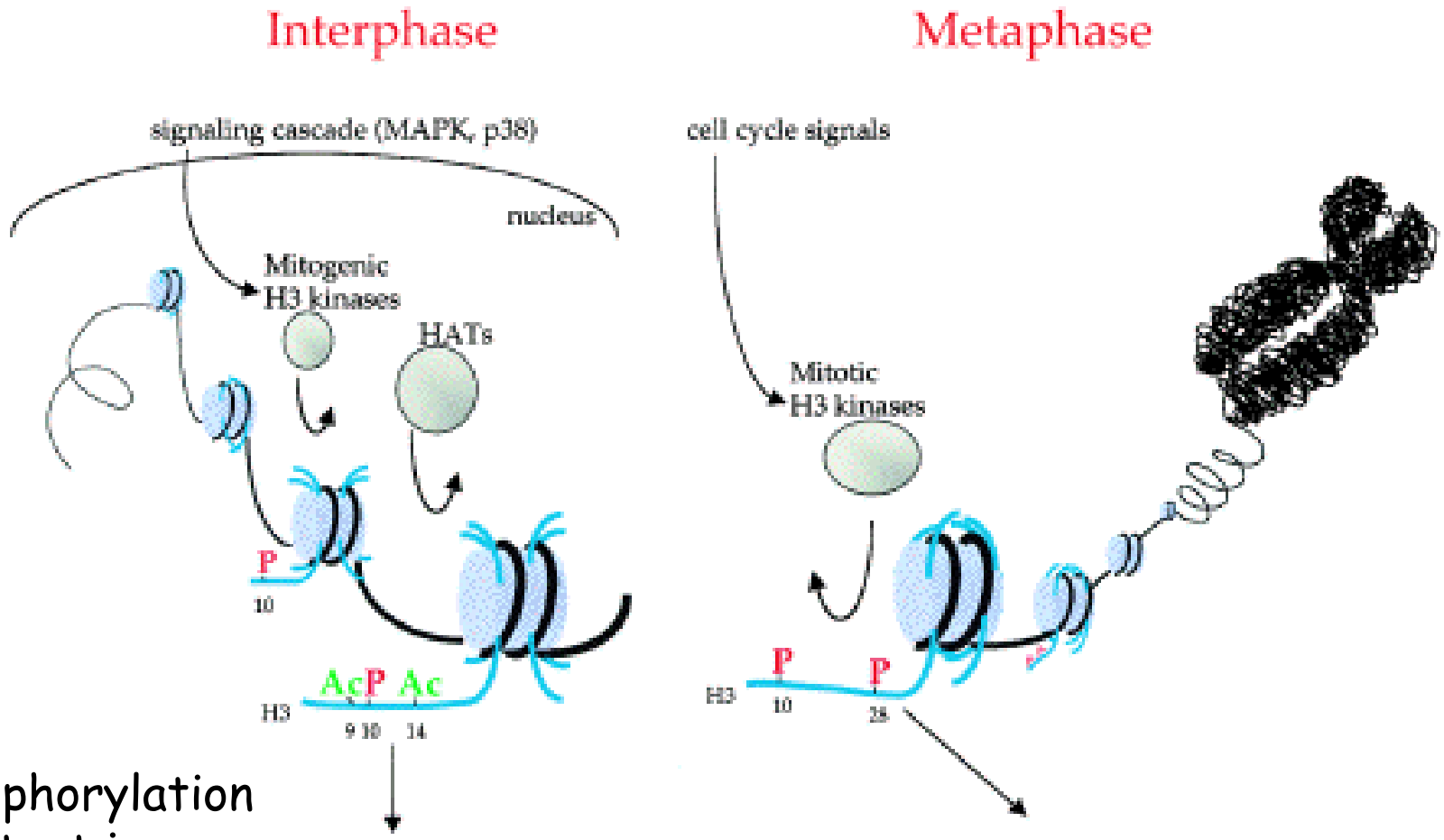


ΔJHD2 STRAIN ONLY SLIGHTLY INCREASES ITS AMOUNT OF TRIMETHYL H3K4

Methylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H1	Lys26	Ezh2	transcriptional silencing	(48,49)
H3	Lys4	Set1 (S. cerevisiae)	permissive euchromatin (di-Me)	(26)
		Set7/9 (vertebrates)	transcriptional activation (tri-Me)	(27)
		MLL, ALL-1	transcriptional activation	(28,29)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
	Arg8	PRMT5	transcriptional repression	(31)
	Lys9	Suv39h,Clr4	transcriptional silencing (tri-Me)	(32,33)
		G9a	transcriptional repression genomic imprinting	(34)
		SETDB1	transcriptional repression (tri-Me)	(35)
		Dim-5 (N.crassa), Kryptonite (A. thaliana)	DNA methylation (tri-Me)	(36,37)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
	Arg17	CARM1	transcriptional activation	(18)
	Lys27	Ezh2	transcriptional silencing	(38)
			X inactivation (tri-Me)	
		G9a	transcriptional silencing	(34)
	Lys36	Set2	transcriptional activation (elongation)	(39)
	Lys79	Dot1	euchromatin	(40)
			transcriptional activation (elongation)	(41)
			checkpoint response	(42)
H4	Arg3	PRMT1	transcriptional activation	(43)
		PRMT5	transcriptional repression	(31)
	Lys20	PR-Set7	transcriptional silencing (mono-Me)	(44)
		Suv4-20h	heterochromatin (tri-Me)	(45)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
		Set9 (S. pombe)	checkpoint response	(46)
	Lys59	unknown	transcriptional silencing	(47)

Phosphorylation



H3 phosphorylation is important in gene activation and mitotic chromosome condensation

Phosphorylation

La fosforilazione sembra essere un processo importante per la capacità di creare un "concentramento di cariche".

La fosforilazione delle zone positive di H1 favorisca la dissociazione di questo dal DNA

La variante istonica H2A.Z è sensibile alla fosforilazione attraverso un meccanismo ancora poco chiaro

H2A.X contiene un sito che viene fosforilato in risposta alle DSBs

Phosphorylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H1	Ser27	unknown	transcriptional activation, chromatin decondensation	(48,49)
H2A	Ser1	unknown	mitosis, chromatin assembly	(50)
		MSK1	transcriptional repression	(51)
	Thr119 (D. melanogaster)	NHK1	mitosis	(52)
	Ser122 (S. cerevisiae)	unknown	DNA repair	(53)
	Ser129 (S. cerevisiae)	Mec1, Tel1	DNA repair	(54,55)
	Ser139 (mammalian H2A.X)	ATR, ATM, DNA-PK	DNA repair	(56-58)
H2B	Ser10 (S. cerevisiae)	Ste20	apoptosis	(59)
	Ser14 (vertebrates)	Mst1	apoptosis	(60)
		unknown	DNA repair	(61)
	Ser33 (D. melanogaster)	TAF1	transcriptional activation	(62)
H3	Thr3	Haspin/Gsg2	mitosis	(63)
	Ser10	Aurora-B kinase	mitosis, meiosis	(64,65)
		MSK1, MSK2	immediate-early gene activation	(66)
		IKK-α	transcriptional activation	(67)
		Snf1	transcriptional activation	(68)
	Thr11 (mammals)	Dlk/Zip	mitosis	(69)
	Ser28 (mammals)	Aurora-B kinase	mitosis	(70)
		MSK1, MSK2	immediate-early activation	(66,71)
H4	Ser1	unknown	mitosis, chromatin assembly	(50)
		CK2	DNA repair	(72)

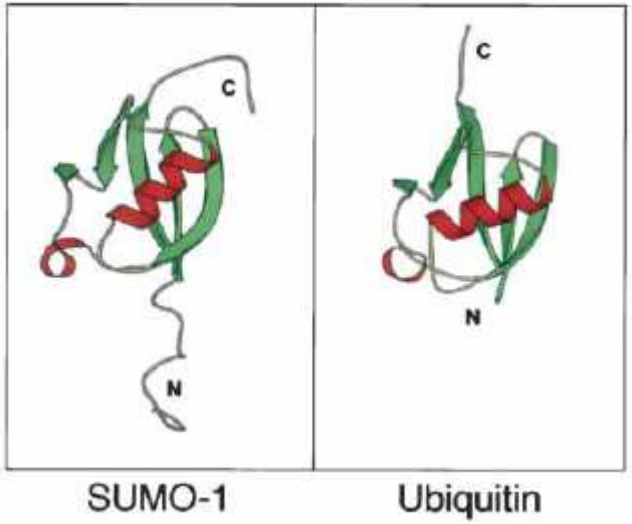
Sumolylation

Sumo is an
ubiquitine-like protein

Histone sumolylation
is associated to:
- Gene expression
- Chromatin structural changes
- Signal transduction
- Genome stability

- Not related to degradation

Substrate: H4 lysine
Associated to gene repression



Ubiquitylation

Associated to:

- DNA repair
- Cell cycle control

- Transcriptional regulation

- Not related to degradation

Substrate: H2B lysine 123

Ligase: RAD6

Ubiquitylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys119 (mammals)	Ring2	spermatogenesis	(73)
H2B	Lys120 (mammals)	UbcH6	meiosis	(74)
	Lys123 (S. cerevisiae)	Rad6	transcriptional activation euchromatin	(75)

Sumoylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys126 (S. cerevisiae)	Ubc9	transcriptional repression	(76)
H2B	Lys6 or Lys7 (S. cerevisiae)	Ubc9	transcriptional repression	(76)
H4	N-terminal tail (S. cerevisiae)	Ubc9	transcriptional repression	(77)

Biotinylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys9	biotinidase	unknown	(78)
	Lys13	biotinidase	unknown	(78)
H3	Lys4	biotinidase	gene expression	(79)
	Lys9	biotinidase	gene expression	(79)
	Lys18	biotinidase	gene expression	(79)
H4	Lys12	biotinidase	DNA damage response	(80,81)

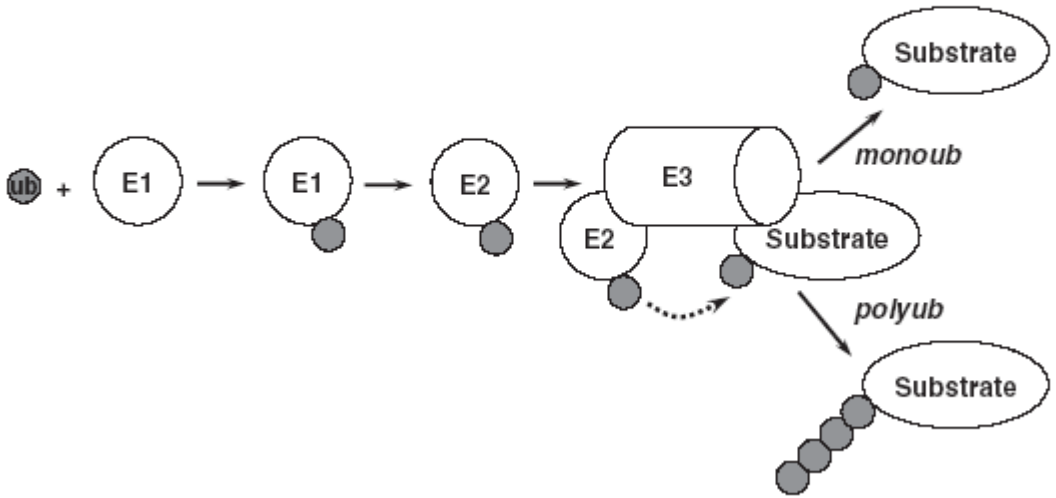
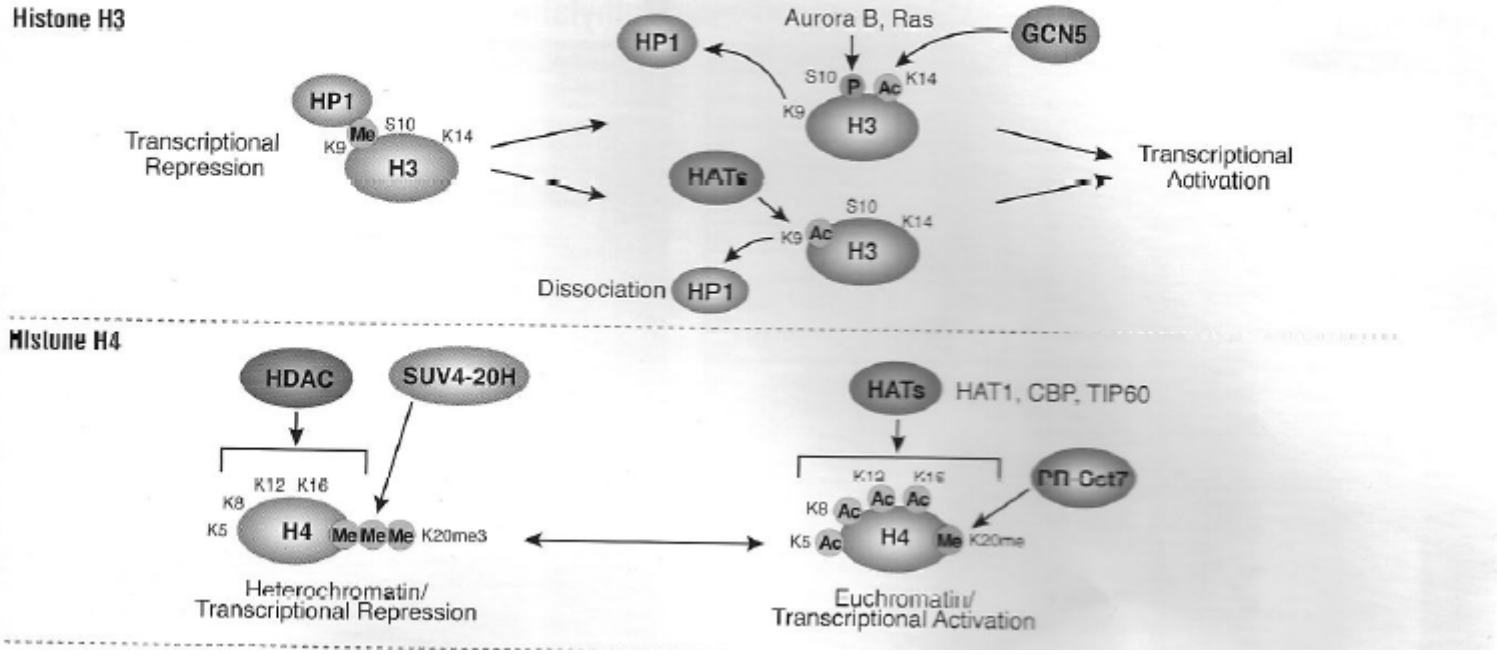


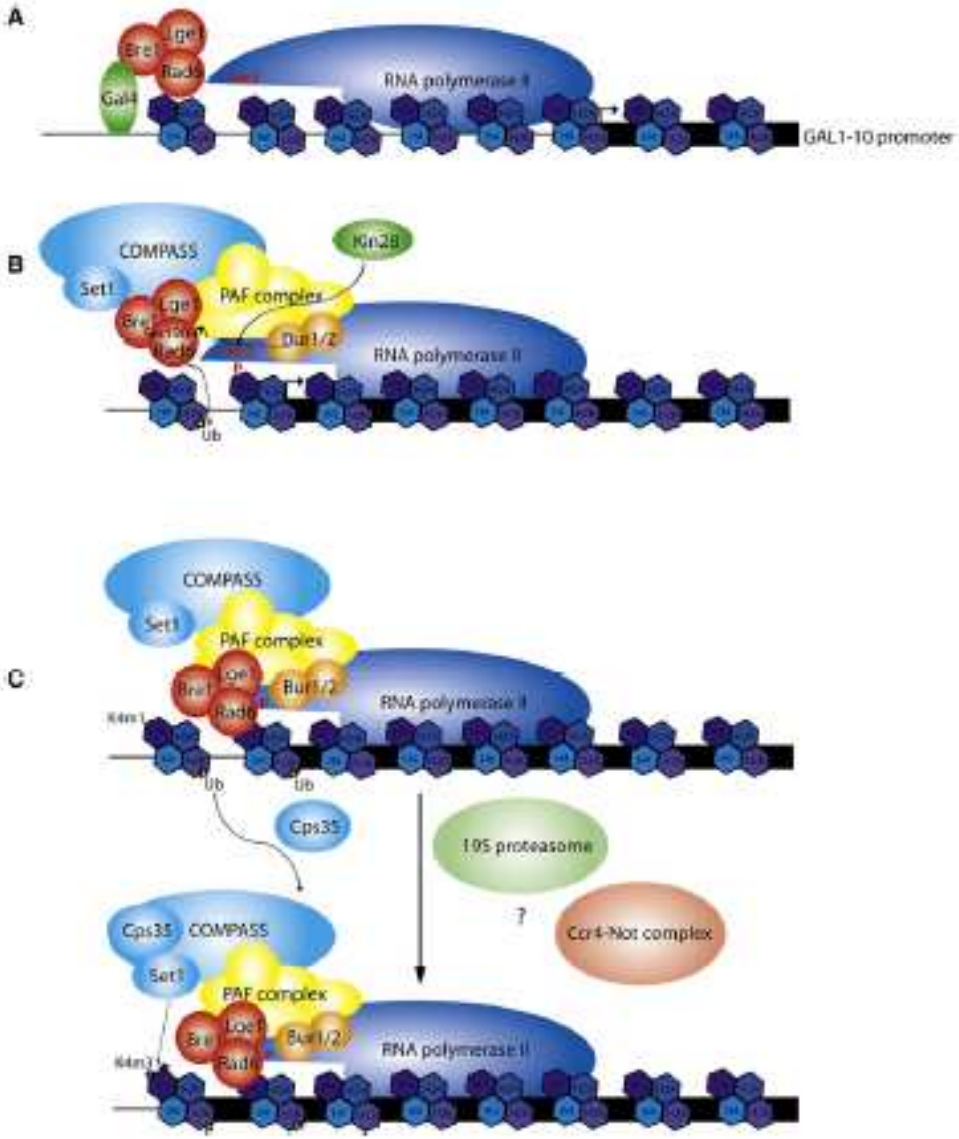
Table 1. Enzymes Involved in H2A and H2B Ubiquitination/Deubiquitination in Different Organisms

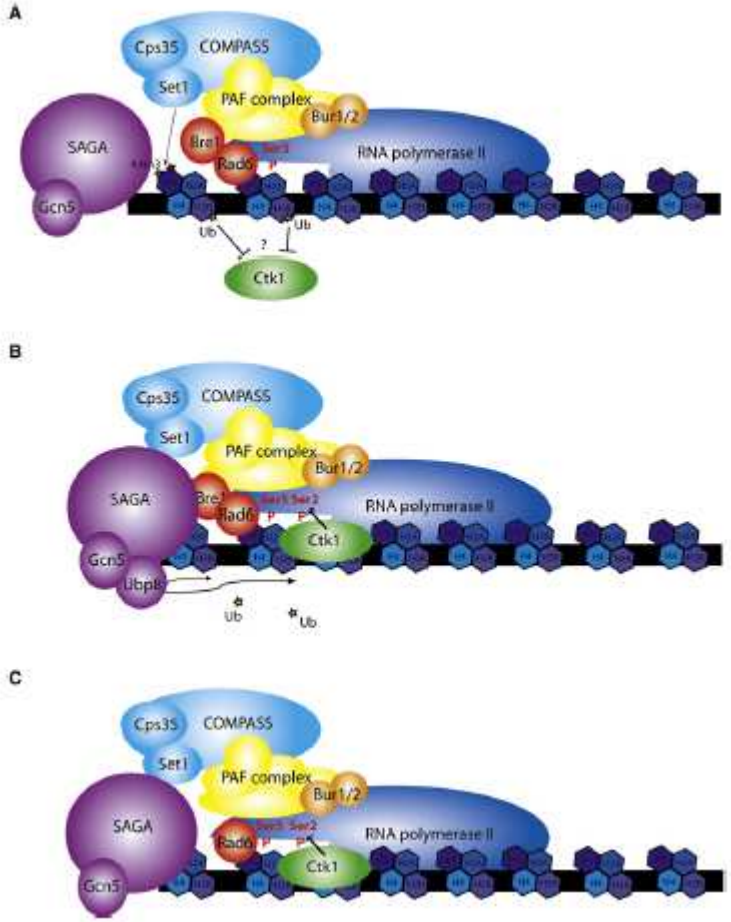
	H2B Ubiquitination		H2B Deubiquitination		H2A Ubiquitination		H2A Deubiquitination
	E2	E3	Transcription	Silencing	E2	E3	
S. cerevisiae	Rad6	Bre1	Ubp8	Ubp10 (Dot4)	–	–	–
S. pombe	Rhp6	Brl1 (Rfp2/Spcc1919.15) Brl2 (Rfp1/Spcc970.10c)			?		
Drosophila	Dhr6	Bre1 (CG10542)	Nonstop	USP7		dRing (Sce)	
Mouse	mHR6A/mHR6B						
Human	hHR6A/hHR6B *UbcH6 ? Mdm2 ?*	RNF20	USP22 *USP3 ?*			Ring1B (Ring2/Rnf2) 2A-HUB (hRUL138)	Ubp-M (USP16) 2A-DUB (MYSM1) USP21 *USP3 ?*
Arabidopsis		HUB1		SUP32 (UBP26)			

Examples of Crosstalk Between Post-translational Modifications

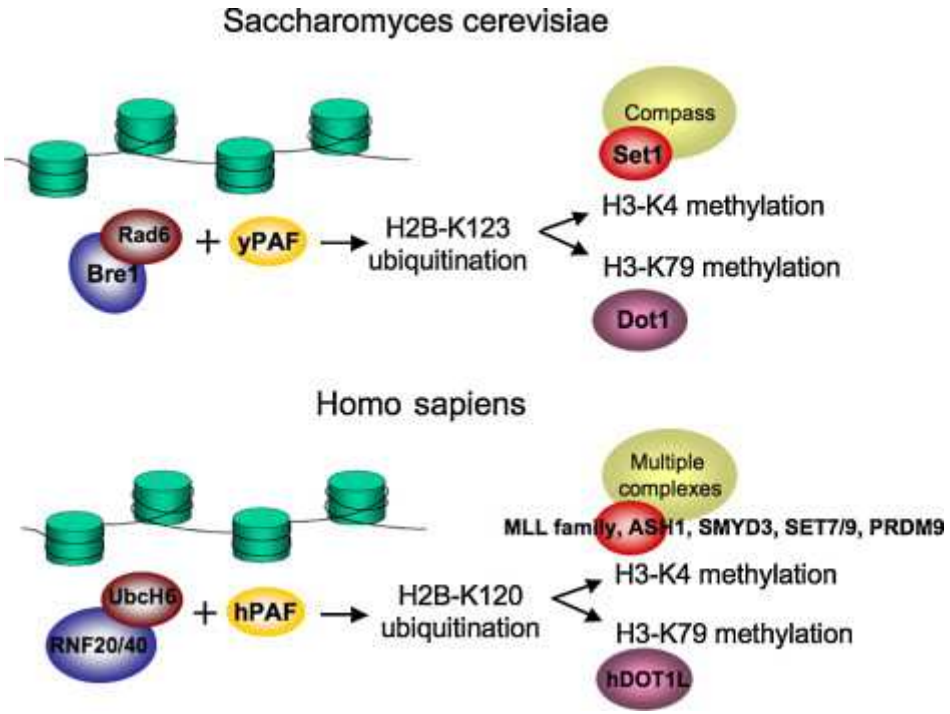


Trans-Tail
Pathway




A
Cps35
COMPASS
Set1
PAF complex
Bur1/2
Bre1
Rad6
SAGA
RNA polymerase II
Gcn5
Ub
?
Ub
Ctk1
B
Cps35
COMPASS
Set1
PAF complex
Bur1/2
SAGA
Bre1
Rad6
RNA polymerase II
Ctk1
Gcn5
Ubp8
Ub
Ub
C
Cps35
COMPASS
Set1
PAF complex
Bur1/2
SAGA
Rad6
RNA polymerase II
Ctk1
Gcn5

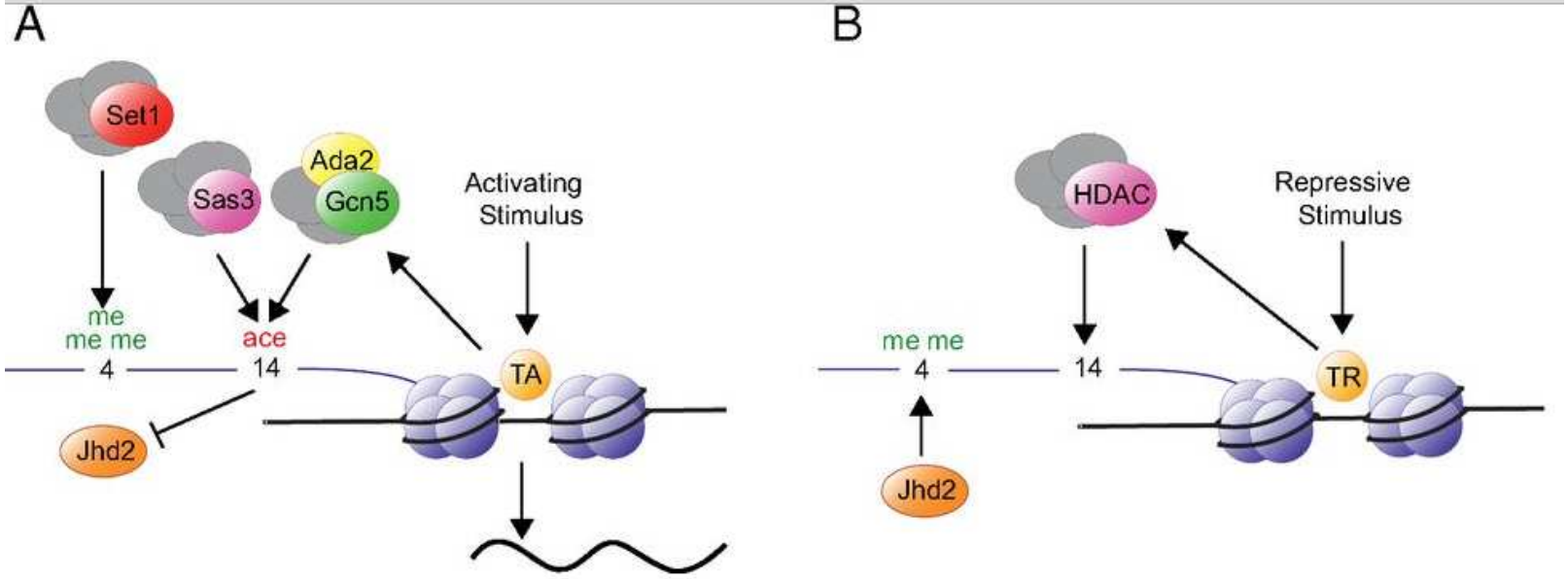
Regulative cross-talks among histone modification



Histone H3K4 demethylation is negatively regulated by histone H3 acetylation in *Saccharomyces cerevisiae*

Vicki E. Maltby[a], Benjamin J. E. Martin[a], Julie Brind'Amour[b,1], Adam T. Chruscicki[a,1], Kristina L. McBurney[a], Julia M. Schulze[c], Ian J. Johnson[a], Mark Hills[d], Thomas Hentrich[c], Michael S. Kobor[c], Matthew C. Lorincz[b], and LeAnn J. Howe[a,2]

Departments of [a]Biochemistry and Molecular Biology and [b]Medical Genetics, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada V6T 1Z3; [c]Center for Molecular Medicine and Therapeutics, Child and Family Research Institute, Vancouver, BC, Canada V5Z 4H4; and [d]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada V5Z 1L3



Molecular Cell
Article

Lysine Acetylation Controls Local Protein Conformation by Influencing Proline Isomerization

Françoise S. Howe,[1] Ivan Boubriak,[1] Matthew J. Sale,[1,2] Anitha Nair,[1] David Clynes,[1,3] Anne Grijzenhout,[1] Struan C. Murray,[1] Ronja Woloszczuk,[1] and Jane Mellor[1,*]
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[2]Present address: Signalling Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK
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*Correspondence: jane.mellor@bioch.ox.ac.uk
http://dx.doi.org/10.1016/j.molcel.2014.07.004

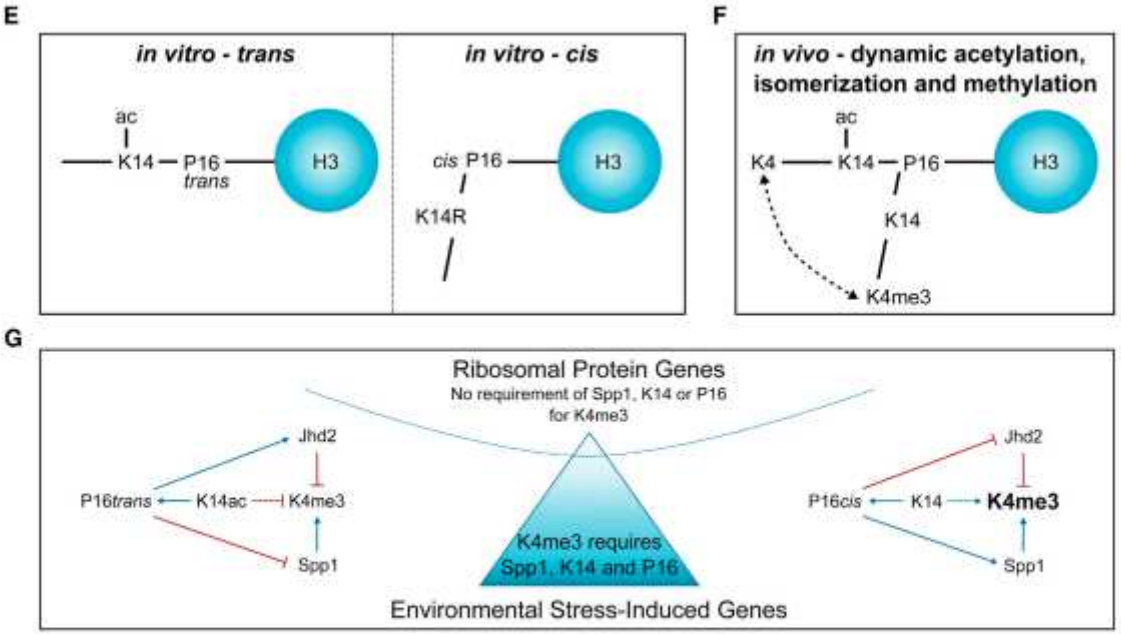
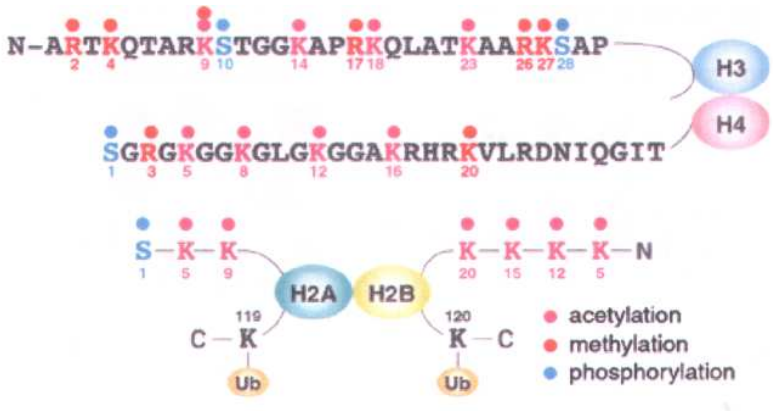


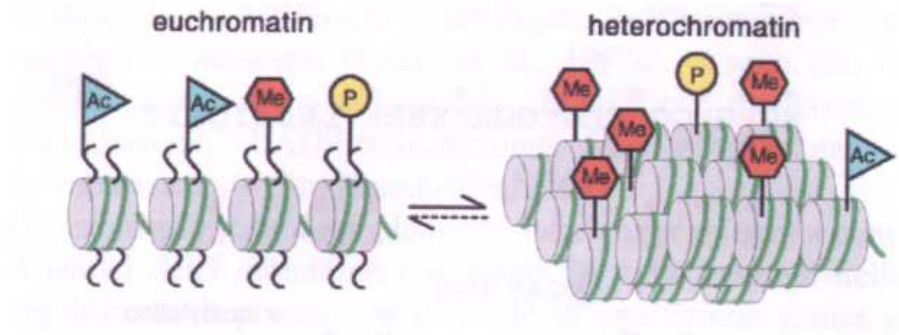
Figure 6. K14ac Promotes P16*trans*

(A) Relative binding ± SEM (n = 2–11) assessed by surface plasmon resonance of purified recombinant Spt7 bromodomain (residues 363–619) to the indicated H3 peptides (11–26).

(B) Coomassie-stained gel showing a pull-down experiment, quantified relative to the K18ac peptide, with the indicated H3 peptides and the purified recombinant Spt7 bromodomain (n = 3).

The histone code





The histone code

Allis proposed that the combination of the different aminoacids modifications on the histone tails formed a real functional code

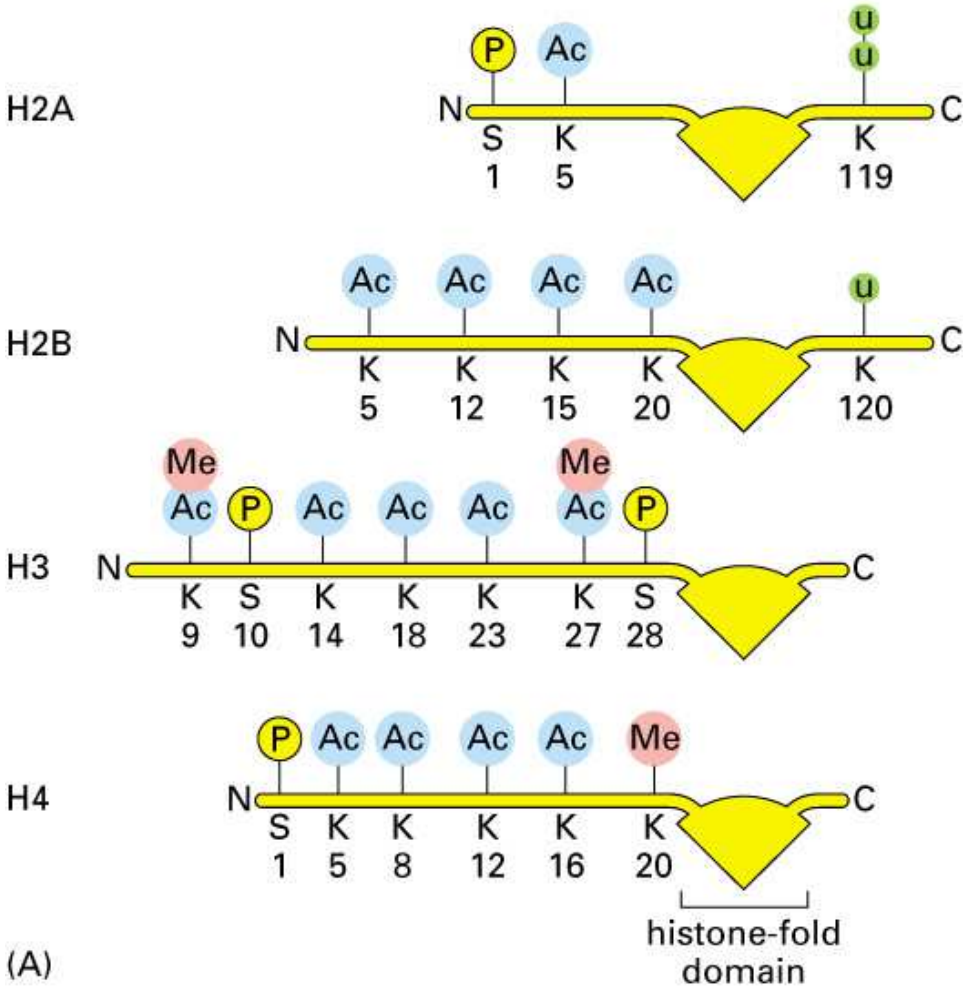
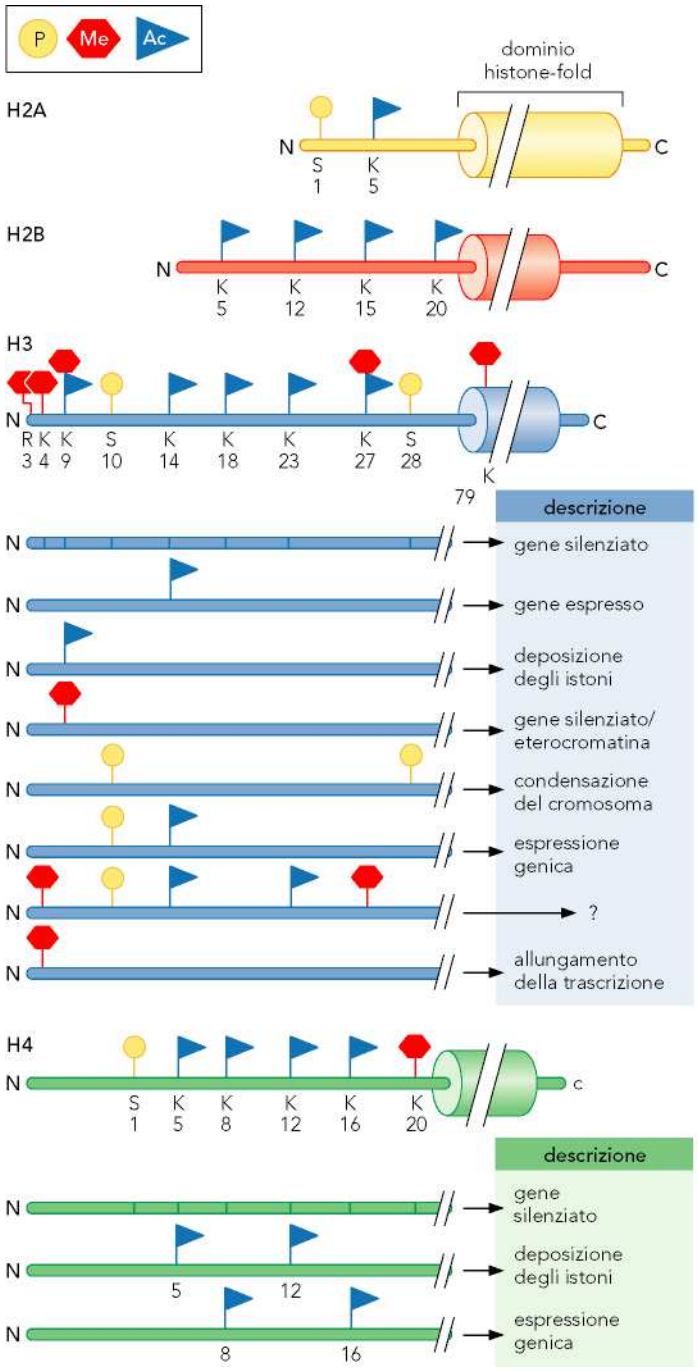
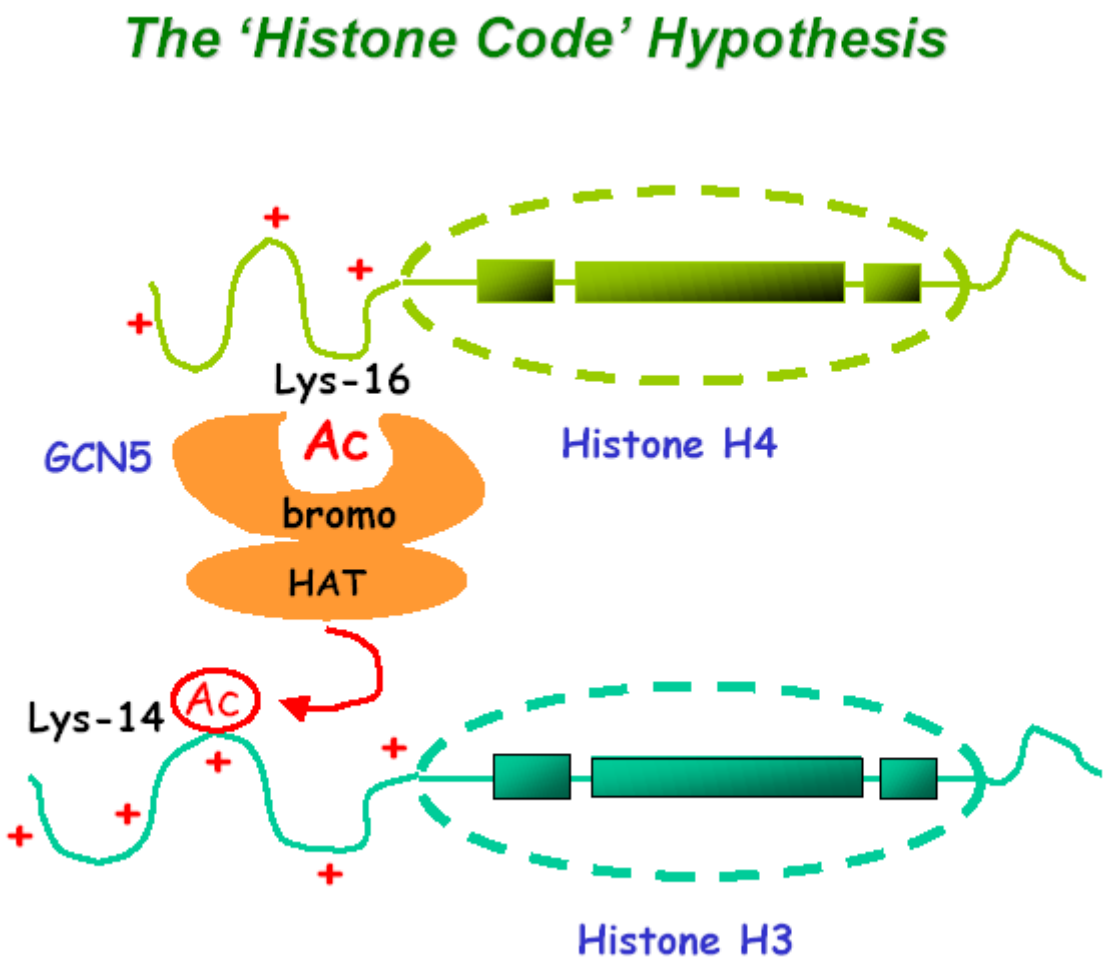


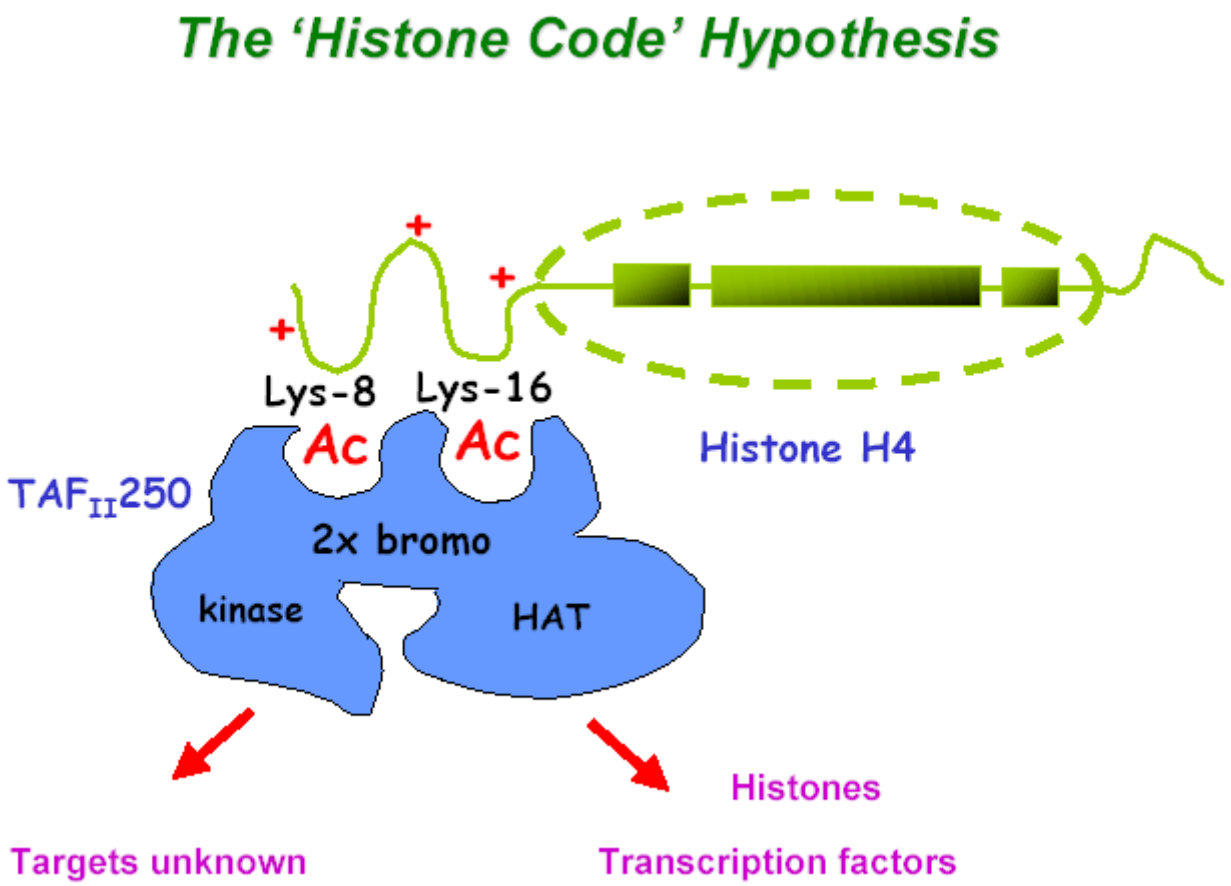
Figure 4–35 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

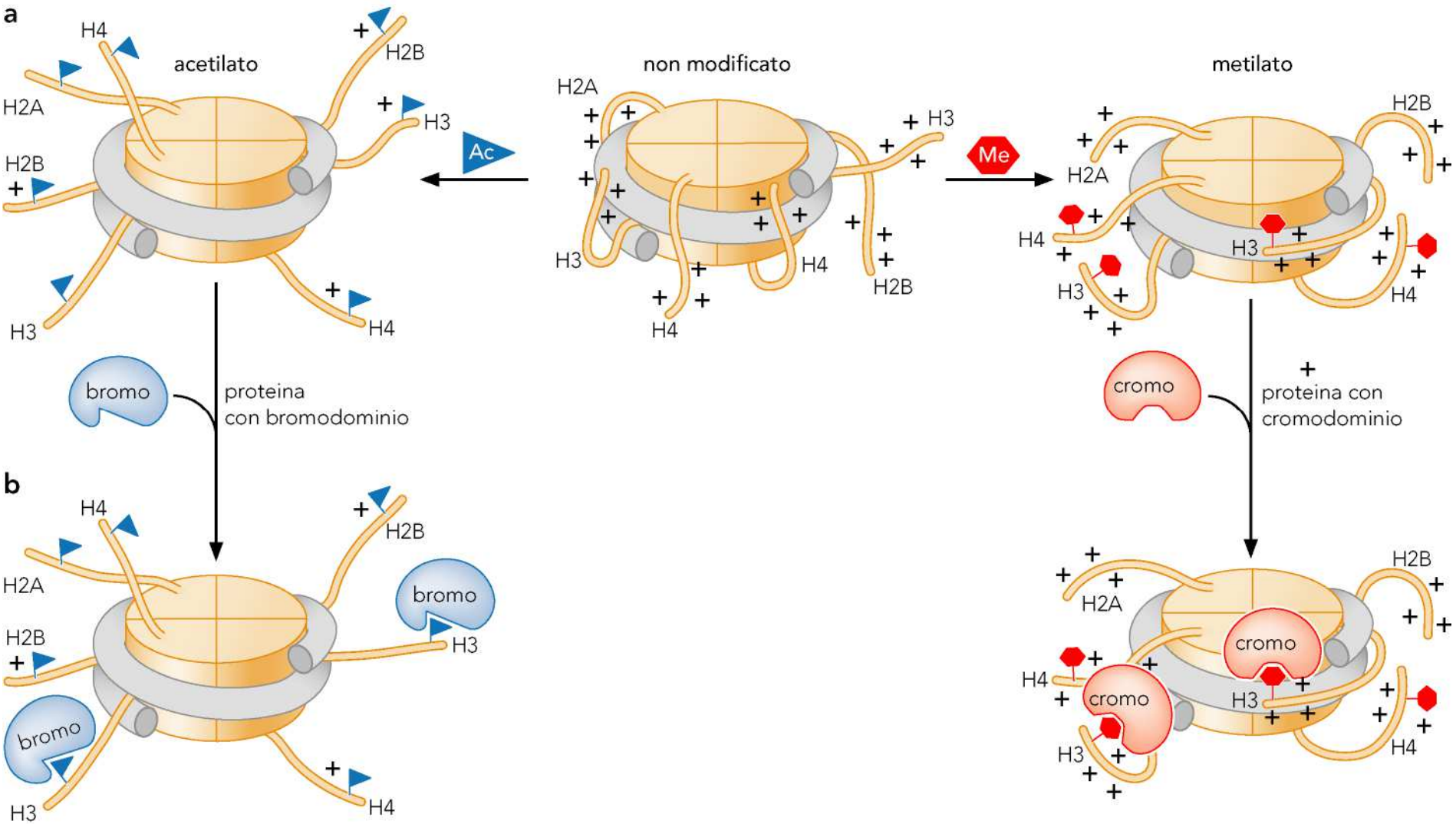

P
Me
Ac
dominio
histone-fold
H2A
N
C
S 1
K 5
H2B
K 5
K 12
K 15
K 20
H3
R K 3 4
K 9
S 10
K 14
K 18
K 23
K 27
S 28
K 79
descrizione
gene silenziato
gene espresso
deposizione degli istoni
gene silenziato/ eterocromatina
condensazione del cromosoma
espressione genica
?
allungamento della trascrizione
H4
c
S 1
K 5
K 8
K 12
K 16
K 20
gene silenziato
deposizione degli istoni
espressione genica

Histone code



Histone code




a
acetilato
H4
H2A
H2B
H3
H3
H4
Ac
non modificato
H2A
H3
H4
H2B
Me
metilato
H2A
H2B
H4
H3
H3
H4
bromo
proteina
con bromodominio
cromo
proteina con
cromodominio
b
H4
H2A
H2B
H3
H4
bromo
bromo
H2A
H2B
H4
H3
H3
H4
cromo
cromo

Nucleosome remodeling

- Chromatin remodelers acquire energy through ATP hydrolysis to remodel chromatin

- Numerous chromatin remodeling complexes exits

- They differ in the core DNA-dependent ATPase subunit

- There is some sharing of factors between complexes and homologs between species

- There are 3 main classes (more could follow)

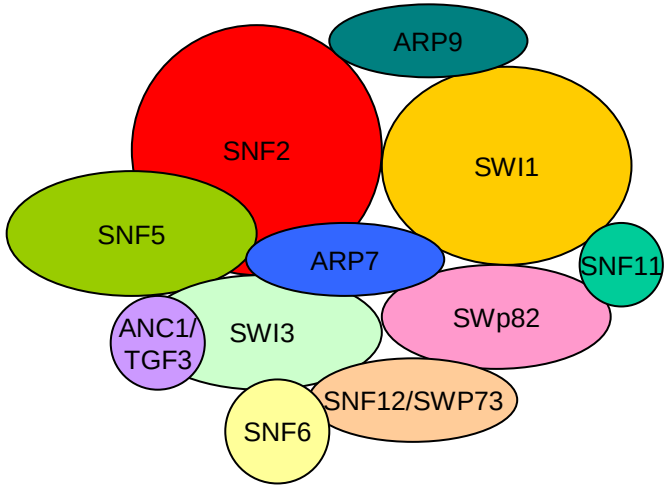
SWI/SNF → snf2

RSC → sth1

NURF → iswi

Nucleosome remodeling

The SWI/SNF complex



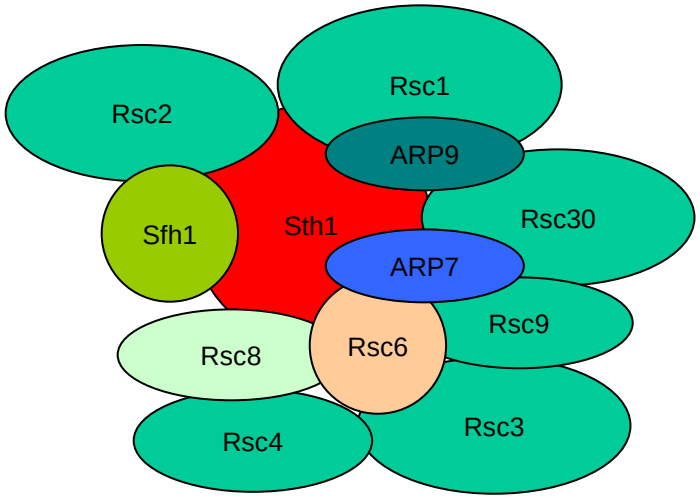
SWI (switch)
SNF (sucrose non fermenting)

- 11 subunits
- ~1 MDa
- Snf2 is an ATPase

Subunit	Size (kDa)	Function
SWI2 / SNF2	194	DNA dependent ATPase
SWI1	148	AT-rich interaction domain (ARID) for non-specific binding, Zn-finger protein
SNF5	103	Assembly and catalytic functions of the complex
SWI3	93	
SWp82p	~82	
SNF12 / SWP73	64	
ARP7	54	Actin-related protein
ARP9	53	Actin-related protein
SNF6	38	
ANC1 / TFG3	27	
SNF11	19	Interacts with the N-terminal D1 region of SWI2

Nucleosome remodeling

The RSC (remodels the structure of chromatin) complex



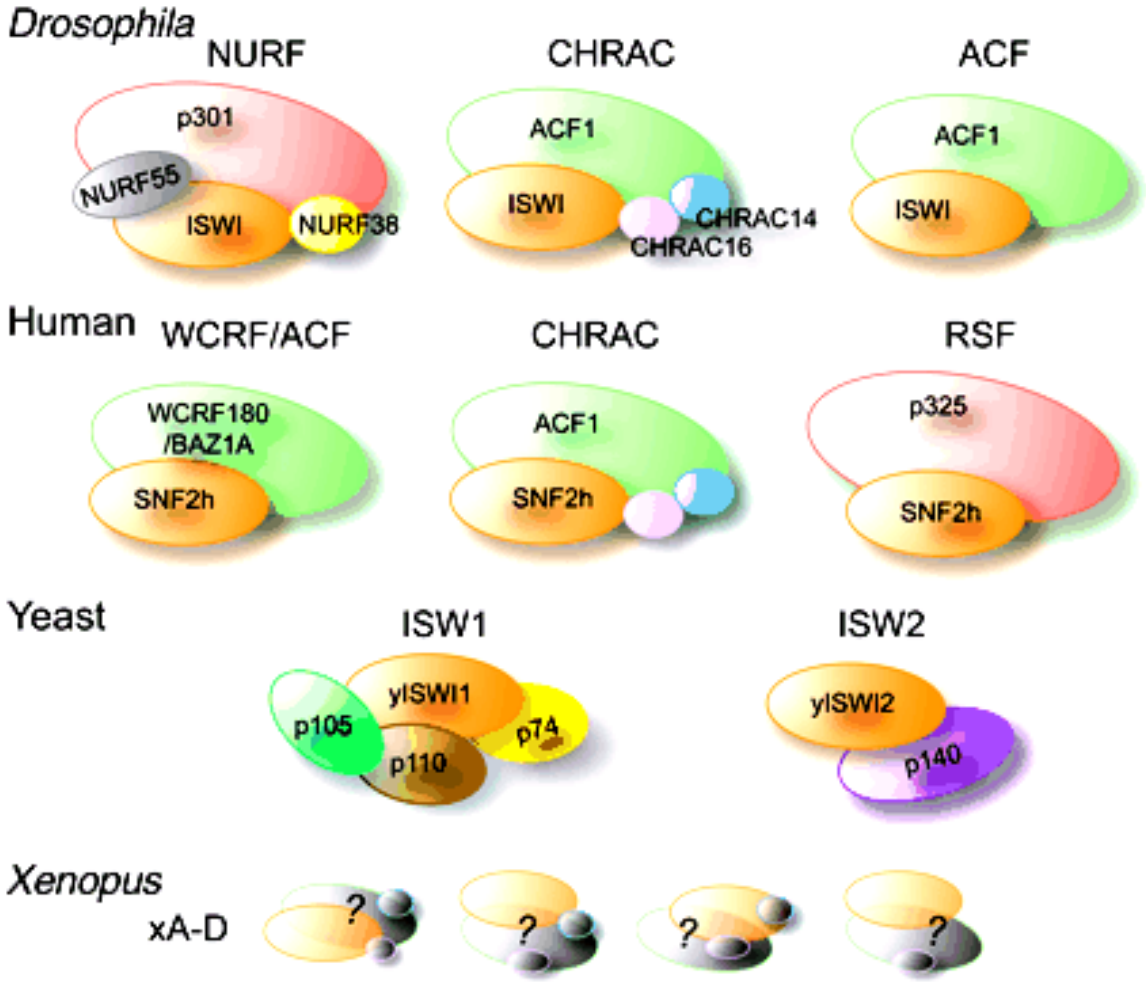
- 12+ subunits
- ~1 MDa
- Sth1 (Snf two homolog) is an ATPase

Subunits with no known homolog in Swi/Snf

Subunit	SWI/SNF homolog	Size (kDa)	Function
Sth1	SWI2 / SNF2	157	DNA dependent ATPase
Rsc1		107	
Rsc2		102	
Rsc3		102	
Rsc30		101	
Rsc4		72	
Rsc8	SWI3	63	Subunit assembly, binds through C-terminal coiled coil domain
Rsc9		65	
Sfh1	SNF5	49	Assembly and catalytic functions of the complex
Rsc6	SWP73	54	
Arp7	ARP7	54	Actin-related protein
Arp9	ARP9	53	Actin-related protein

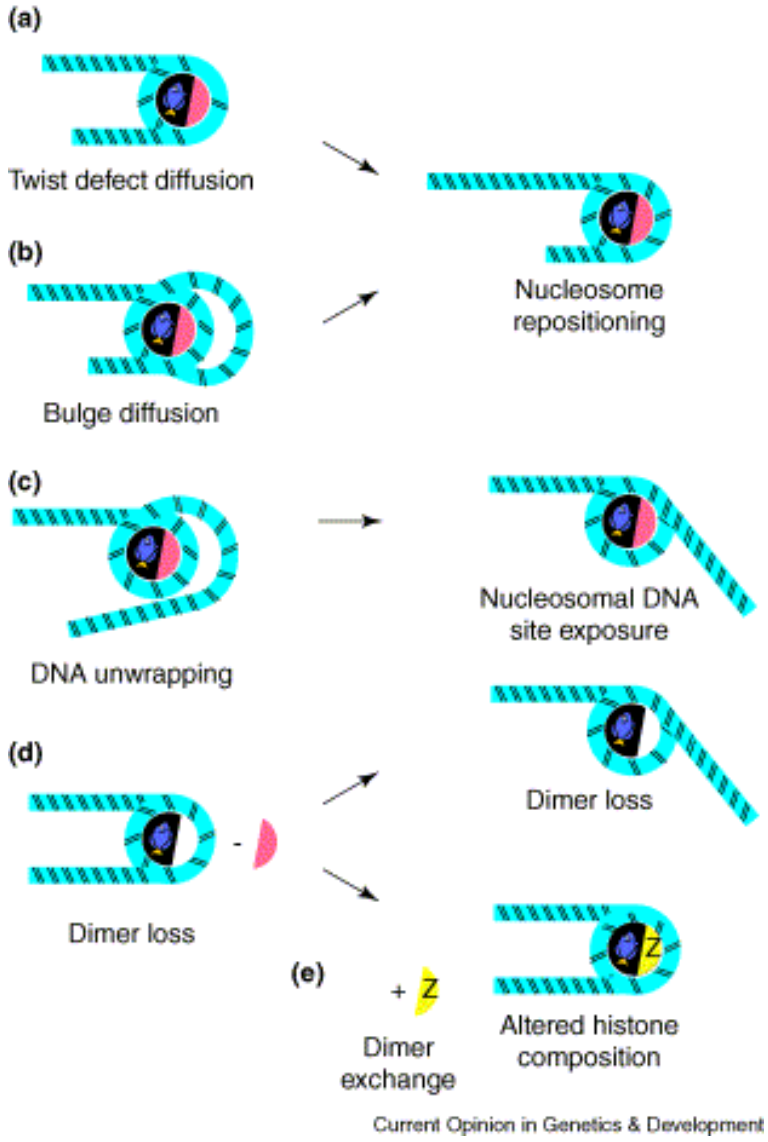
Nucleosome remodeling

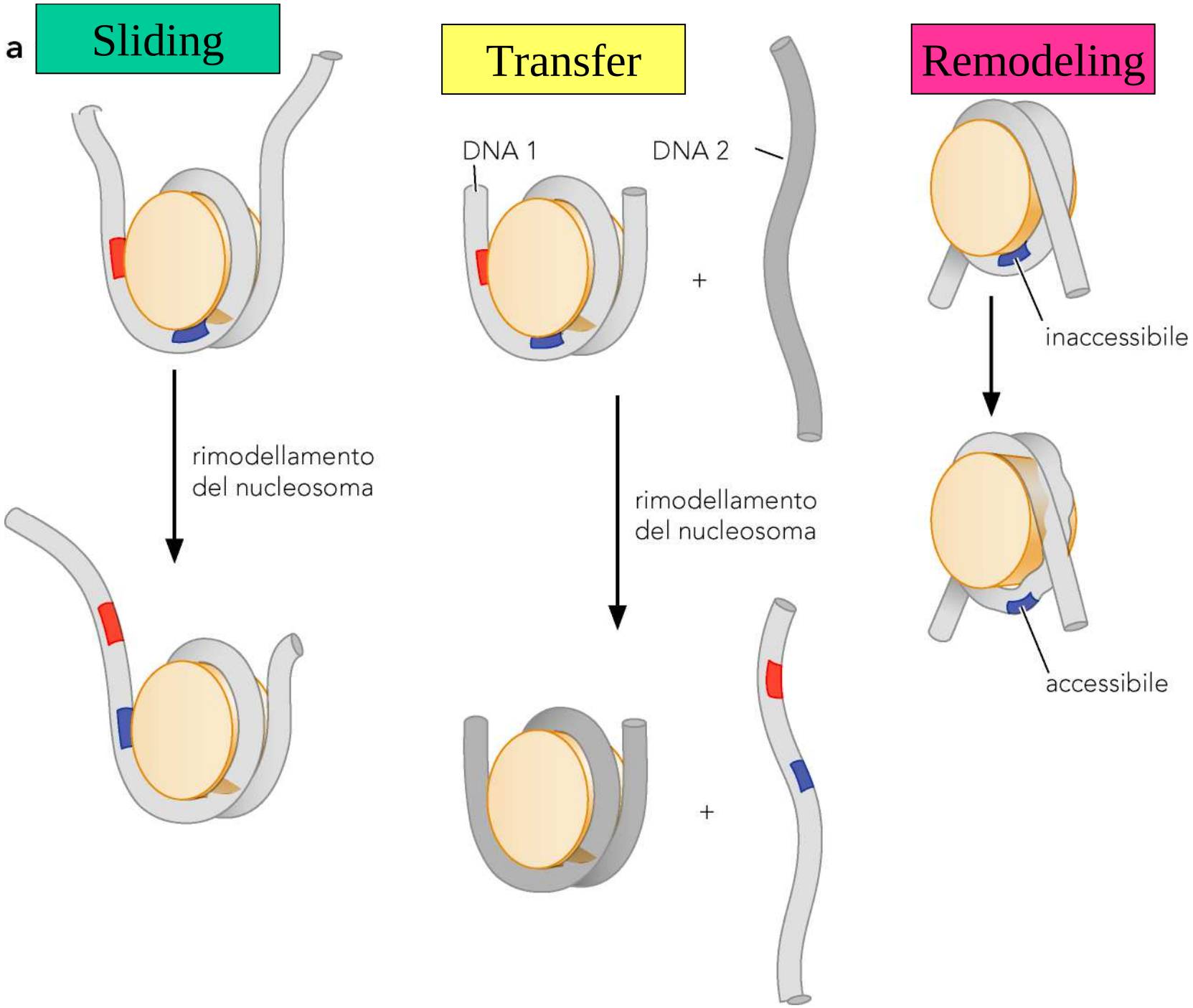
Multiple types of ISWI complexes exits



Nucleosome remodeling

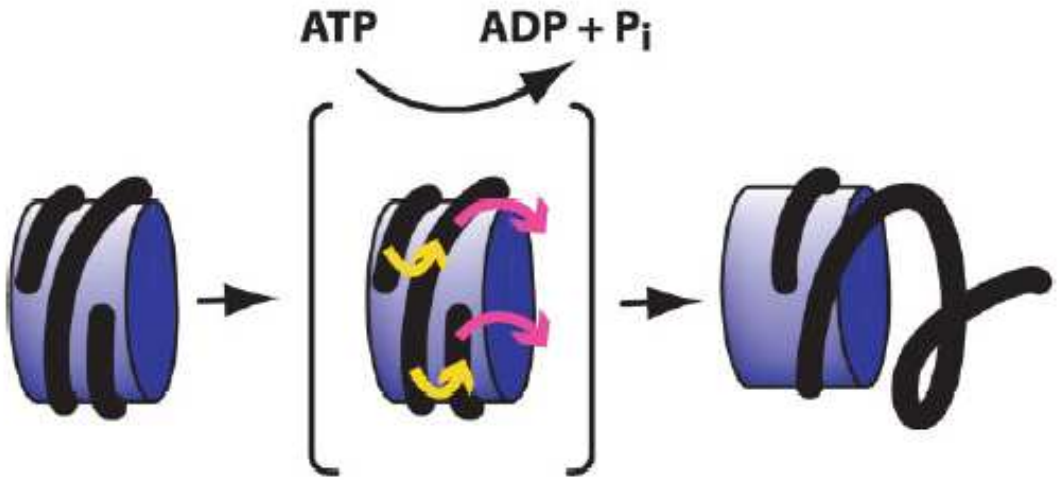
How does a chromatin remodeler function?



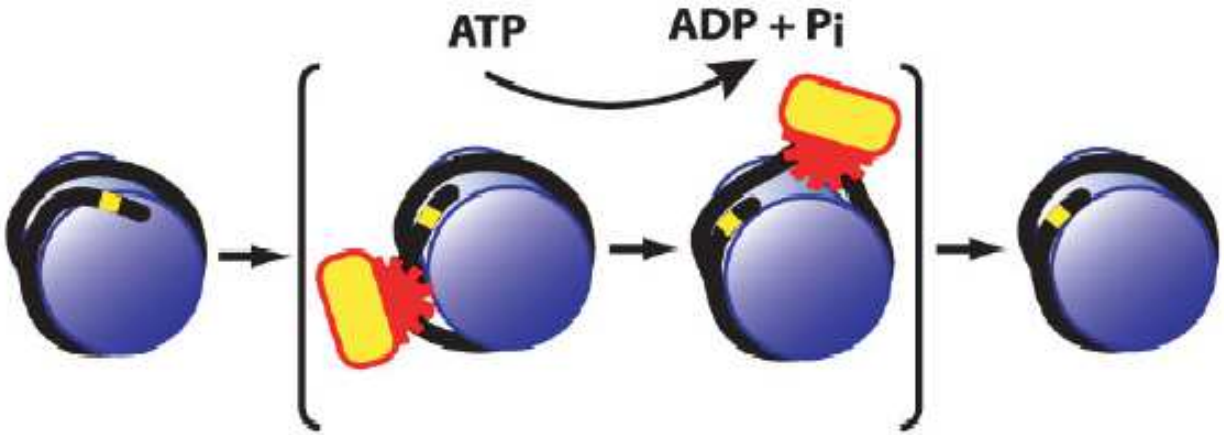

a
Sliding
rimodellamento
del nucleosoma
Transfer
DNA 1
DNA 2
+
rimodellamento
del nucleosoma
+
Remodeling
inaccessibile
accessibile

Nucleosome remodeling

SNF

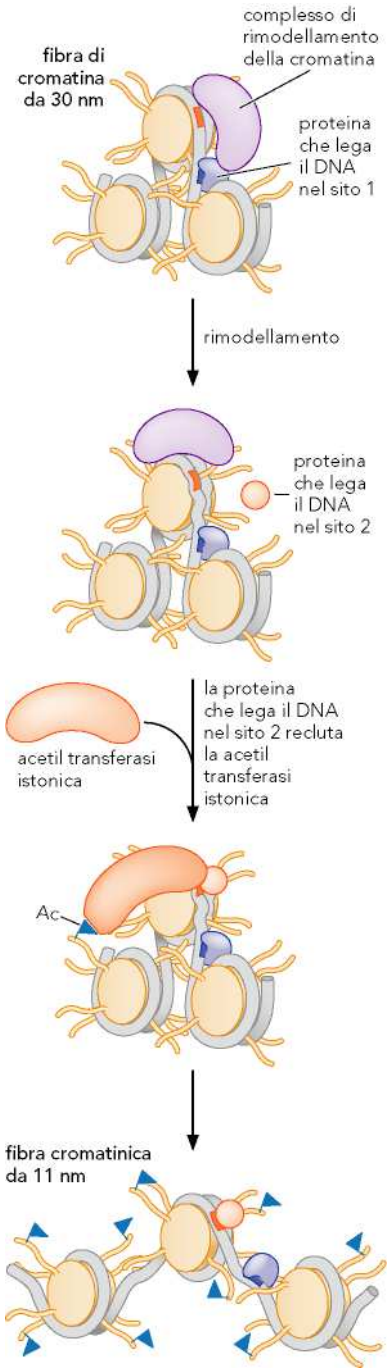


ISWI



Alexandra Lusser and James T. Kadonaga 2003 Wiley Periodicals,

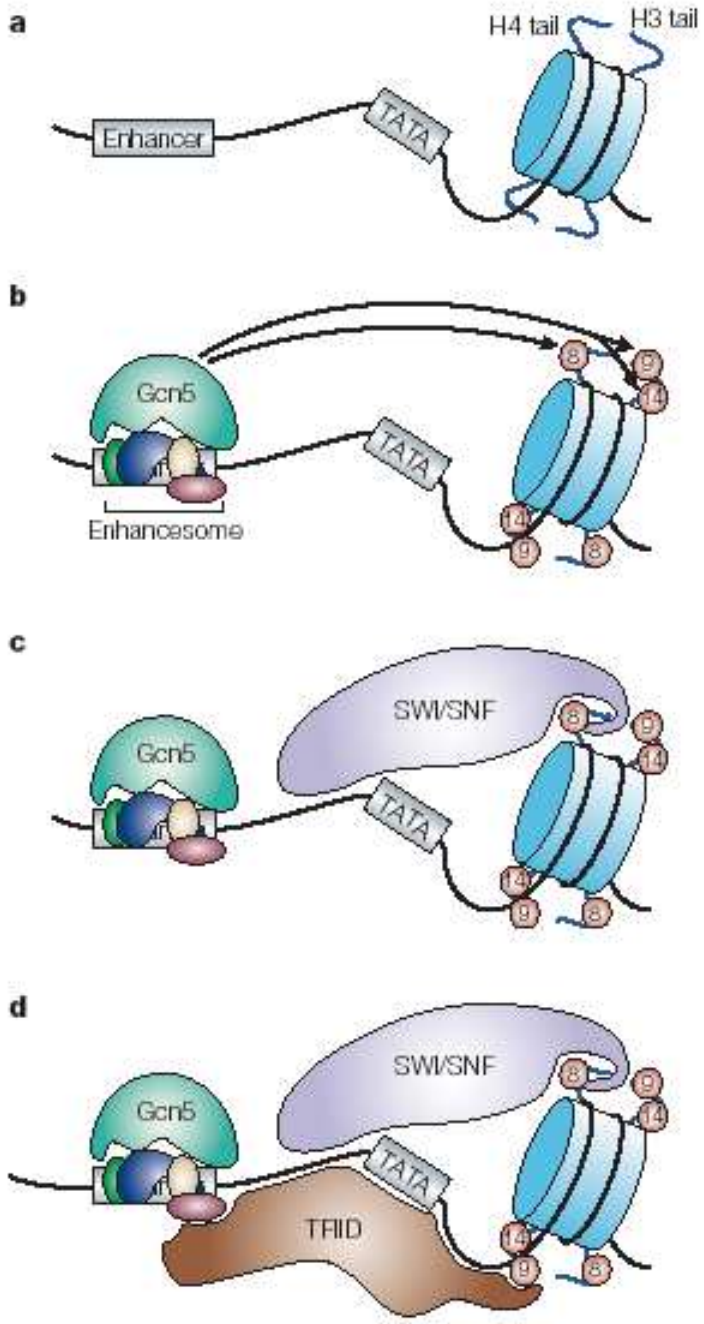
A cascade of events leading to chromatin opening



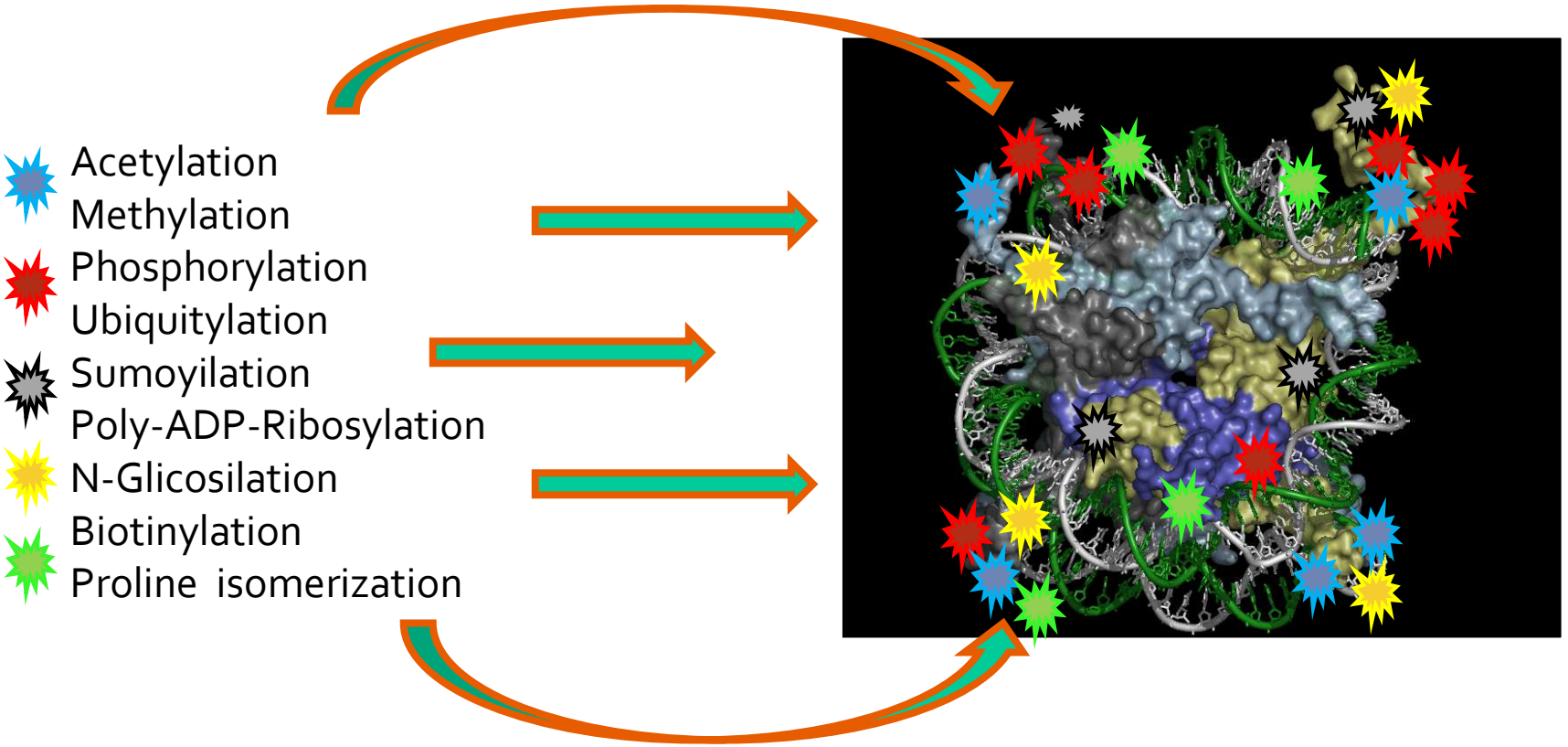
Control of chromatin structure.

Histone modifications

Nucleosome remodeling



HISTONE MODIFICATIONS



POST TRANSLATIONAL HISTONE MODIFICATIONS → **CHROMATIN DYNAMICS** → **DIFFERENT BIOLOGICAL OUTCOMES**

W. Højfeldt **et al., 2014**

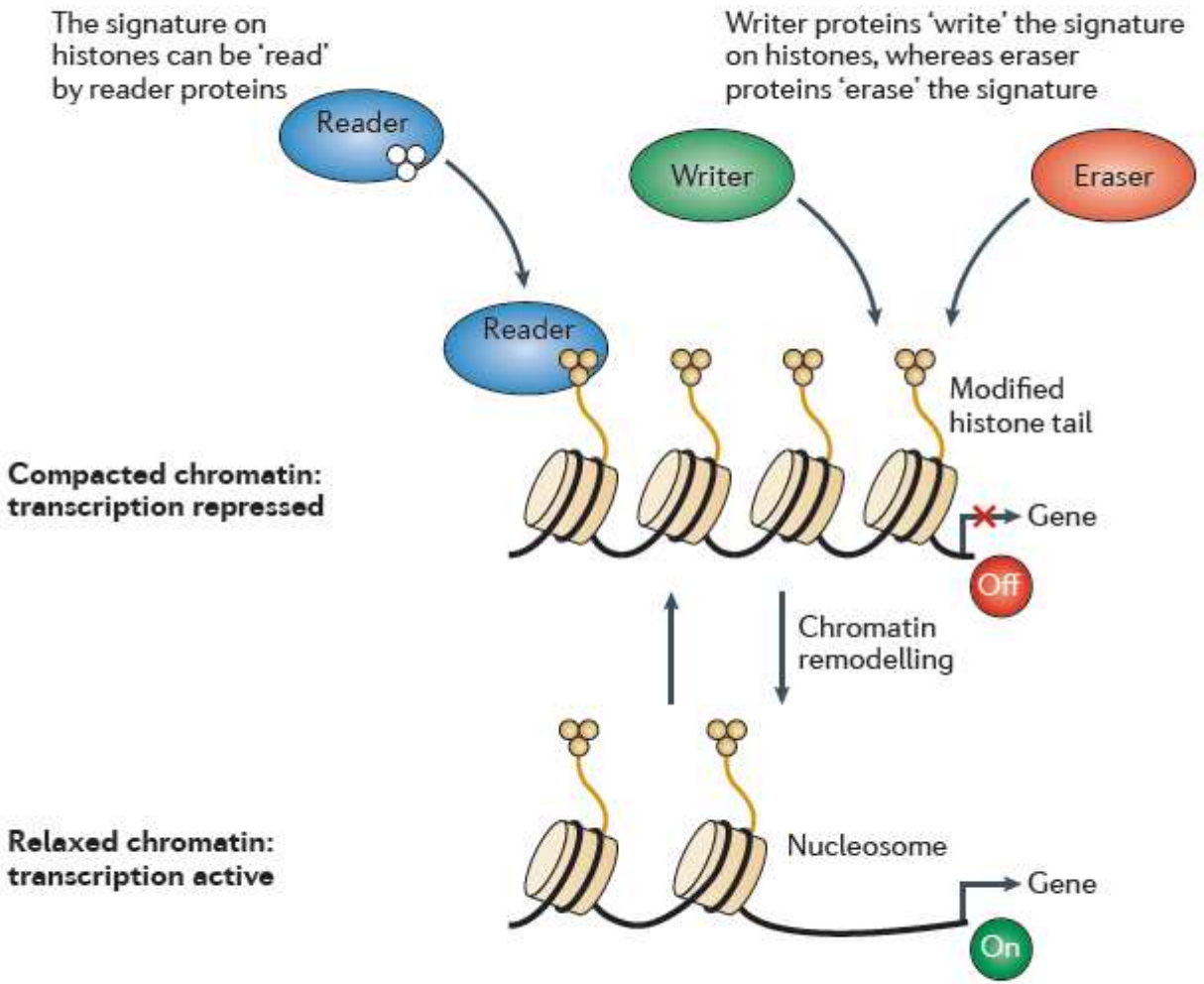
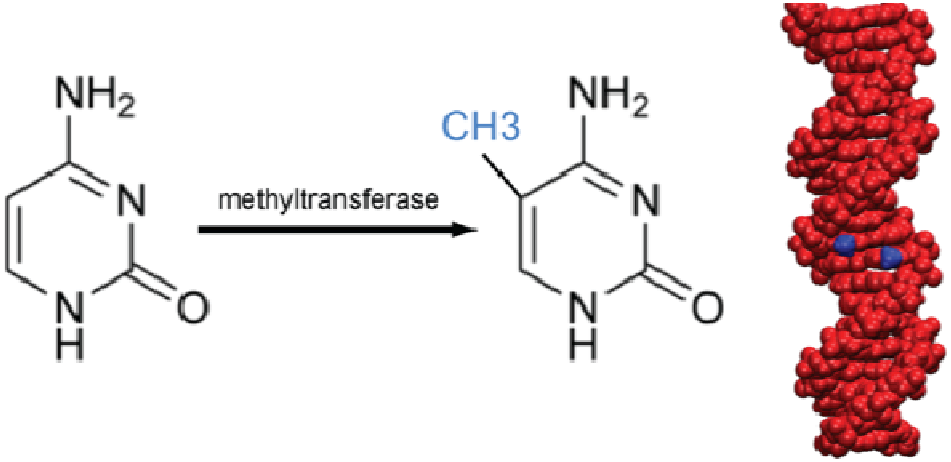


Figure 1 | **Readers, writers and erasers.** The model shown depicts how epigenetic information present in the chromatin structure is interpreted and modified. See BOX 1 for a more precise explanation of the different parts of the figure.

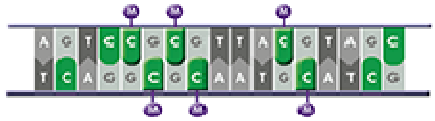
DNA methylation

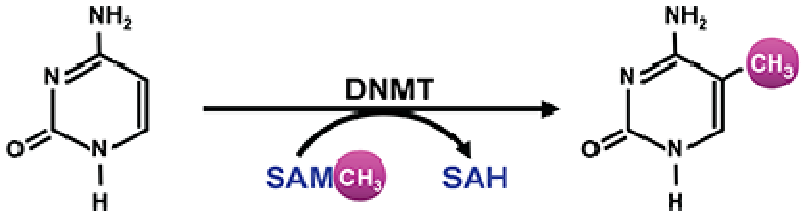
Present in Mammalian and other vertebrates (prevalentemently at CpG) and Plants CpG, CpHpG e siti CpHpH (H = A, C or T)
limited in insects and absent in the yeast *S.cerevisiae*



DNA Methylation

Methylating the cytosine of a CpG motif silences genes



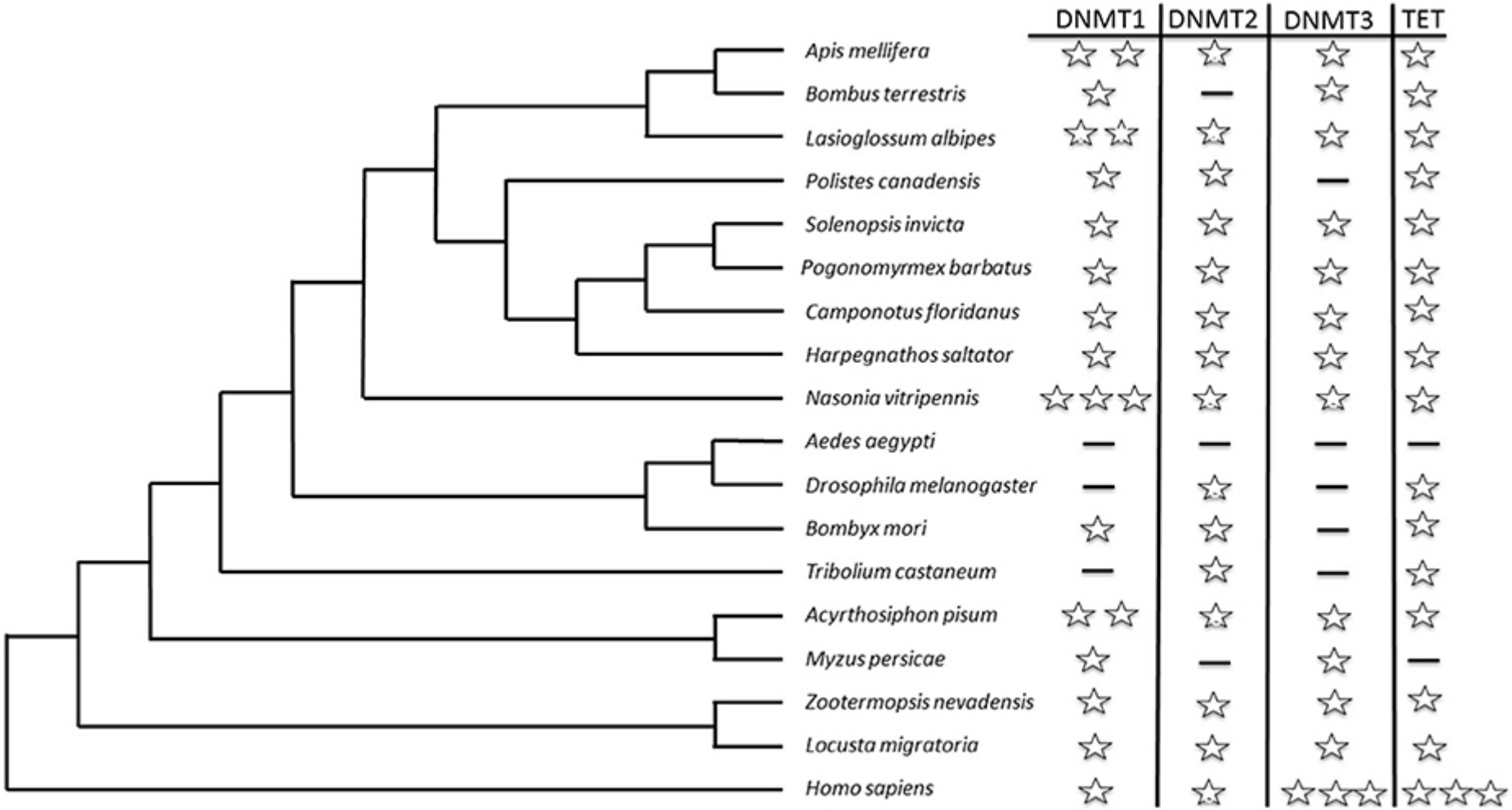


Cytosine

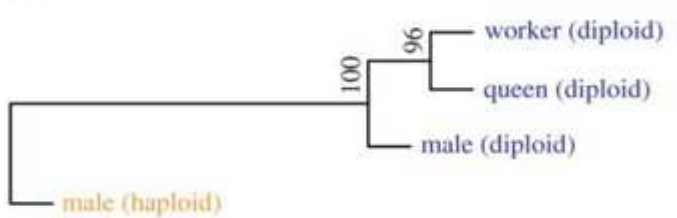
5' Methyl-cytosine

Metilazione delle citosine

Mus musculus 7,5%
Arabidopsis 14% (non solo CpG ma anche CpH e CpHpH)
Fungi 0,1-0,5%
(assente in Saccharomyces e Scizosaccharomyces ma 5% in Neurospora)
Drosophila 0,04%
Presente nelle api nelle sequenze codificanti (effetto sullo slicing)


DNMT1
DNMT2
DNMT3
TET
Apis mellifera
Bombus terrestris
Lasioglossum albipes
Polistes canadensis
Solenopsis invicta
Pogonomyrmex barbatus
Camponotus floridanus
Harpegnathos saltator
Nasonia vitripennis
Aedes aegypti
Drosophila melanogaster
Bombyx mori
Tribolium castaneum
Acyrthosiphon pisum
Myzus persicae
Zootermopsis nevadensis
Locusta migratoria
Homo sapiens

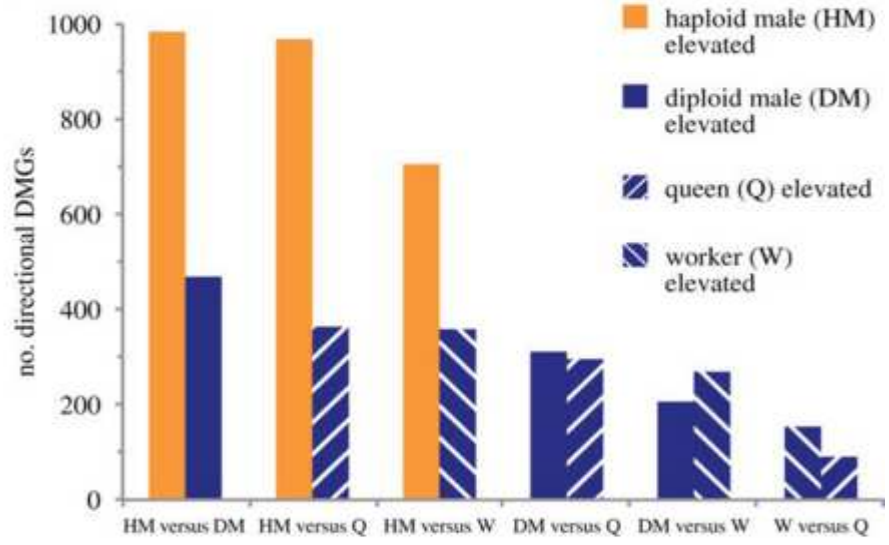
(*a*)



(*b*)

	male (haploid)	male (diploid)	queen (diploid)
worker (diploid)	1134	485	248
queen (diploid)	1453	630	
male (diploid)	1583		

(*c*)

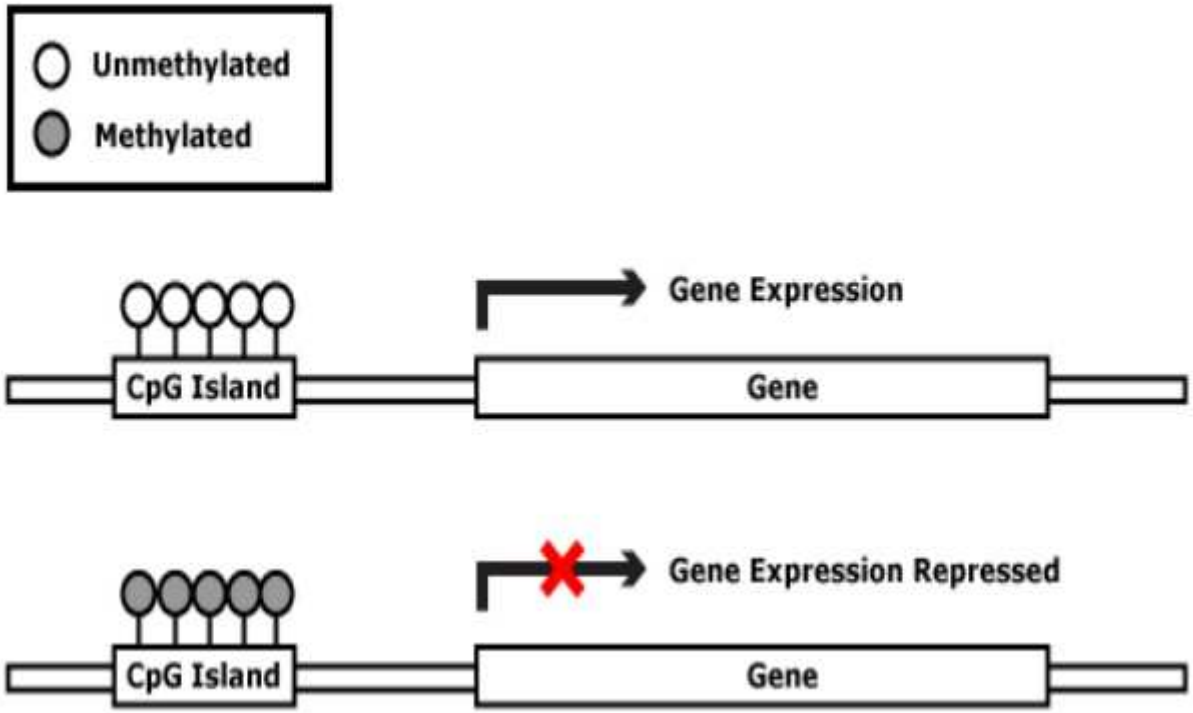


Le principali funzioni della metilazione sono collegate alla repressione della trascrizione:

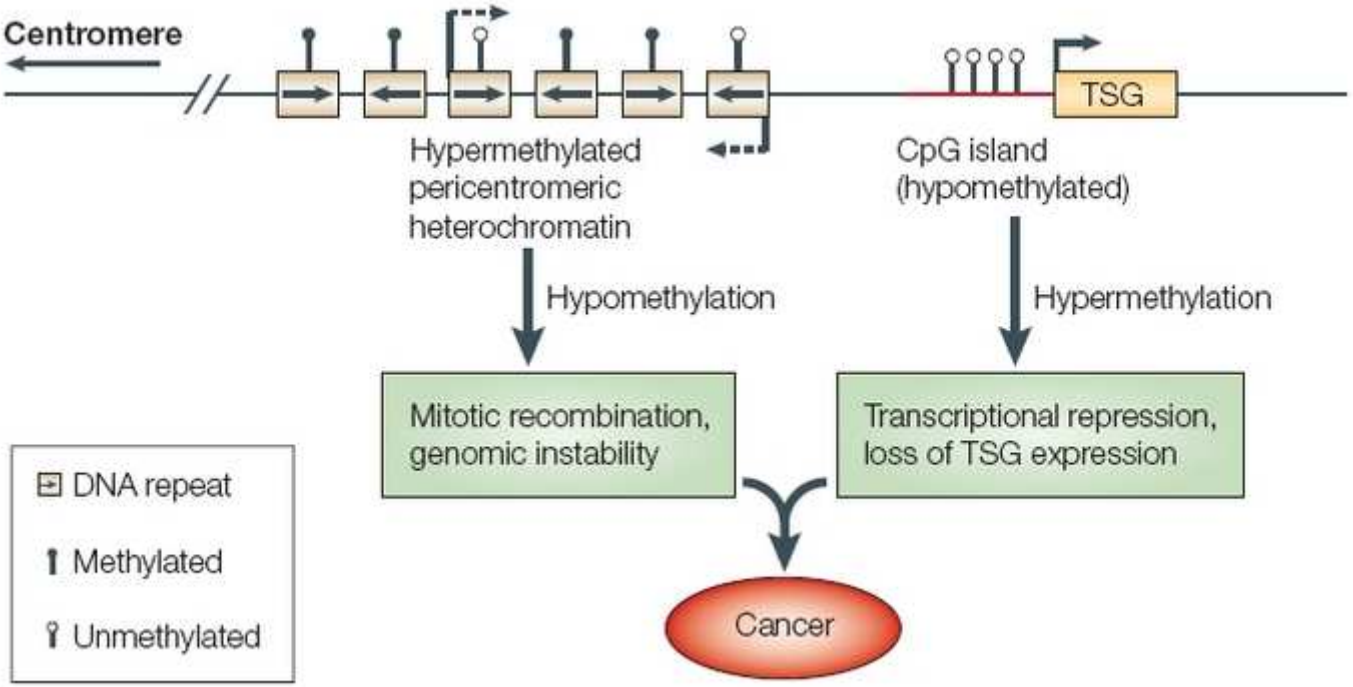
- **Difesa contro i trasposoni**: la metilazione e' fondamentale per mantenere silenti i genomi dei trasposoni e dei retrotrasposoni

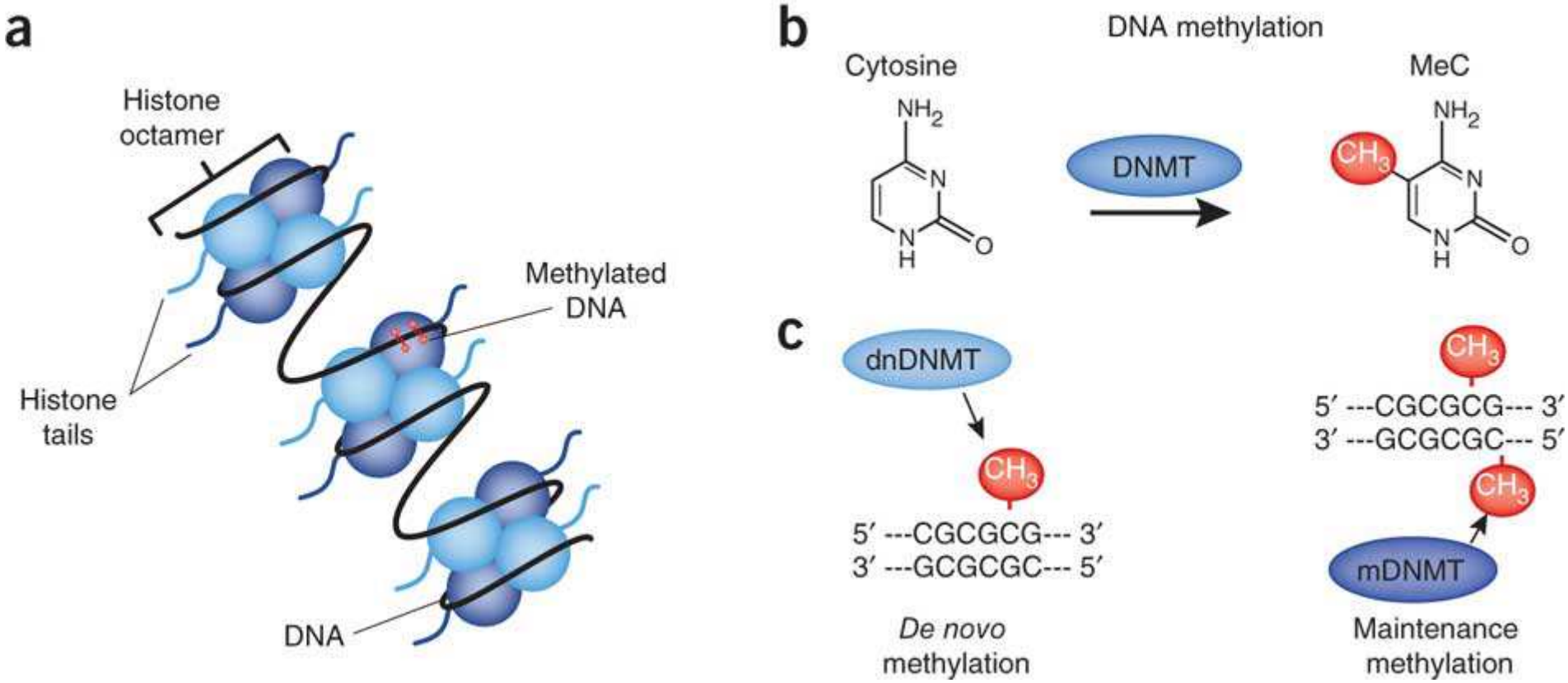
- **Regolazione genica:** la metilazione contribuisce a stabilire e mantenere uno stato trascrizionalmente inattivo (eterocromatina)

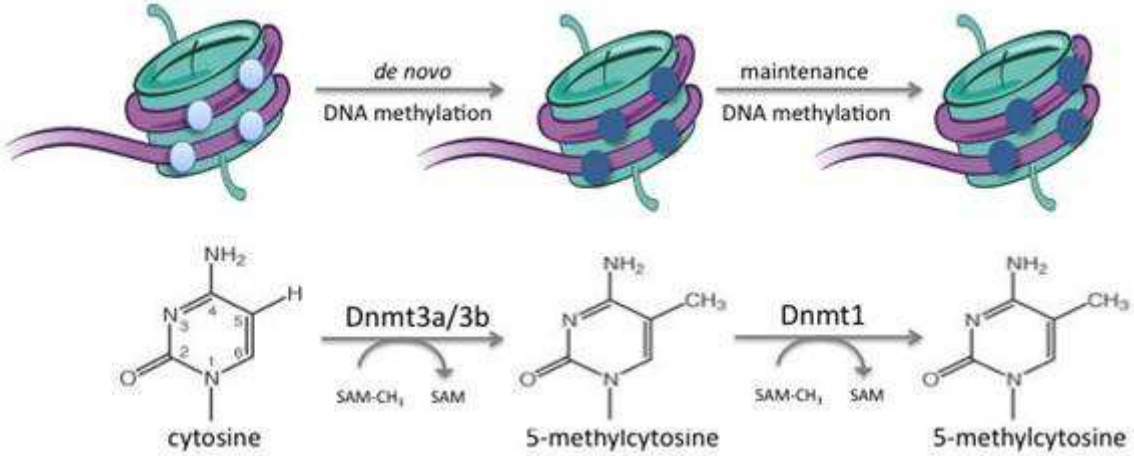
DNA Cytosine Methylation



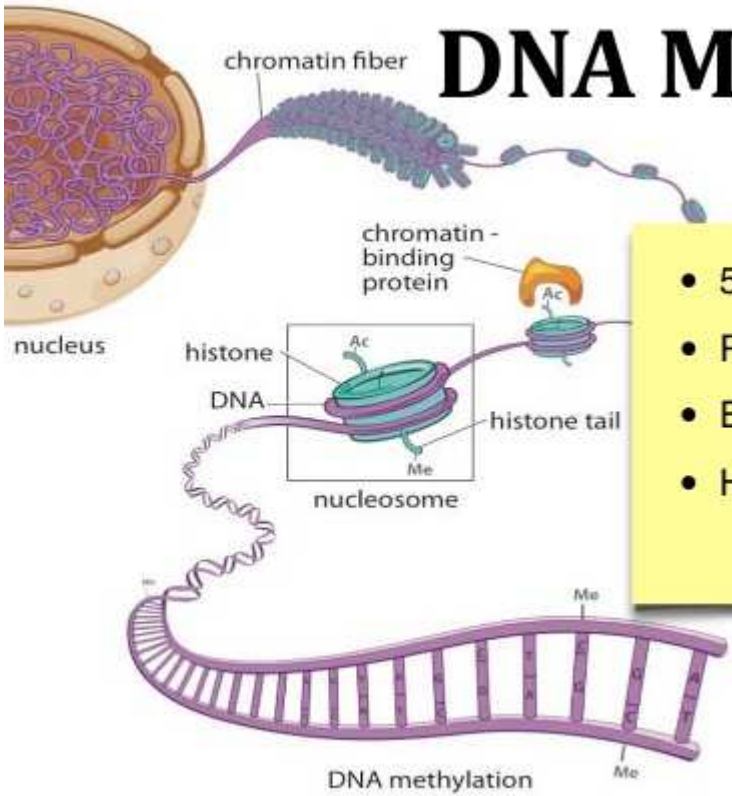
. Metivier, R. *et al.* Cyclical DNA methylation of a transcriptionally active promoter. *Nature* **452**, 45–50 (2008).


Centromere
TSG
Hypermethylated
pericentromeric
heterochromatin
CpG island
(hypomethylated)
Hypomethylation
Hypermethylation
Mitotic recombination,
genomic instability
Transcriptional repression,
loss of TSG expression
Cancer
DNA repeat
Methylated
Unmethylated

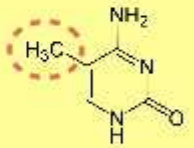

a
Histone
octamer
Methylated
DNA
Histone
tails
DNA
b
DNA methylation
Cytosine
MeC
NH2
N
N
H
O
DNMT
CH3
c
dnDNMT
5′ ---CGCGCG--- 3′
3′ ---GCGCGC--- 5′
De novo
methylation
mDNMT
Maintenance
methylation

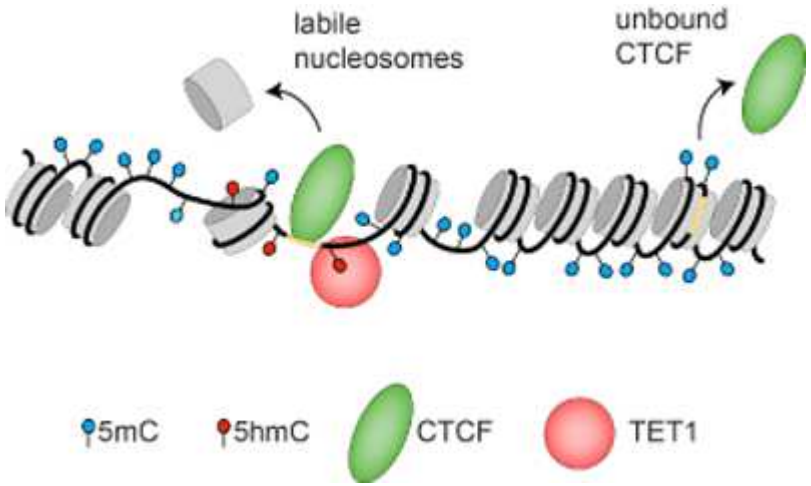

de novo
DNA methylation
maintenance
DNA methylation
Dnmt3a/3b
SAM-CH3
SAM
Dnmt1
SAM-CH3
SAM
cytosine
5-methylcytosine
5-methylcytosine

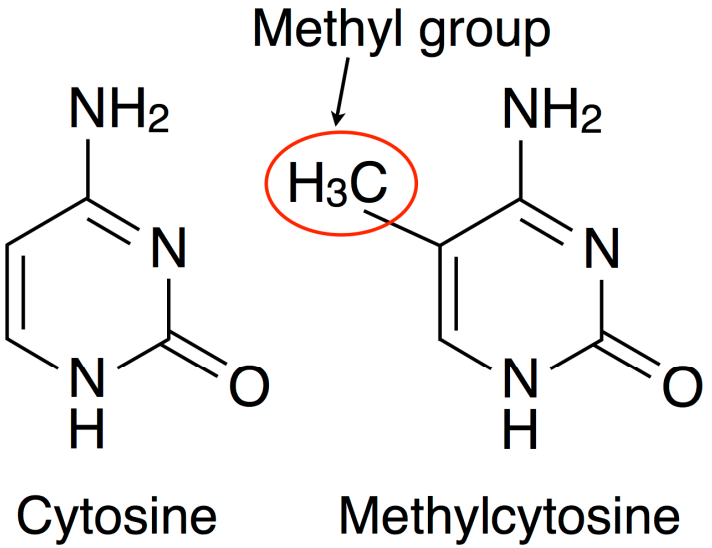
DNA Methylation



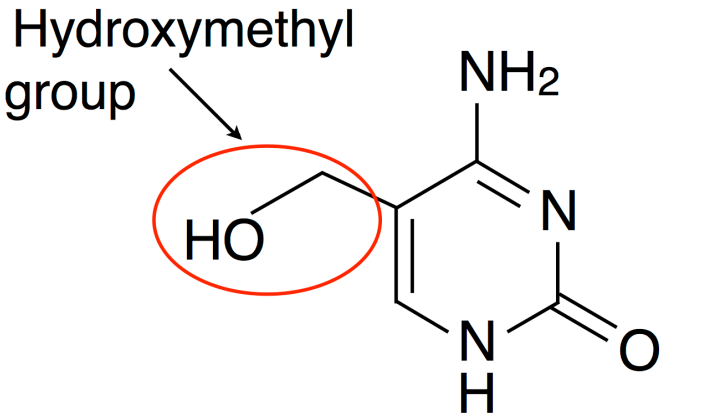
- 5 position of cytosine
- Regulation of gene expression
- Environment effect
- Heritable mark





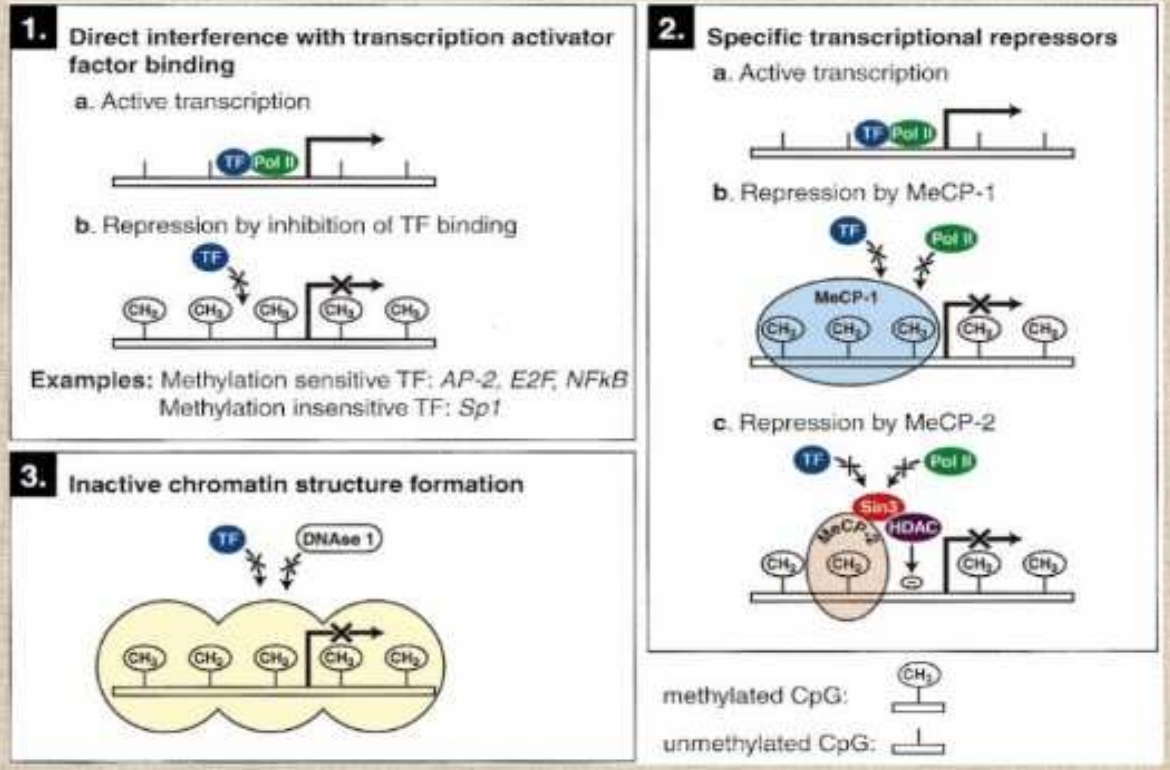


Cytosine Methylcytosine

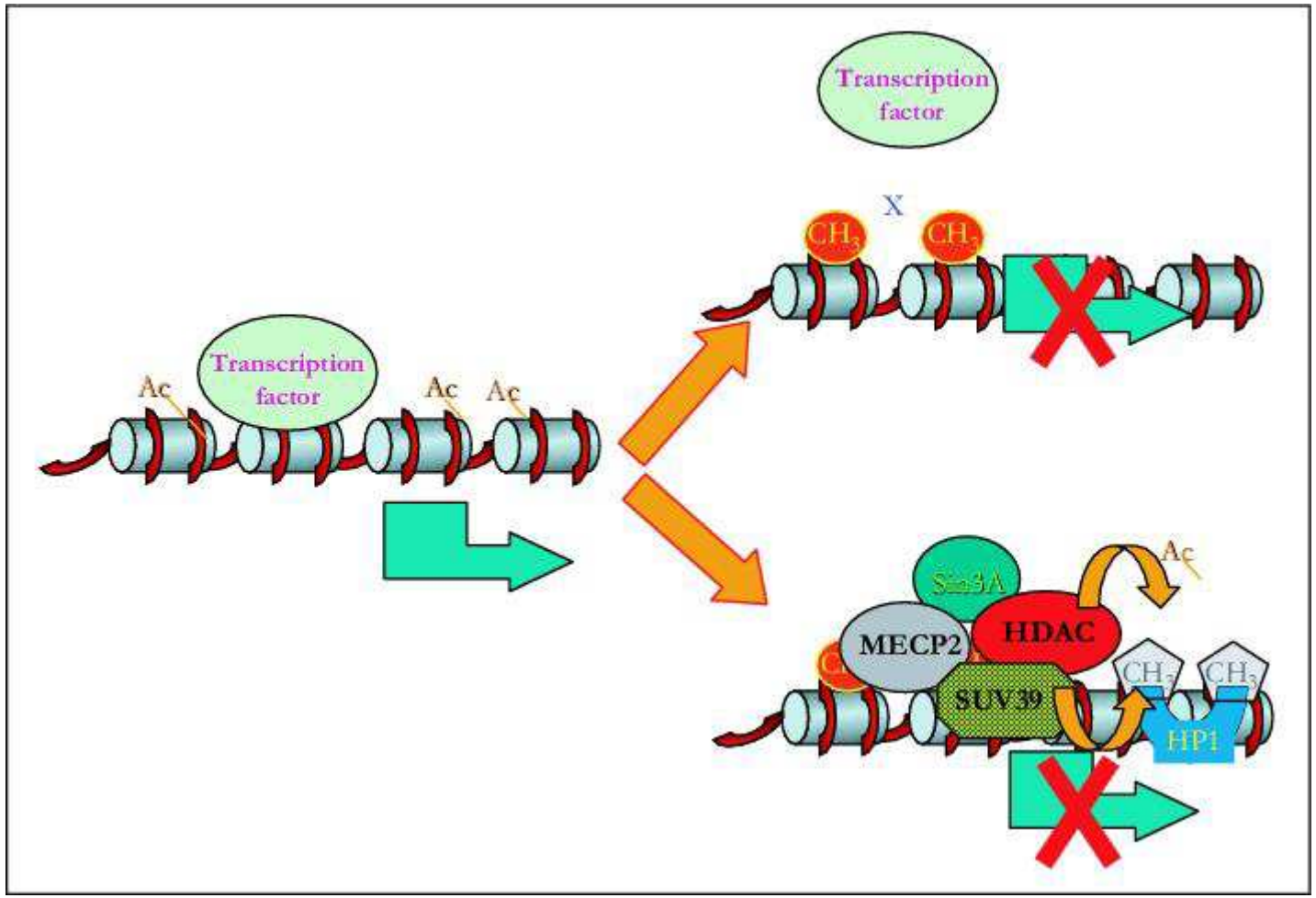


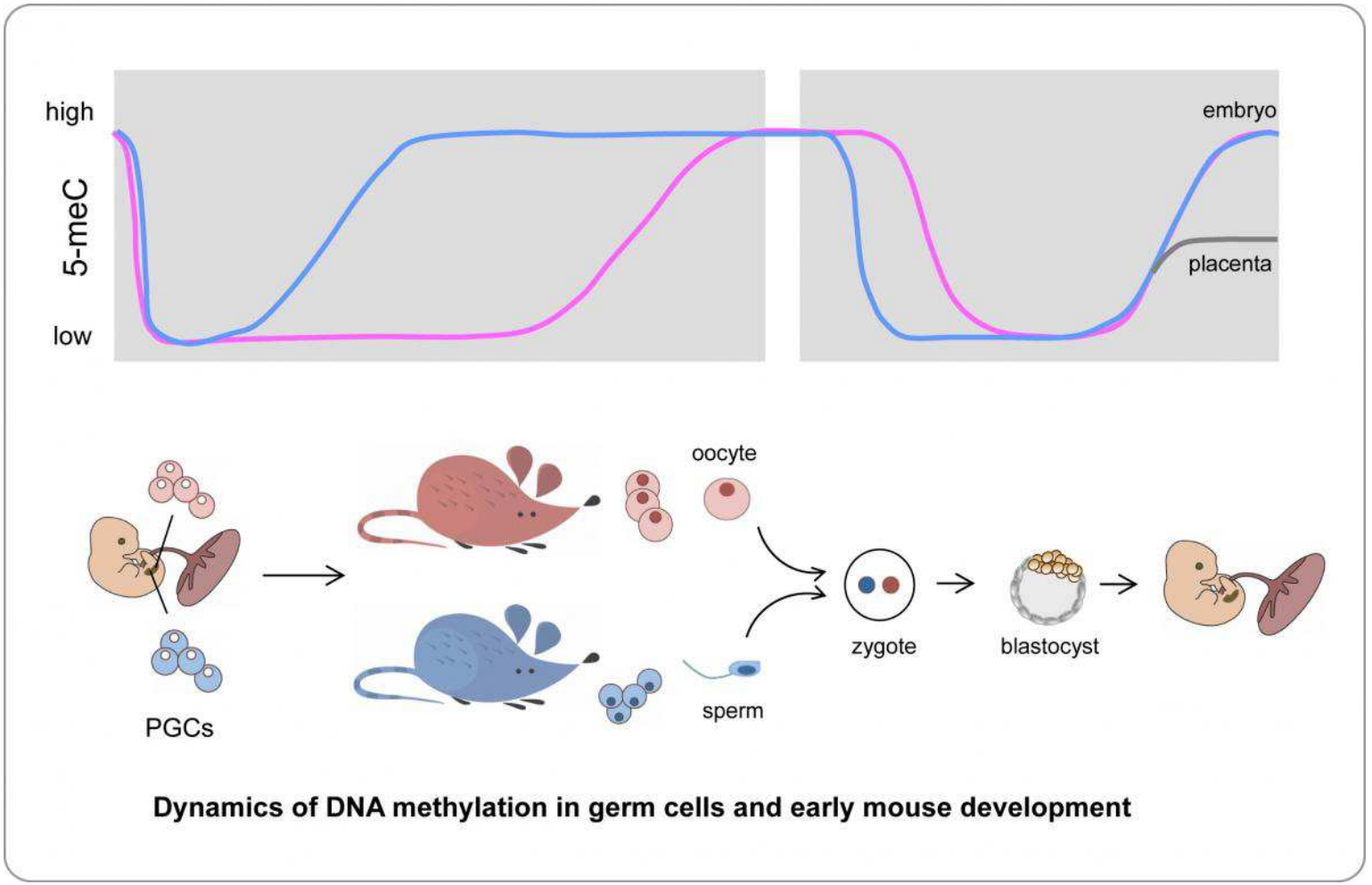
Hydroxymethylcytosine

DNA methylation and transcriptional repression

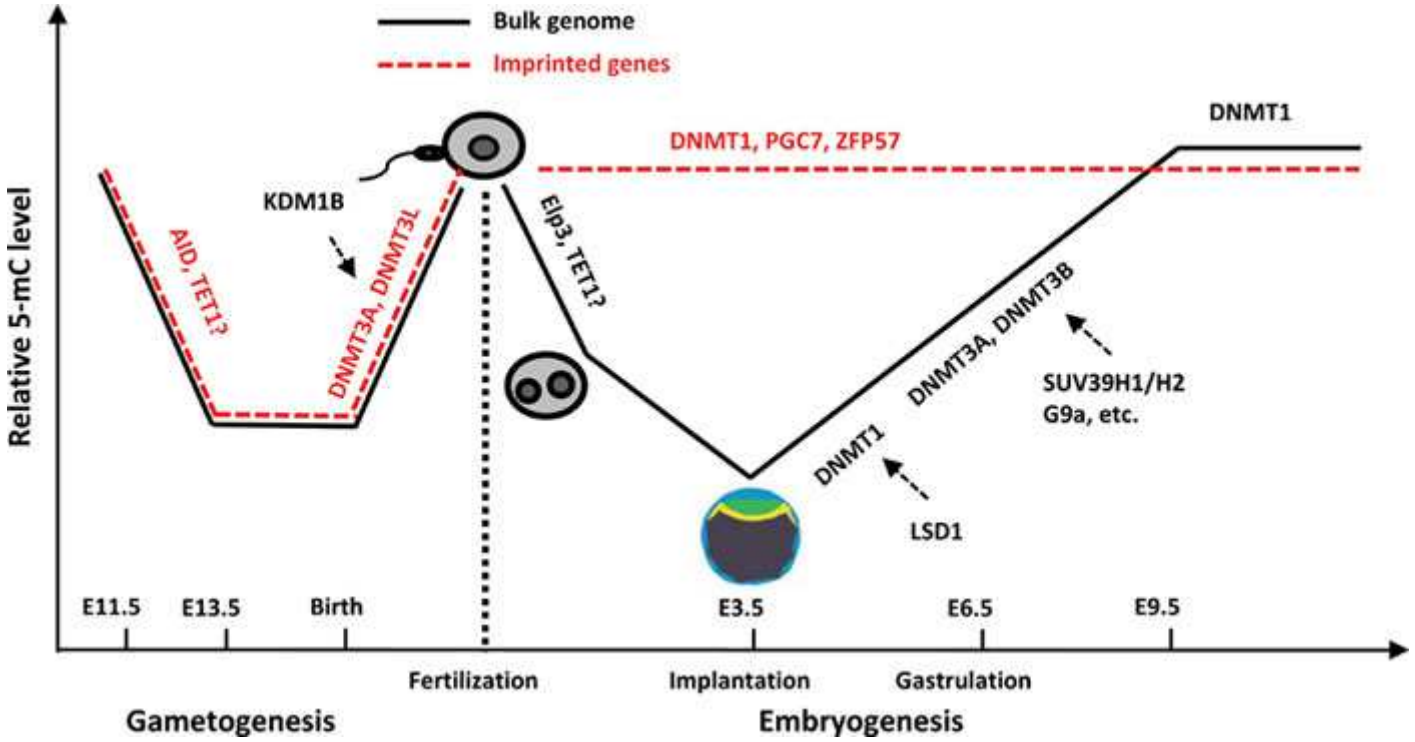


Singal and Ginder, 1999


Transcription factor
X
CH3
CH3
Transcription factor
Ac
Ac
Ac
Sin3A
HDAC
Ac
MECP2
CH3
CH3
SUV39
HP1

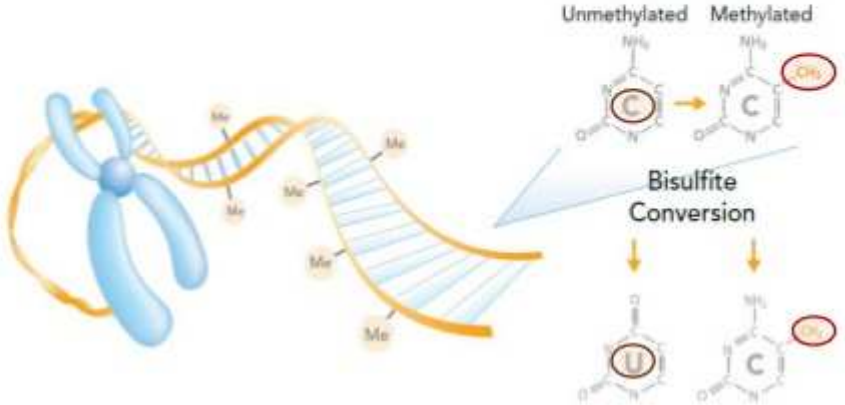


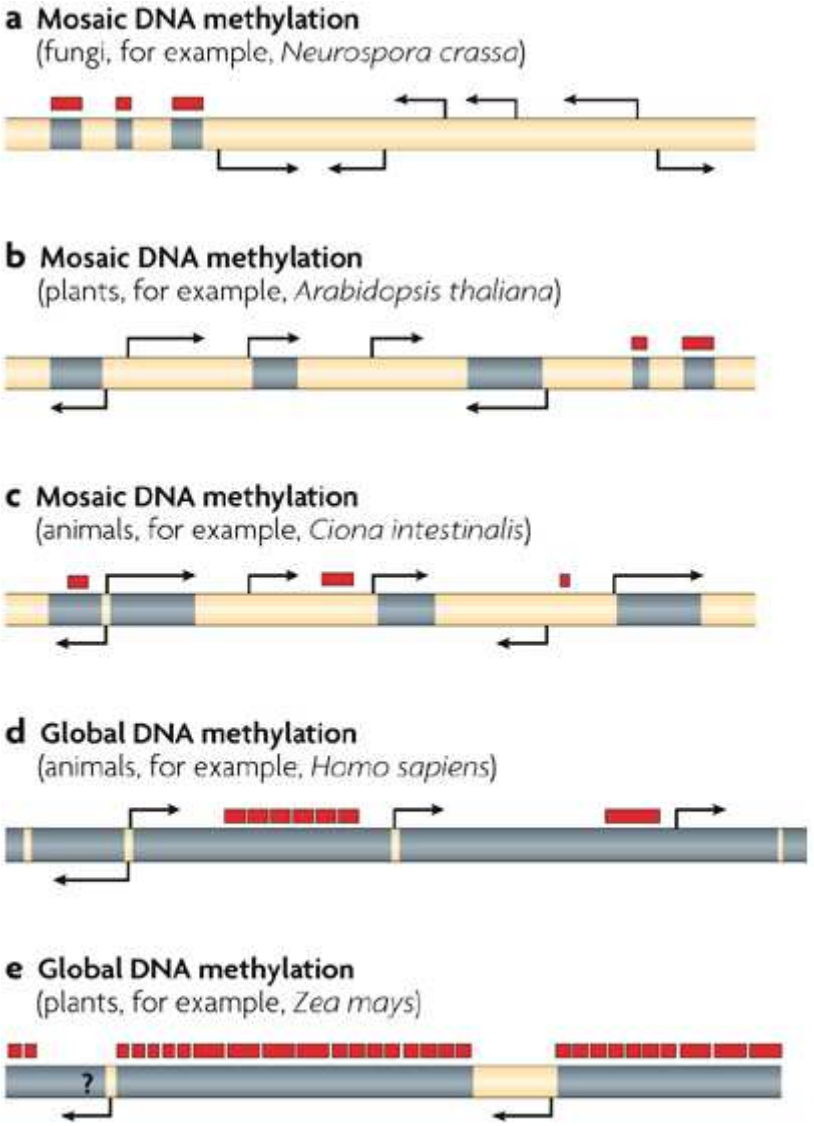
Dynamics of DNA methylation in germ cells and early mouse development


Bulk genome
Imprinted genes
Relative 5-mC level
KDM1B
AID, TET1?
DNMT3A, DNMT3L
DNMT1, PGC7, ZFP57
Elp3, TET1?
DNMT1
DNMT3A, DNMT3B
SUV39H1/H2
G9a, etc.
DNMT1
LSD1
E11.5
E13.5
Birth
E3.5
E6.5
E9.5
Fertilization
Implantation
Gastrulation
Gametogenesis
Embryogenesis

Introduction

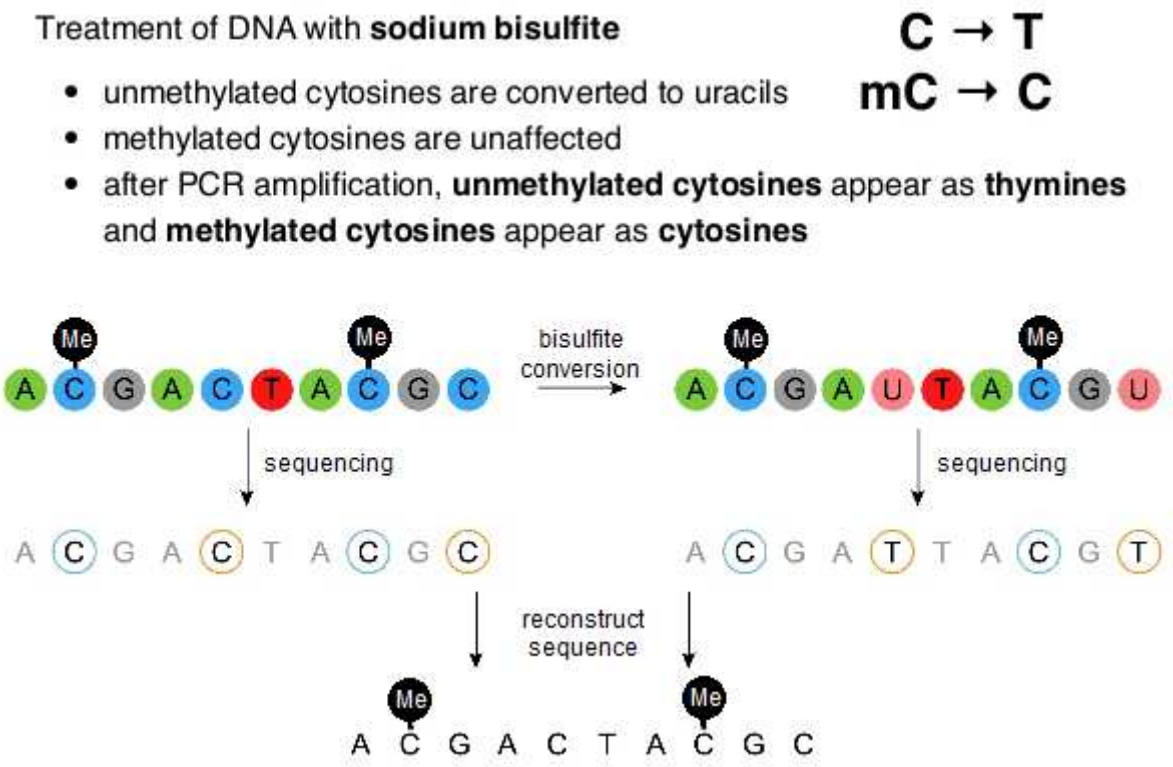
- Measuring DNA Methylation





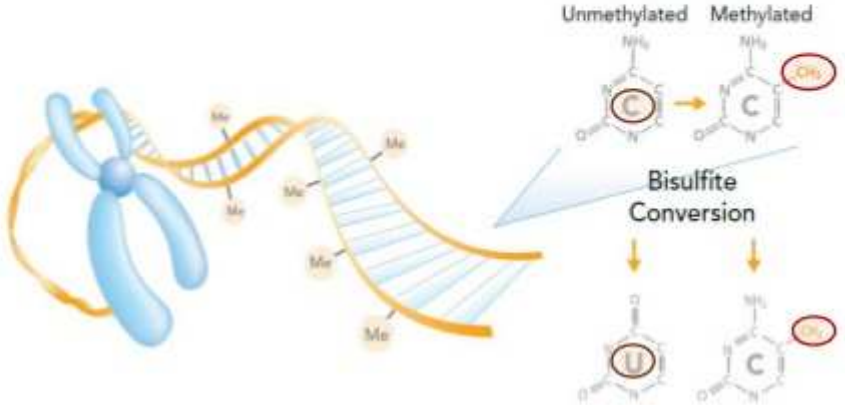
Nature Reviews | Genetics

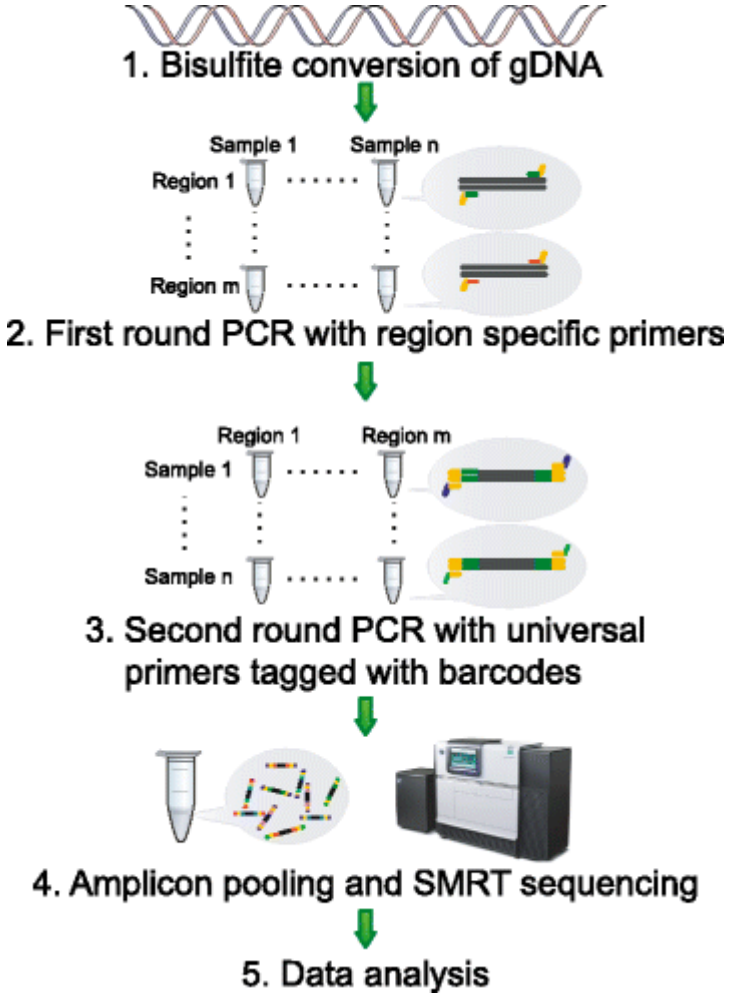
- Sodium bisulfite mapping

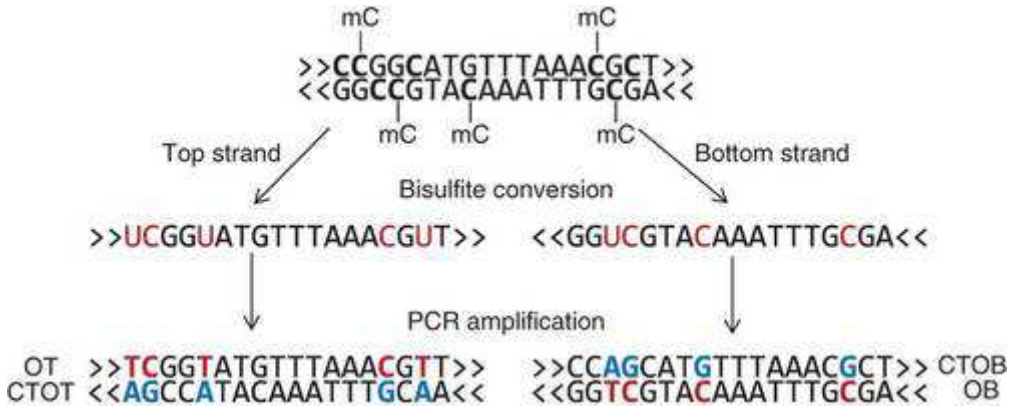


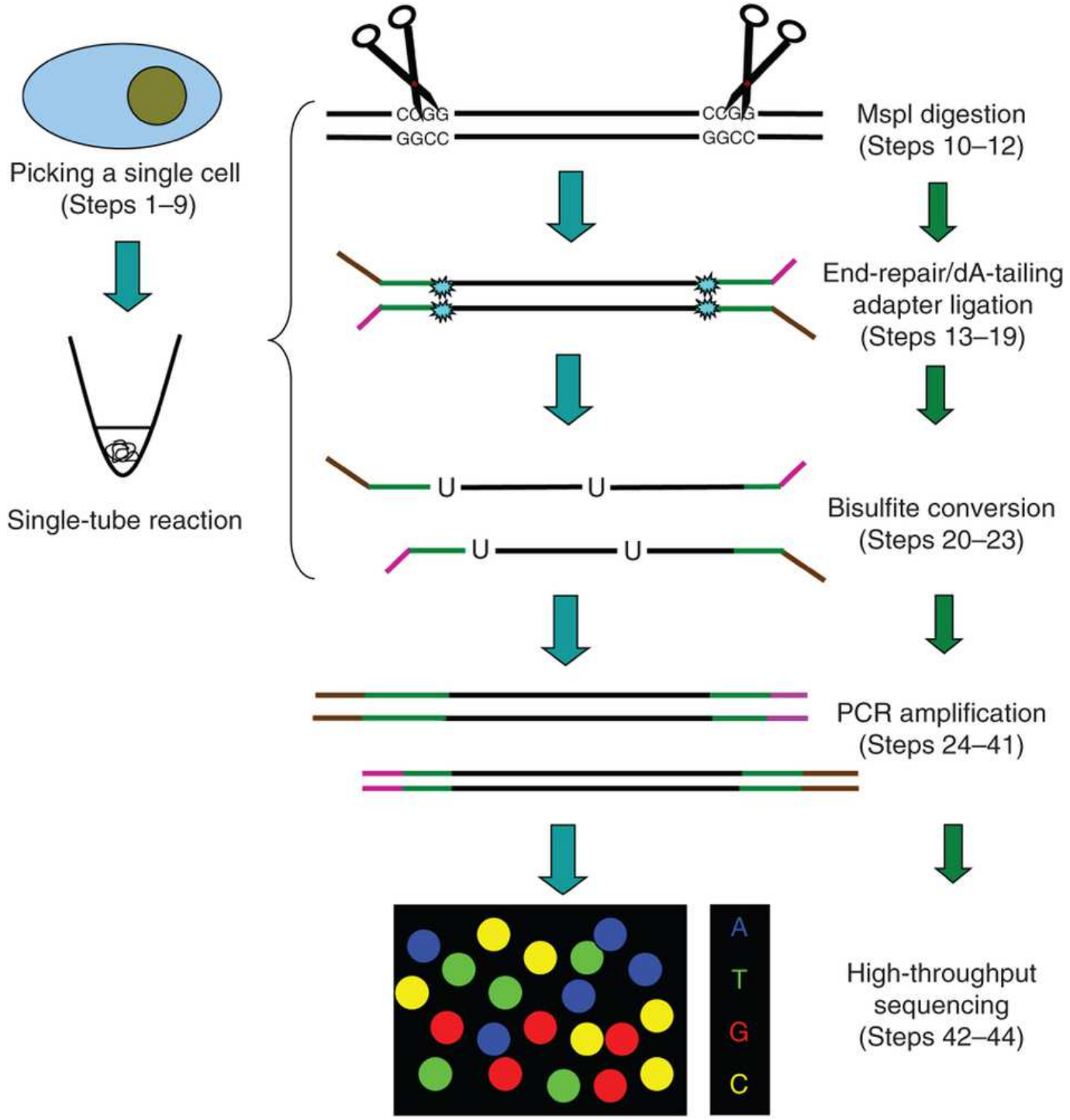
Introduction

- Measuring DNA Methylation

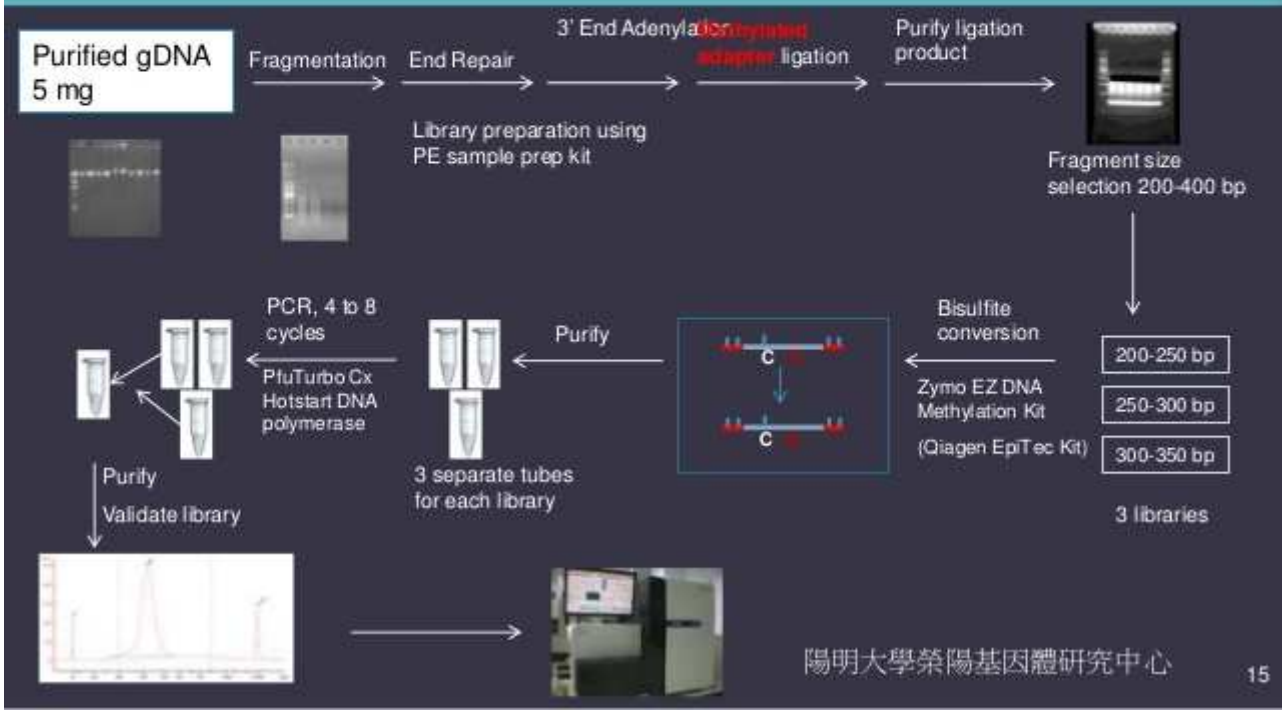


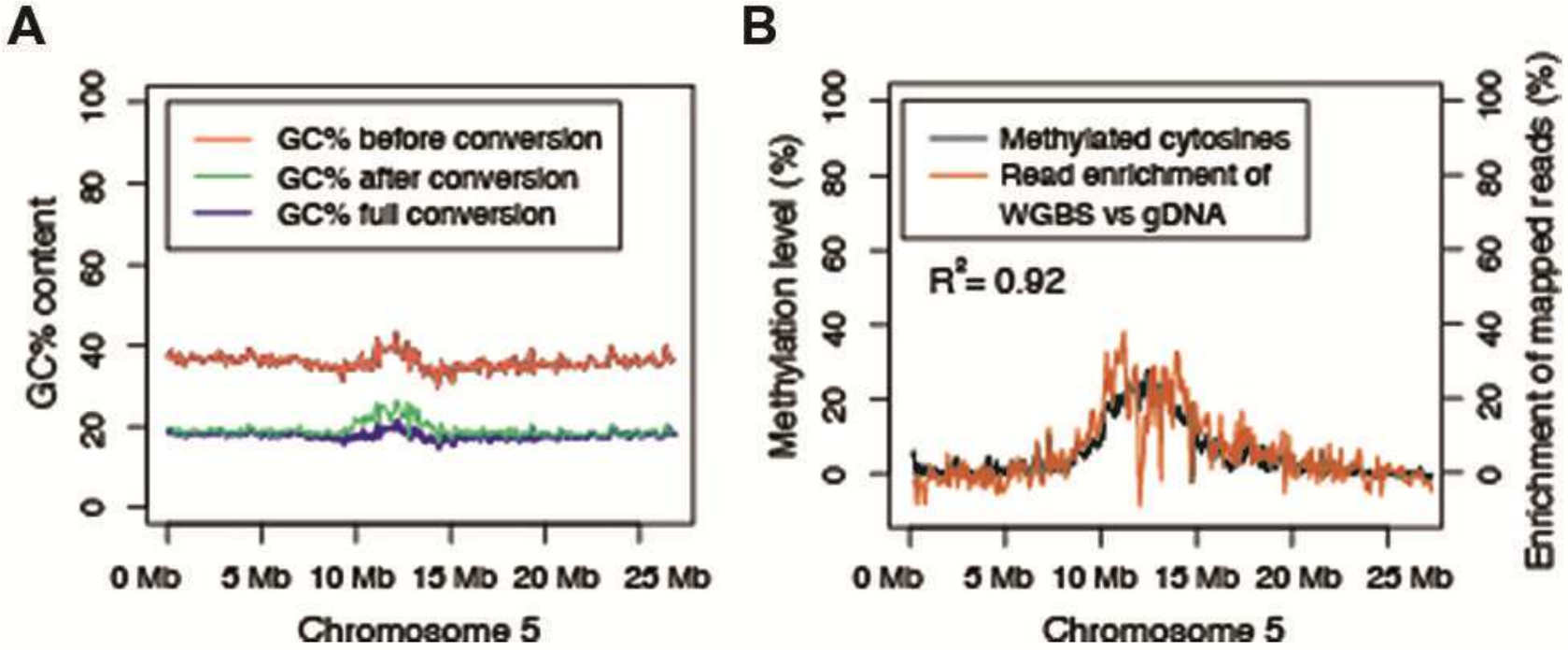

1. Bisulfite conversion of gDNA
Sample 1
Sample n
Region 1
Region m
2. First round PCR with region specific primers
Region 1
Region m
Sample 1
Sample n
3. Second round PCR with universal primers tagged with barcodes
4. Amplicon pooling and SMRT sequencing
5. Data analysis


mC
mC
>>CCGGCATGTTTAAACGCT>>
<<GGCCGTACAAATTTGCGA<<
mC
mC
mC
Top strand
Bottom strand
Bisulfite conversion
>>UCGGUATGTTTAAACGUT>>
<<GGUCGTACAAATTTGCGA<<
PCR amplification
OT
CTOT
>>TCGGTATGTTTAAACGTT>>
<<AGCCATACAAATTTGCAA<<
>>CCAGCATGTTTAAACGCT>>
<<GGTCGTACAAATTTGCGA<<
CTOB
OB

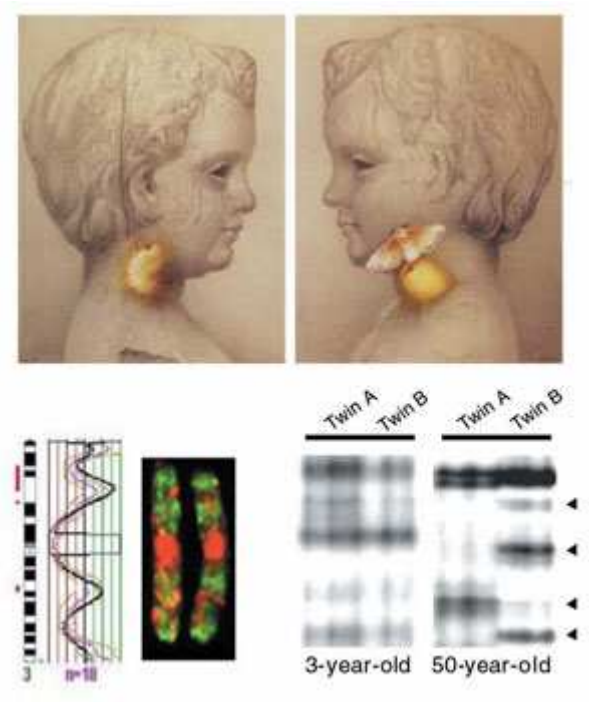

Picking a single cell
(Steps 1–9)
Single-tube reaction
CCGG
GGCC
CCGG
GGCC
MspI digestion
(Steps 10–12)
End-repair/dA-tailing
adapter ligation
(Steps 13–19)
U
U
U
U
Bisulfite conversion
(Steps 20–23)
PCR amplification
(Steps 24–41)
A
T
G
C
High-throughput
sequencing
(Steps 42–44)

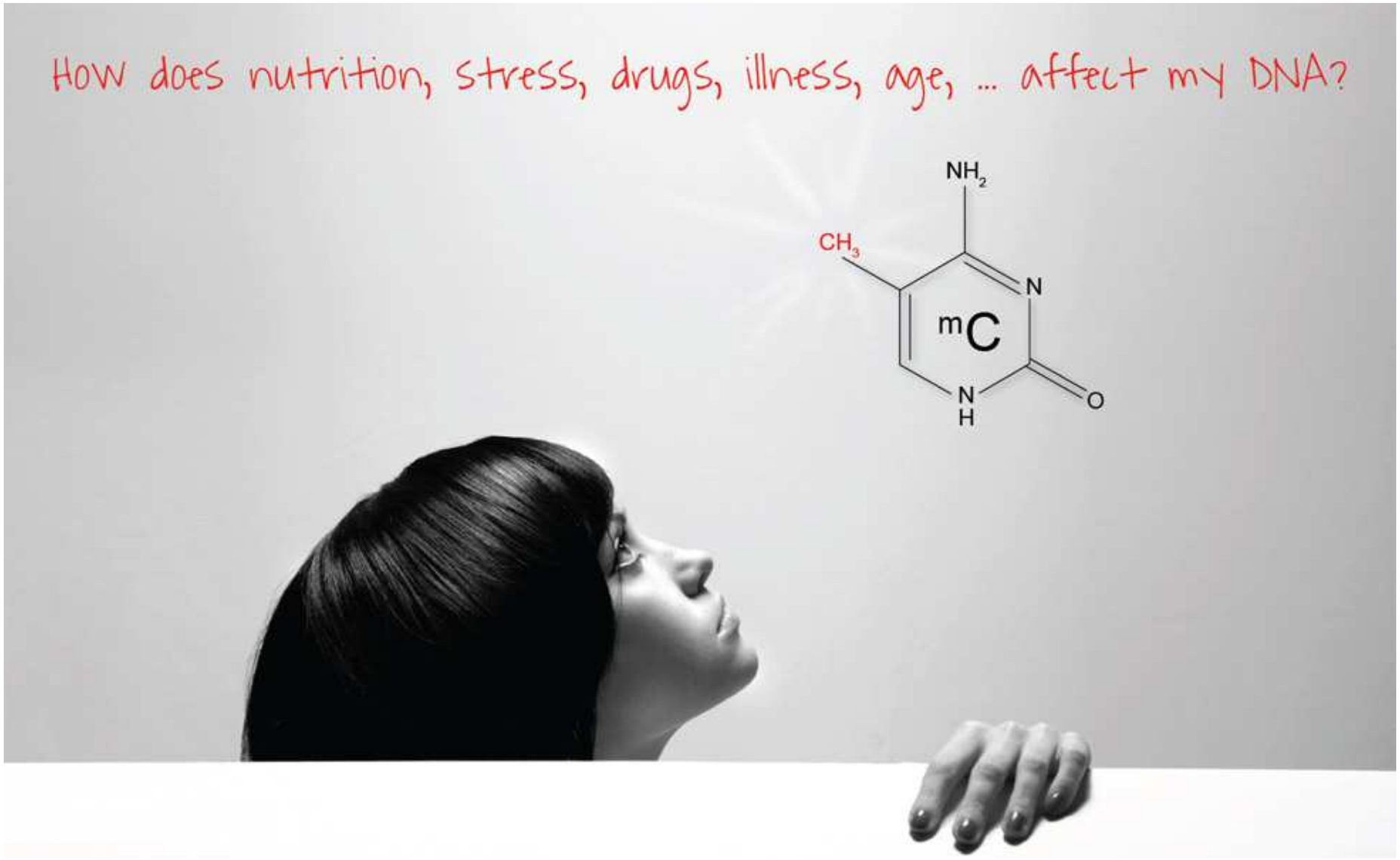
Whole Genome Bisulfite Sequencing Library Construction



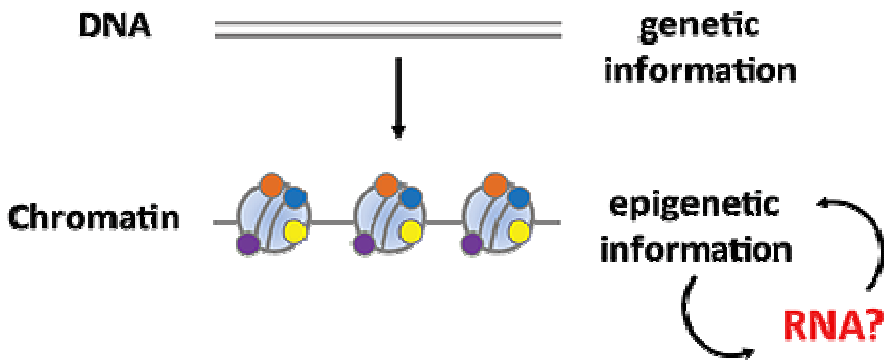

A
GC% content
0
20
40
60
80
100
GC% before conversion
GC% after conversion
GC% full conversion
0 Mb
5 Mb
10 Mb
15 Mb
20 Mb
25 Mb
Chromosome 5
B
Methylation level (%)
Enrichment of mapped reads (%)
Methylated cytosines
Read enrichment of WGBS vs gDNA
R² = 0.92
Chromosome 5

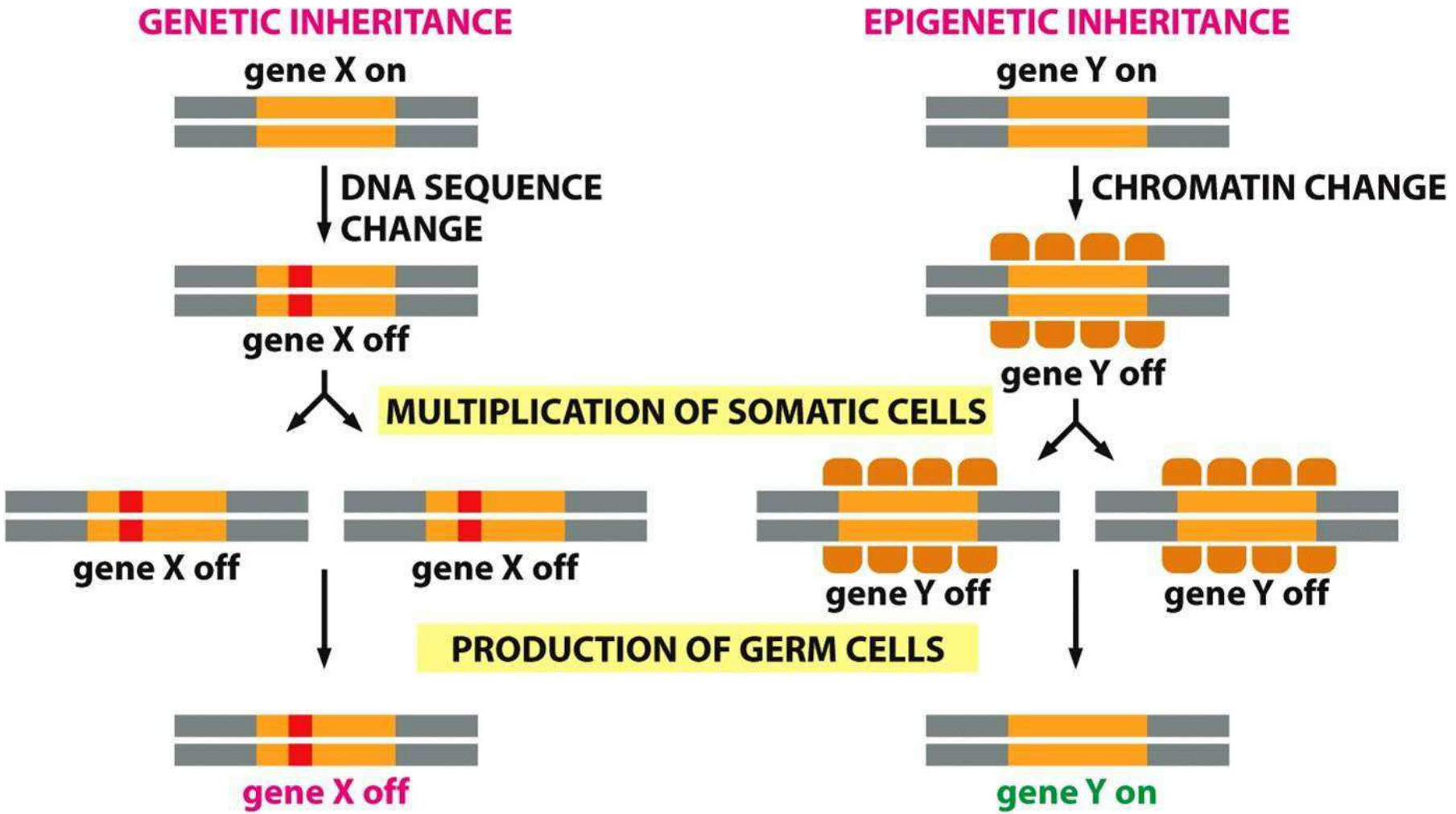
Epigenetics are stable heritable traits (or "phenotypes") that cannot be explained by changes in DNA sequence. The Greek prefix epi- (ἐπι- "over, outside of, around") in epigenetics implies features that are "on top of" or "in addition to" the traditional genetic basis for inheritance.

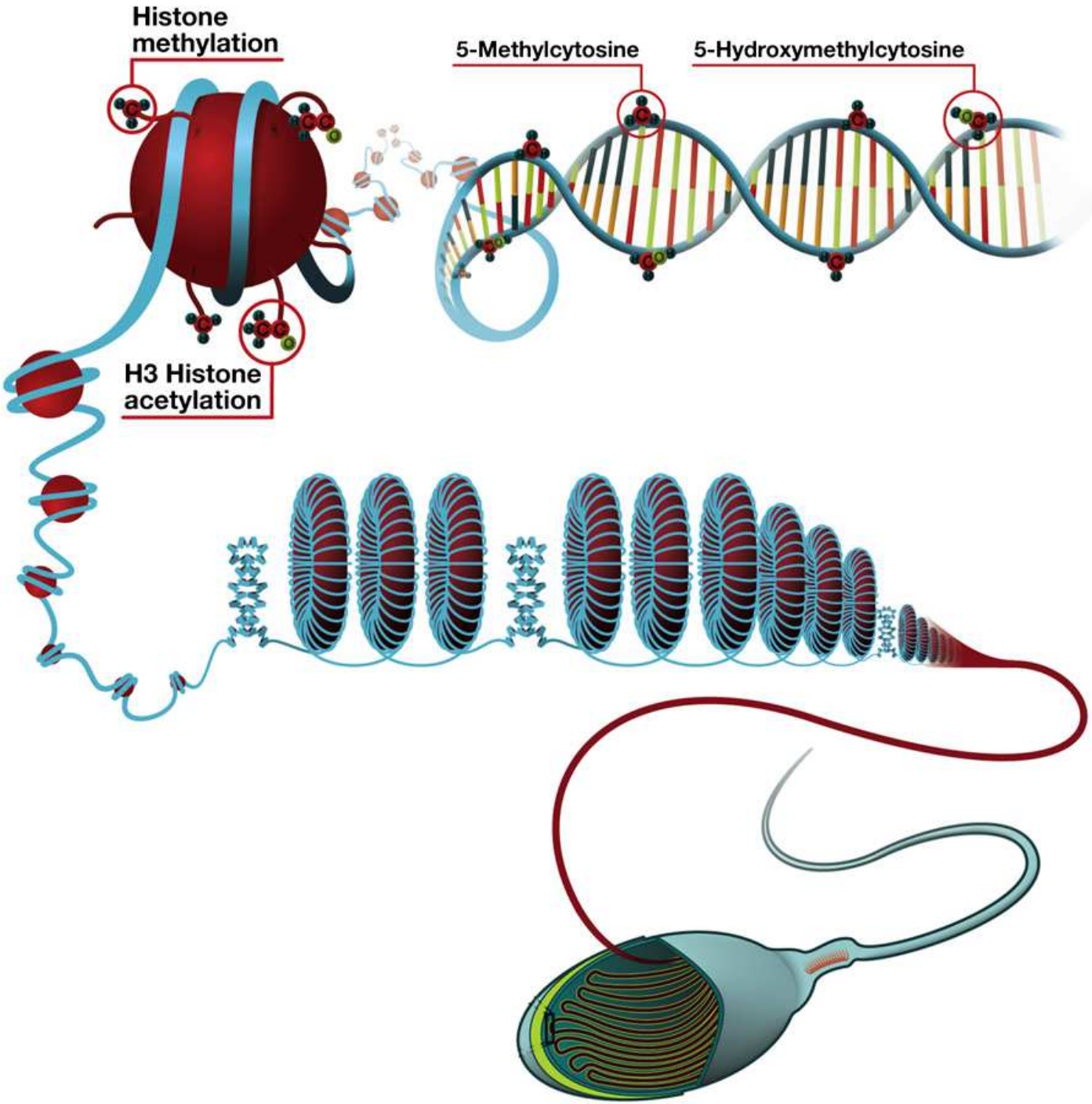


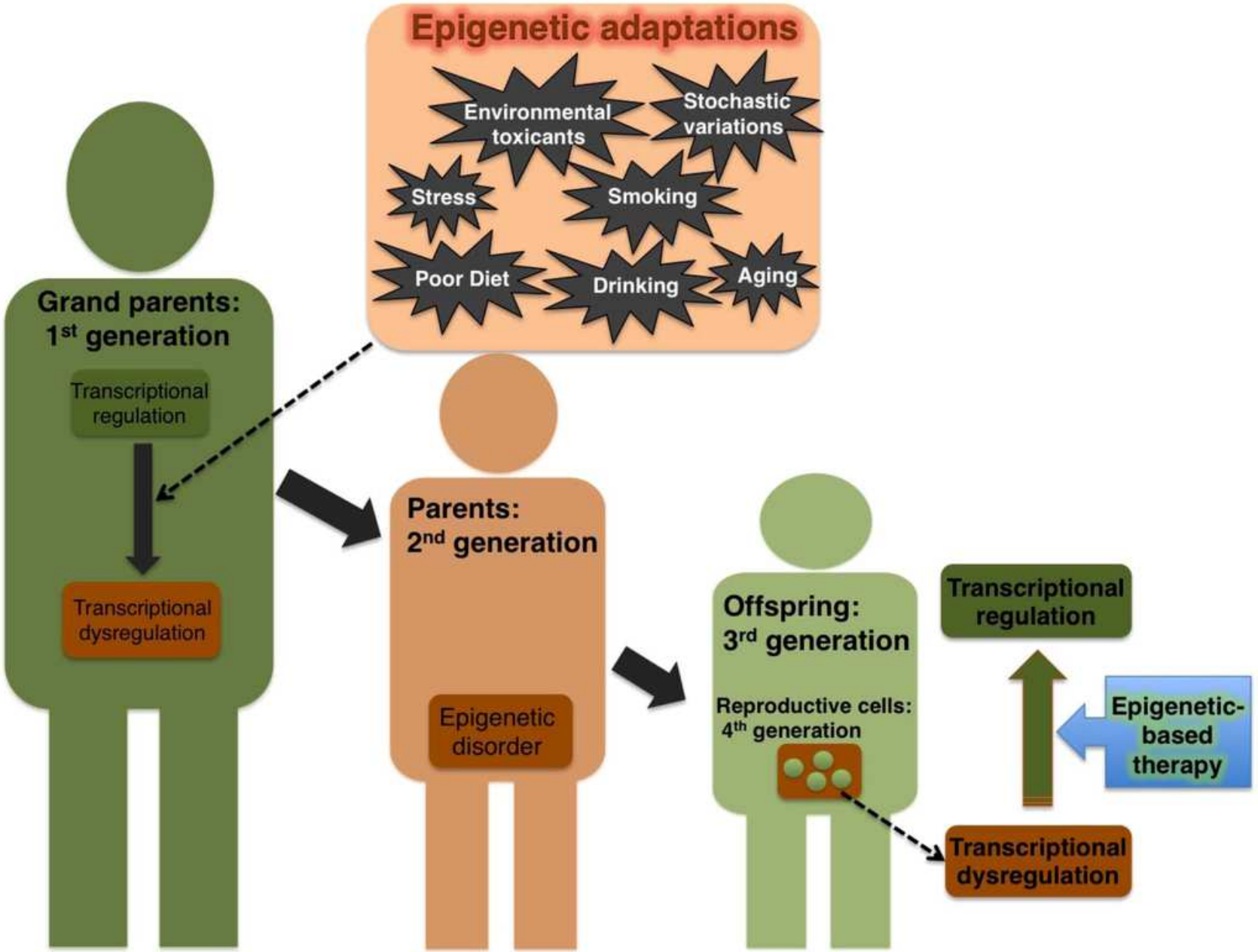

How does nutrition, stress, drugs, illness, age, ... affect my DNA?
NH2
CH3
N
mC
N
H
O

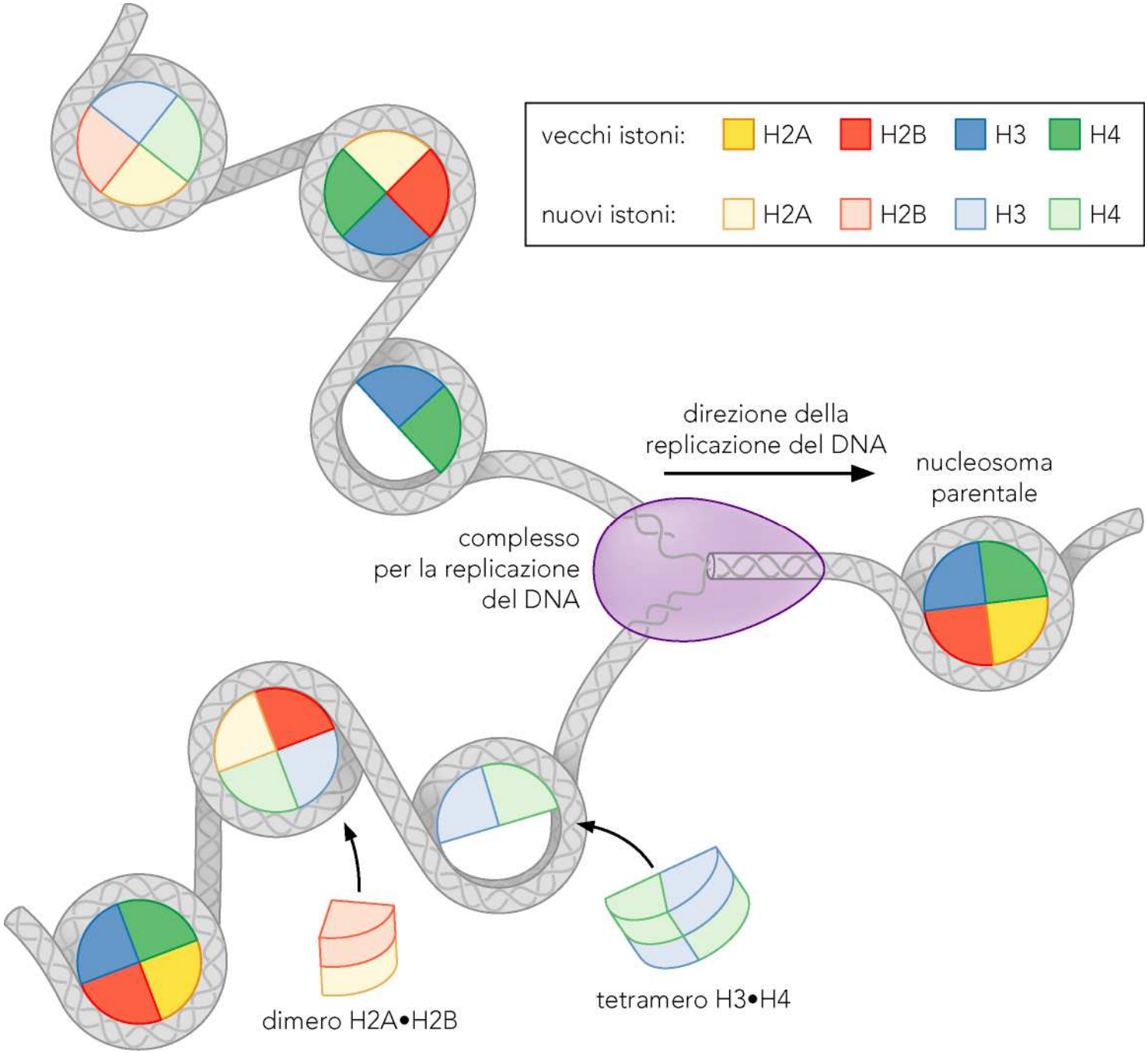

JANUARY 18, 2010
Joe Klein: The CIA's Afghan Disaster
Yemen: The New Center Of Terror
Why the Recession Hasn't Been Cool To Teens
TIME
WHY YOUR DNA ISN'T YOUR DESTINY
The new science of epigenetics reveals how the choices you make can change your genes —and those of your kids
BY JOHN CLOUD
www.time.com

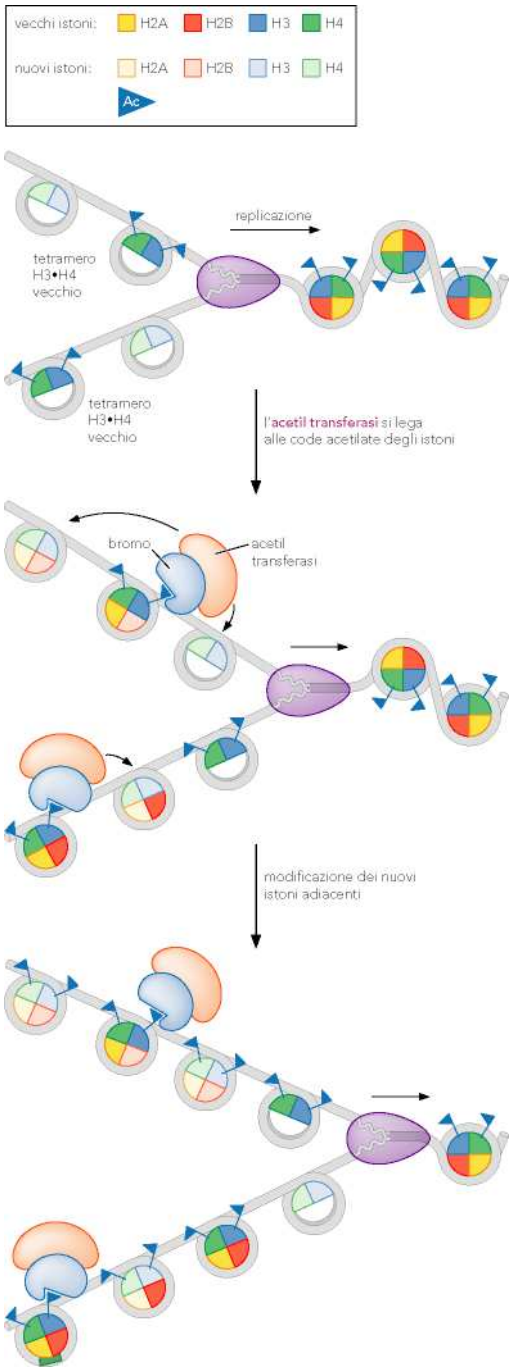

DNA
genetic
information
Chromatin
epigenetic
information
RNA?

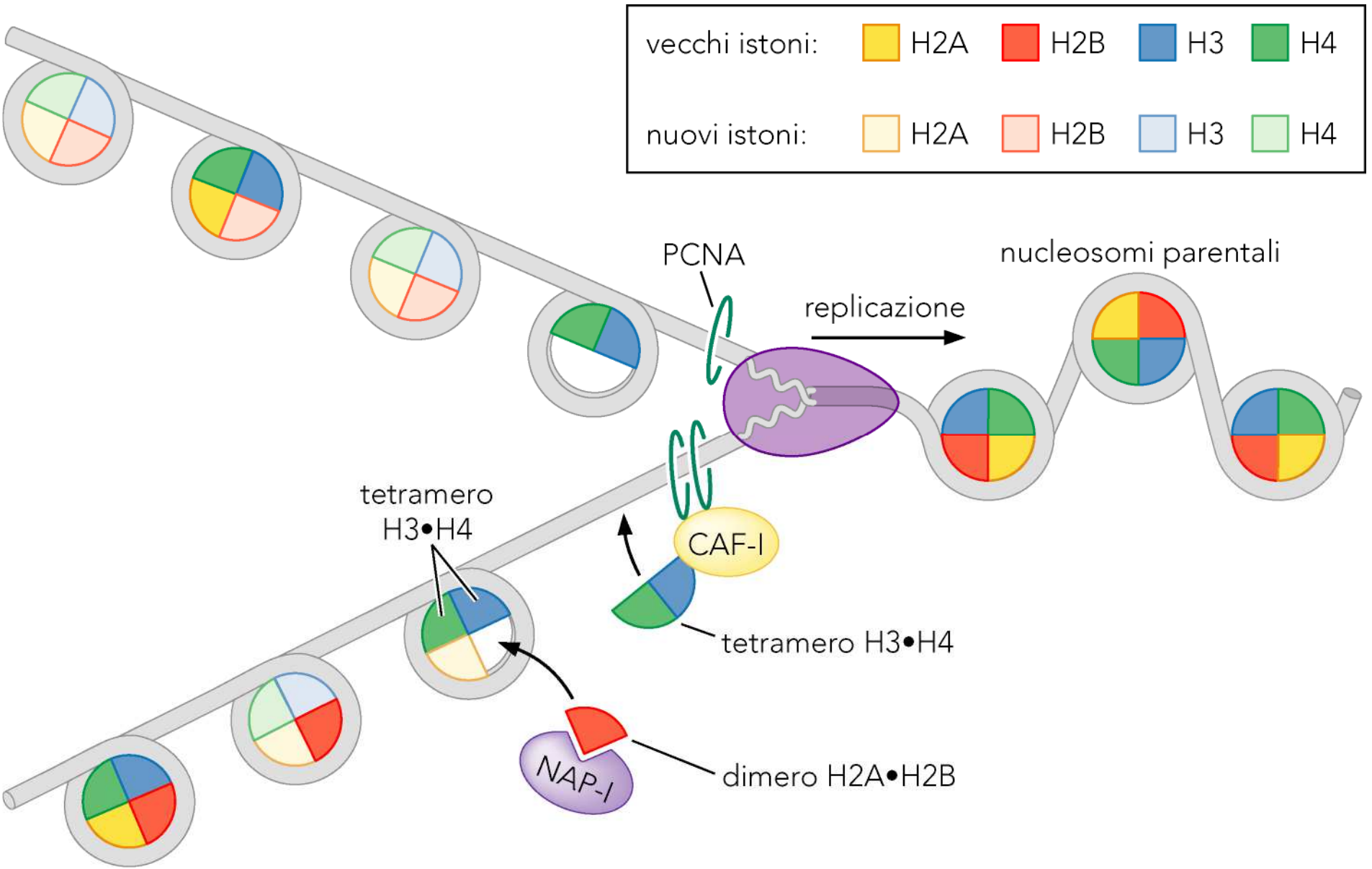

GENETIC INHERITANCE
gene X on
DNA SEQUENCE CHANGE
gene X off
MULTIPLICATION OF SOMATIC CELLS
gene X off
gene X off
PRODUCTION OF GERM CELLS
gene X off
EPIGENETIC INHERITANCE
gene Y on
CHROMATIN CHANGE
gene Y off
gene Y off
gene Y off
gene Y on


Histone
methylation
5-Methylcytosine
5-Hydroxymethylcytosine
H3 Histone
acetylation


Epigenetic adaptations
Environmental toxicants
Stochastic variations
Stress
Smoking
Poor Diet
Drinking
Aging
Grand parents: 1st generation
Transcriptional regulation
Transcriptional dysregulation
Parents: 2nd generation
Epigenetic disorder
Offspring: 3rd generation
Reproductive cells: 4th generation
Transcriptional regulation
Epigenetic-based therapy
Transcriptional dysregulation


vecchi istoni:
H2A
H2B
H3
H4
nuovi istoni:
H2A
H2B
H3
H4
direzione della
replicazione del DNA
nucleosoma
parentale
complesso
per la replicazione
del DNA
dimero H2A•H2B
tetramero H3•H4


vecchi istoni:
H2A
H2B
H3
H4
nuovi istoni:
H2A
H2B
H3
H4
Ac
replicazione
tetramero
H3•H4
vecchio
tetramero
H3•H4
vecchio
l'acetil transferasi si lega
alle code acetilate degli istoni
bromo
acetil
transferasi
modificazione dei nuovi
istoni adiacenti



PCNA à il bookmark degli strand in attiva replicazione

Grandma's Experiences Leave a Mark on Your Genes

Your ancestors' lousy childhoods or excellent adventures might change your personality, bequeathing anxiety or resilience by altering the epigenetic expressions of genes in the brain.

By Dan Hurley | Thursday, June 25, 2015

RELATED TAGS: GENES & HEALTH

Alison Mackey/DISCOVER

[This article originally appeared in print as "Trait vs. Fate"]

Michael Meaney
McGill University

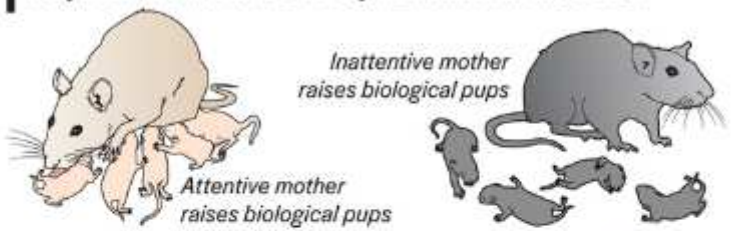
Moshe Szyf
McGill University

Actually, they ended up doing a series of elaborate experiments. With the assistance of postdoctoral researchers, they began by selecting mother rats who were either highly attentive or highly inattentive. Once a pup had grown up into adulthood, the team examined its hippocampus, a brain region essential for regulating the stress response. In the pups of inattentive mothers, they found that genes regulating the production of glucocorticoid receptors, which regulate sensitivity to stress hormones, were highly methylated; in the pups of conscientious moms, the genes for the glucocorticoid receptors were rarely methylated.

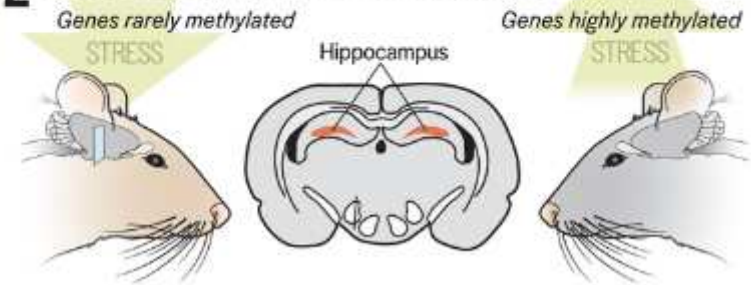
Thinkstock

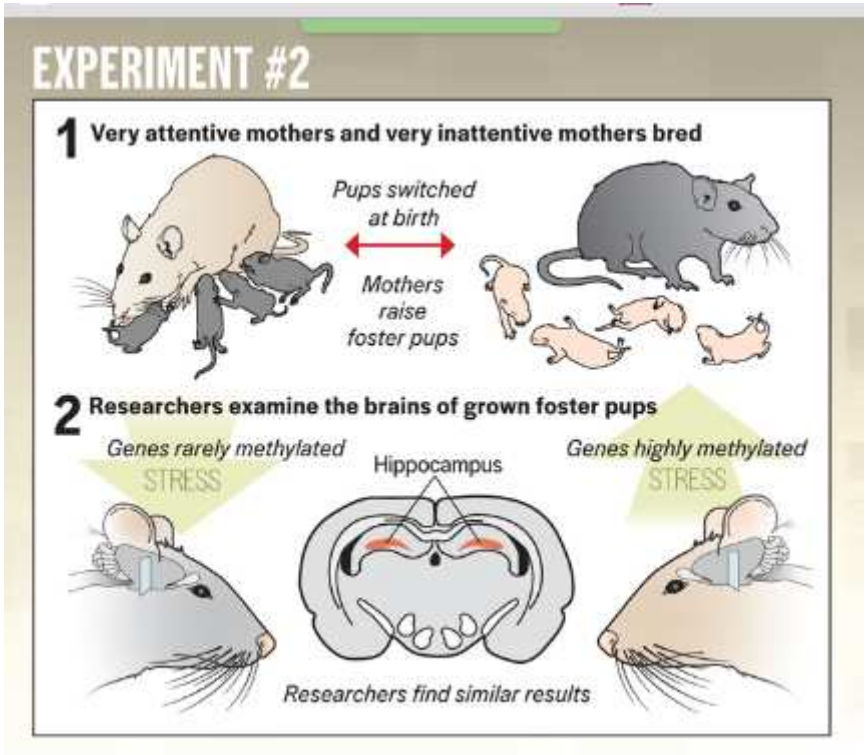
EXPERIMENT #1

1 **Very attentive mothers and very inattentive mothers bred**



2 **Researchers examine the brains of grown pups**




EXPERIMENT #2
1 Very attentive mothers and very inattentive mothers bred
Pups switched at birth
Mothers raise foster pups
2 Researchers examine the brains of grown foster pups
Genes rarely methylated
STRESS
Hippocampus
Genes highly methylated
STRESS
Researchers find similar results

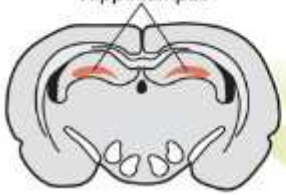
EXPERIMENT #3

1 **Inattentive mothers bred**

Inattentive mother raises biological pups

2 **Brains of "damaged" pups treated with trichostatin A, a drug that removes methyl groups**

Hippocampus



3 **Epigenetic changes disappear**

STRESS

Genes rarely methylated

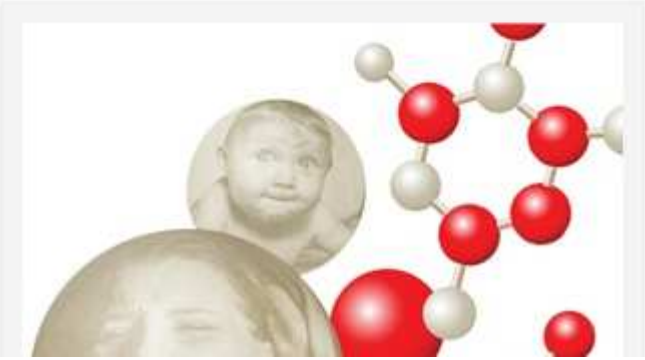
The Mark Of Cain

The message that a mother's love can make all the difference in a child's life is nothing new. But the ability of epigenetic change to persist across generations remains the subject of debate. Is methylation transmitted directly through the fertilized egg, or is each infant born pure, a methylated virgin, with the attachments of methyl groups slathered on solely by parents after birth?

Neuroscientist Eric Nestler of the Icahn School of Medicine at Mount Sinai in New York has been seeking an answer for years. In one study, he exposed male mice to 10 days of bullying by larger, more aggressive mice. At the end of the experiment, the bullied mice were socially withdrawn.

To test whether such effects could be transmitted to the next generation, Nestler took another group of bullied mice and bred them with females, but kept them from ever meeting their offspring.

Despite having no contact with their depressed fathers, the offspring grew up to be hypersensitive to stress. "It was not a subtle effect; the offspring were dramatically more susceptible to developing signs of depression," he says.



In further testing, Nestler took sperm from defeated males and impregnated females through in vitro fertilization. The offspring did not show most of the behavioral abnormalities, suggesting that epigenetic transmission may not be at the root. Instead, Nestler proposes, "the female might know she had sex with a loser. She knows it's a tainted male she had sex with, so she cares for her pups differently," accounting for the results.

Alison Mackey/DISCOVER

Despite his findings, no consensus has yet emerged. The latest evidence, published in the Jan. 25 issue of the journal *Science*, suggests that epigenetic changes in mice are usually erased, but not always. The erasure is imperfect, and sometimes the affected genes may make it through to the next generation, setting the stage for transmission of the altered traits in descendants as well.

What's Next?

nature neuroscience

Epigenetic programming by maternal behavior

Ian C G Weaver[1,2], Nadia Cervoni[3], Frances A Champagne[1,2], Ana C D'Alessio[3], Shakti Sharma[1], Jonathan R Seckl[4], Sergiy Dymov[3], Moshe Szyf[2,3] & Michael J Meaney[1,2]

Here we report that increased pup licking and grooming (LG) and arched-back nursing (ABN) by rat mothers altered the offspring epigenome at a glucocorticoid receptor (GR) gene promoter in the hippocampus. Offspring of mothers that showed high levels of LG and ABN were found to have differences in DNA methylation, as compared to offspring of 'low-LG-ABN' mothers. These differences emerged over the first week of life, were reversed with cross-fostering, persisted into adulthood and were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter. Central infusion of a histone deacetylase inhibitor removed the group differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal (HPA) responses to stress, suggesting a causal relation among epigenomic state, GR expression and the maternal effect on stress responses in the offspring. Thus we show that an epigenomic state of a gene can be established through behavioral programming, and it is potentially reversible.

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Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

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Abstract

Maternal care influences hypothalamic-pituitary-adrenal (HPA) function in the rat through epigenetic programming of glucocorticoid receptor expression. In humans, childhood abuse alters HPA stress responses and increases the risk of suicide. We examined epigenetic differences in a neuron-specific glucocorticoid receptor (*NR3C1*) promoter between postmortem hippocampus obtained from suicide victims with a history of childhood abuse and those from either suicide victims with no childhood abuse or controls. We found decreased levels of glucocorticoid receptor mRNA, as well as mRNA transcripts bearing the glucocorticoid receptor 1_F splice variant and increased cytosine methylation of an *NR3C1* promoter. Patch-methylated *NR3C1* promoter constructs that mimicked the methylation state in samples from abused suicide victims showed decreased NGFI-A transcription factor binding and NGFI-A–inducible gene transcription. These findings translate previous results from rat to humans and suggest a common effect of parental care on the epigenetic regulation of hippocampal glucocorticoid receptor expression.

Volume 170 Issue 5, May 2013, pp. 511-520

New Research

Genome-Wide Methylation Changes in the Brains of Suicide Completers

Benoit Labonté, M.Sc., Matt Suderman, Ph.D., Gilles Maussion, Ph.D., Juan Pablo Lopez, B.Sc., Luis Navarro-Sánchez, M.Sc., Volodymyr Yerko, Ph.D., Naguib Mechawar, Ph.D., Moshe Szyf, Ph.D., Michael J. Meaney, Ph.D., and Gustavo Turecki, M.D., Ph.D.

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Accepted: November 26, 2012
Published online: May 01, 2013 | http://dx.doi.org/10.1176/appi.ajp.2012.12050627

Abstract

Objective
Gene expression changes have been reported in the brains of suicide completers. More recently, differences in promoter DNA methylation between suicide completers and comparison subjects in specific genes have been associated with these changes in gene expression patterns, implicating DNA methylation alterations as a plausible component of the pathophysiology of suicide. The authors used a genome-wide approach to investigate the extent of DNA methylation alterations in the brains of suicide completers.

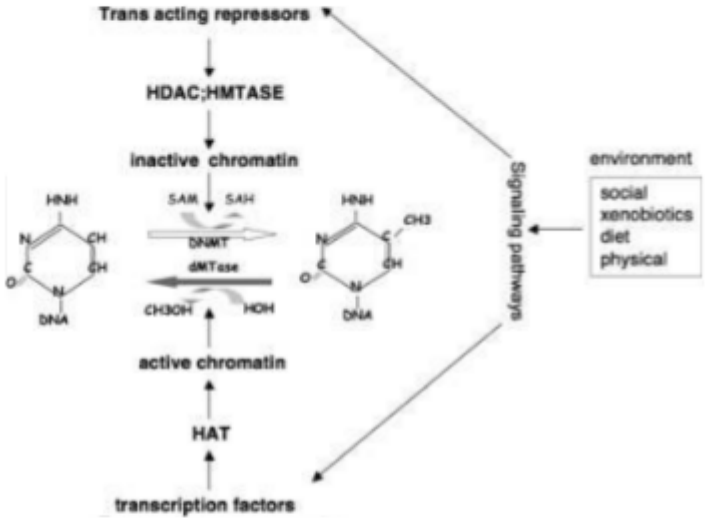

Fig. 1. The dynamic and responsive DNA methylation pattern; a model. A balance of methylation and demethylation reactions determines the DNA methylation state. Active chromatin facilitates DNA demethylation while inactive chromatin facilitates methylation. Different environmental signals trigger pathways in the cell that activate sequence specific factors which recruit chromatin modifying enzymes to specific loci resulting in either activation or inactivation of chromatin.

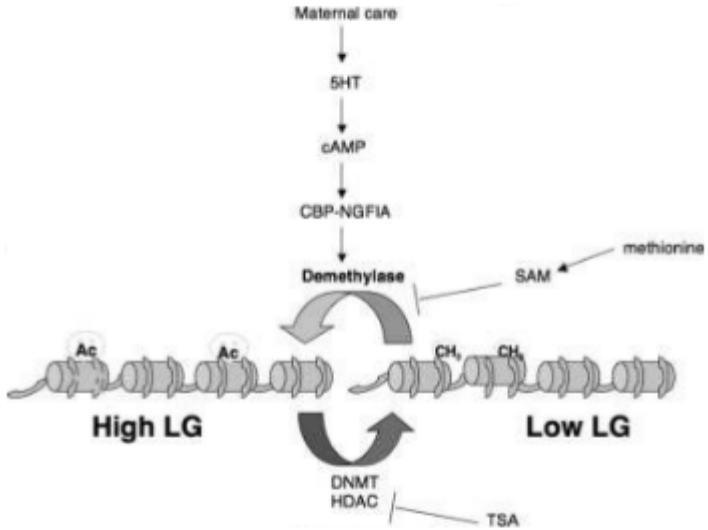

Fig. 2. Epigenetic reprogramming by maternal care; a model. Maternal licking and grooming in the rat triggers activation of 5HT receptor in the hippocampus leading to increase in intracellular cAMP, activation of the transcription factor NGFIA and recruitment of the HAT CBP to the GR exon 1_7 promoter. Acetylation of histone tails facilitates demethylation. In offspring of Low licking and grooming mothers this process is reduced in comparison with offspring of High licking and grooming mothers leading to differential epigenetic programming of the GR promoter. In the adult rat the epigenetic state is reversible. TSA a HDAC inhibitor increases histone acetylation and facilitates demethylation and epigenetic activation of the gene in the offspring of the Low licking and grooming mothers. Conversely, injection of methionine to adult offspring of the High licking and grooming mothers leads to increased SAM, inhibition of demethylation, increased DNA methylation, and reduced activity of the GR exon 1_7 promoter gene.

Indeed, in contrast to DNMTs, which are recruited by chromatin silencing enzymes such as SUV39 [Fuks et al., 2003a] and EZH2 [Vire et al., 2006; 2007], demethylation is facilitated by histone acetylation [Cervoni and Szyf, 2001; Cervoni et al., 2002]. Pharmacological acetylation using HDAC inhibitors such as TSA [Cervoni and Szyf, 2001] or valproic acid [Detich et al., 2003a] trigger rapi-

Epigenetic differences arise during the lifetime of monozygotic twins

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Edited by Stanley M. Gartler, University of Washington, Seattle, WA, and approved May 23, 2005 (received for review January 17, 2005)

Monozygous twins share a common genotype. However, most monozygotic twin pairs are not identical; several types of phenotypic discordance may be observed, such as differences in susceptibilities to disease and a wide range of anthropomorphic features. There are several possible explanations for these observations, but one is the existence of epigenetic differences. To address this issue, we examined the global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins. We found that, although twins are epigenetically indistinguishable during the early years of life, older monozygous twins exhibited remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, affecting their gene-expression portrait. These findings indicate how an appreciation of epigenetics is missing from our understanding of how different phenotypes can be originated from the same genotype.

Materials and Methods

Subjects. Eighty volunteer Caucasian twins from Spain were recruited in the study, including 30 male and 50 female subjects. Their mean (±SD) age was 30.6 (±14.2) years (range, 3–74 years). Twins studied included monochorionic and dichorionic. All subjects, or in the case of children, the parents, gave their informed written consent to be included in the study. Lymphocyte cells were purified by standard procedures and stored at −80°C. In eight cases, epithelial skin cells were obtained from buccal smears. Muscle biopsy tissues ($n = 14$) from the vastus lateralis muscle and s.c. abdominal tissue ($n = 4$) were obtained by needle suction under local anesthesia from volunteer MZ twins from Denmark and the United Kingdom, respectively. Homozygosity was determined by using highly polymorphic short tandem-repeat loci. With five markers, the probability that any twin pair was MZ if all markers were concordant was 99% (5).

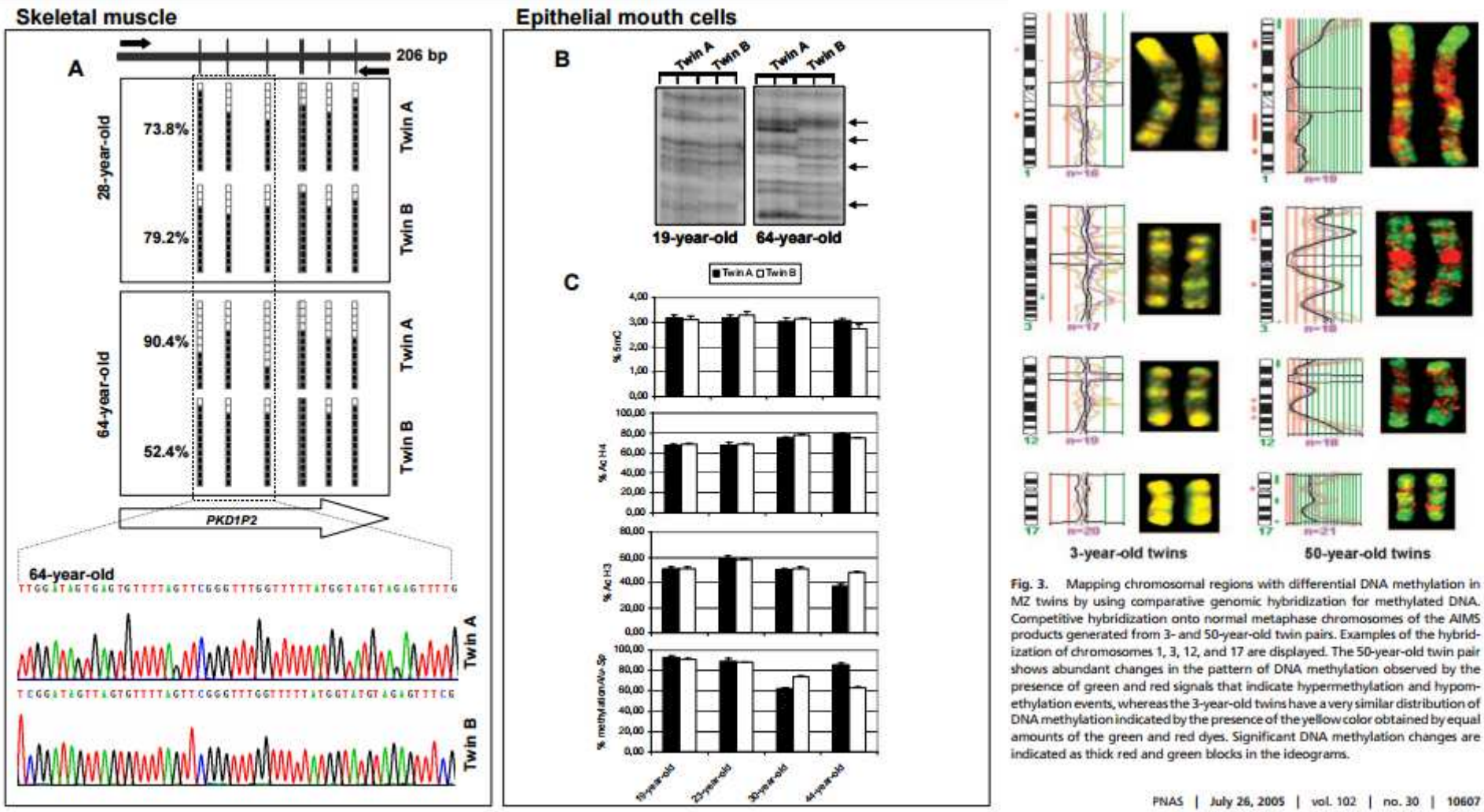
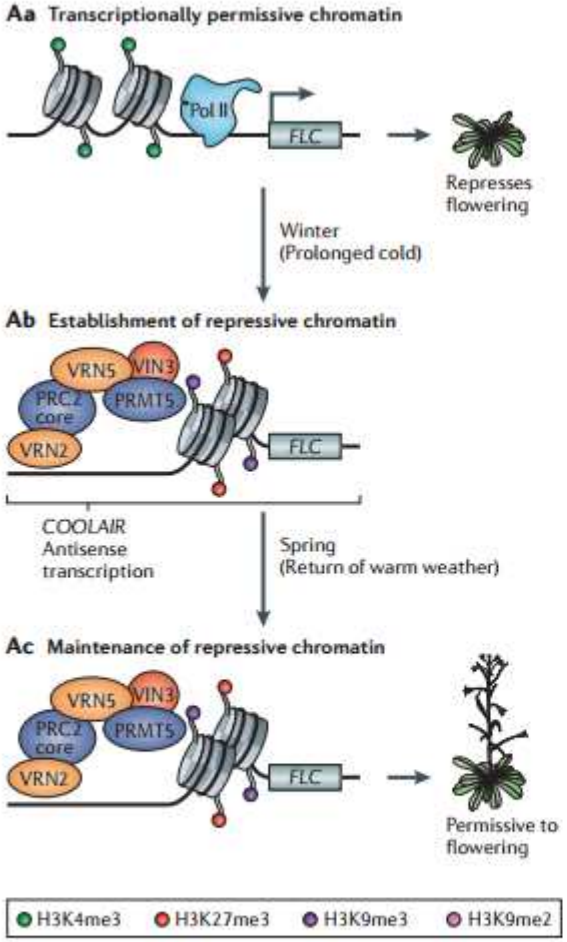
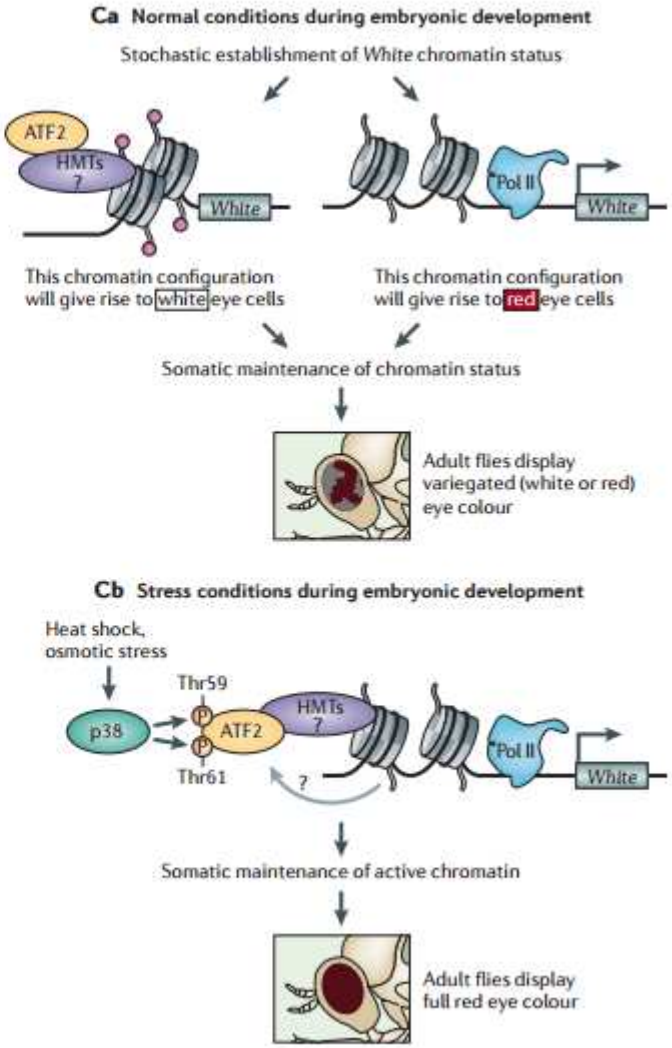


Fig. 3. Mapping chromosomal regions with differential DNA methylation in MZ twins by using comparative genomic hybridization for methylated DNA. Competitive hybridization onto normal metaphase chromosomes of the AIMS products generated from 3- and 50-year-old twin pairs. Examples of the hybridization of chromosomes 1, 3, 12, and 17 are displayed. The 50-year-old twin pair shows abundant changes in the pattern of DNA methylation observed by the presence of green and red signals that indicate hypermethylation and hypomethylation events, whereas the 3-year-old twins have a very similar distribution of DNA methylation indicated by the presence of the yellow color obtained by equal amounts of the green and red dyes. Significant DNA methylation changes are indicated as thick red and green blocks in the ideograms.

Epigenetics and the environment: emerging patterns and implications

Robert Feil[1] and Mario F. Fraga[2,3]





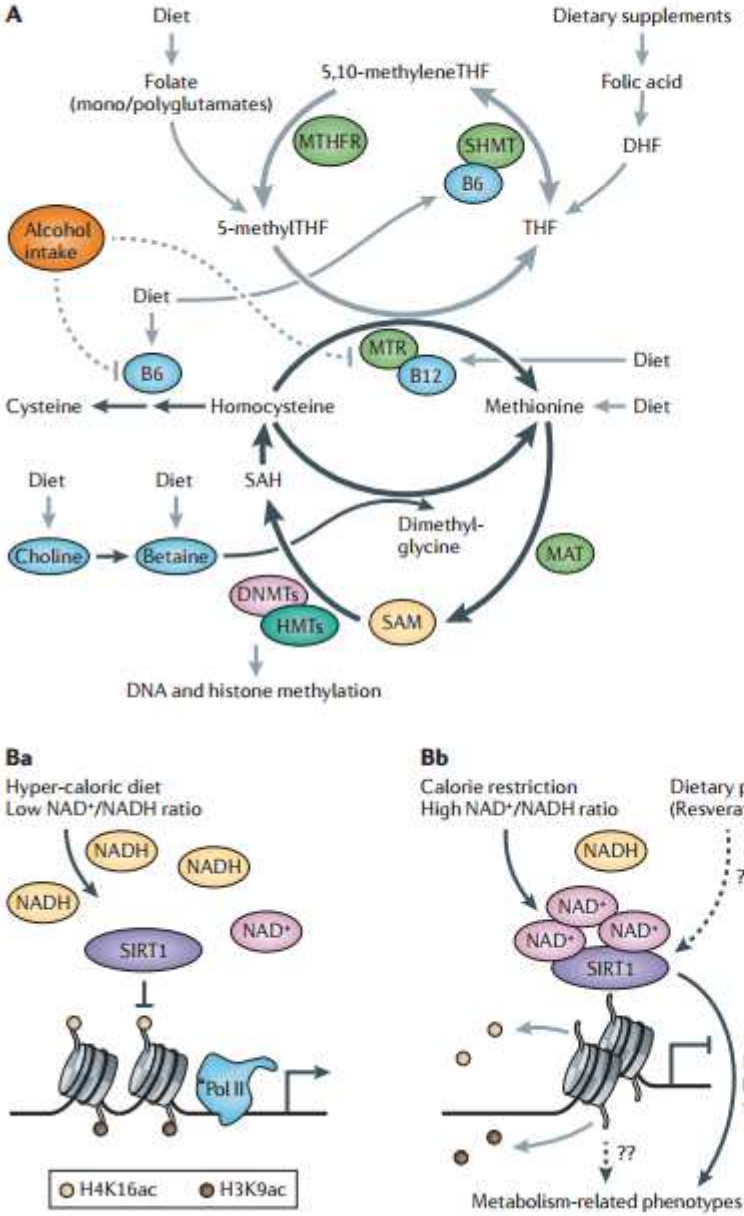


Figure 4 | **Molecular mechanisms that mediate environmental effects. A** | Levels of *S*-adenosylmethionine (SAM) affect global DNA and histone methylation. In cells, SAM is generated by the methionine cycle (also known as the one-carbon cycle; thick black arrows). The cycle incorporates methyl groups 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].

Epigenetics and the environment: emerging patterns and implications

Robert Feil[1] and Mario F. Fraga[2,3]

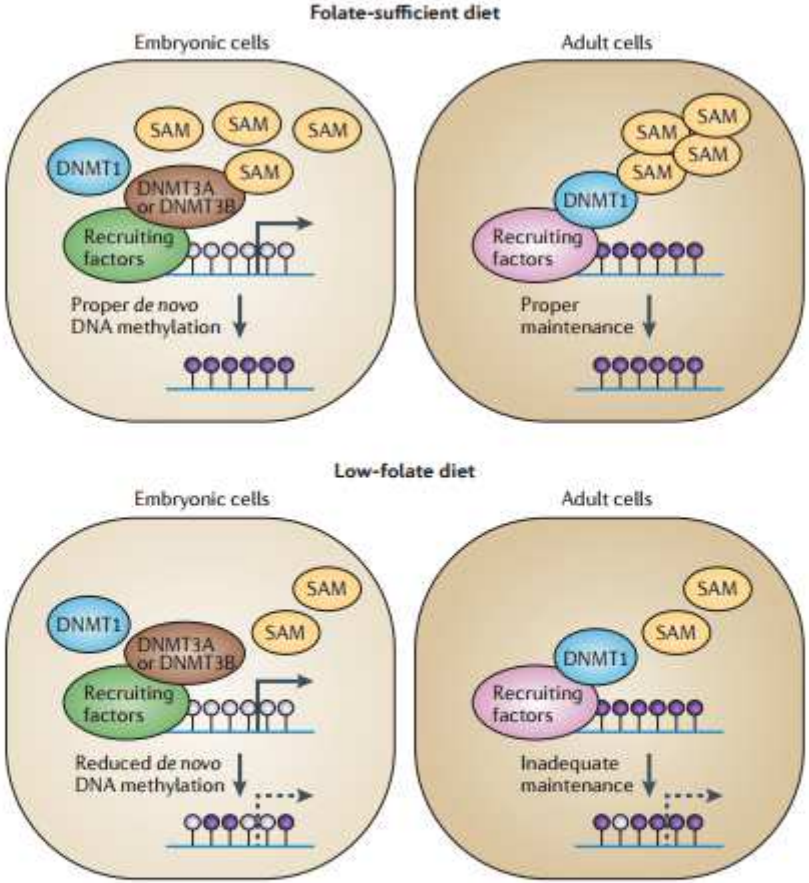
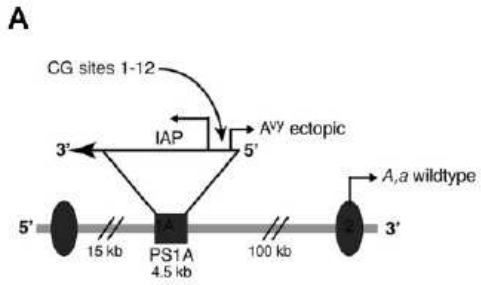


Figure 3 | **Time windows of environmental susceptibility in mammals.** Epigenetic transitions play crucial roles in development and in the differentiation of stem cells and primordial germ cells. Concordantly, the regulating enzymes are generally highly expressed in these pluripotent cells[6]. For example, the *de novo* methyltransferases, DNMT3A and DNMT3B, are highly expressed in the early embryonic cells in which *de novo* methylation is acquired. Low amounts of external methyl donor groups from dietary sources can reduce the concentrations of the universal methyl donor, S-adenosylmethionine (SAM), and can readily affect *de novo* DNA methylation. Also, aberrant gains of methylation may occur in early embryonic cells owing to other external triggers. In adult cells, the maintenance of DNA methylation is performed mainly by the maintenance methyltransferase, DNMT1, in a process that seems less sensitive to diet-induced changes in the abundance of methyl donors.



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TTCTATTTTAAAGAGAACAACCATTGCCTAGGGCGCATCACTCCCTGATTG
CTGCAGCCCATGGCCGAGCTGACGTTCACGGGAAAAACAGGTACAAGTG
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ACAGGATGTCAGCGCCATCTTGTGACGGCGAATGTGGGGGCGGCTTCCC
ACACCATTTCCCACCAGTCTGAGTCCTTGAGCCTCTGCAGCCTCAGAAGAG
GGAGTCATCAGCTGAAACCTCCAGGAACCACCGGGGGGTCCCAGAAGGTAT
GTGCTAA

MOLECULAR AND CELLULAR BIOLOGY, Aug. 2003, p. 5293–5300
0270-7306/03/$08.00+0 DOI: 10.1128/MCB.23.15.5293–5300.2003

Vol. 23, No. 15

Transposable Elements: Targets for Early Nutritional Effects on Epigenetic Gene Regulation

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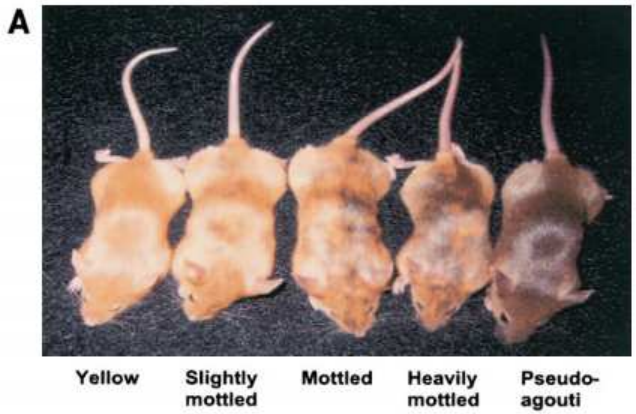
Early nutrition affects adult metabolism in humans and other mammals, potentially via persistent alterations in DNA methylation. With viable yellow agouti (A^{vy}) mice, which harbor a transposable element in the *agouti* gene, we tested the hypothesis that the metastable methylation status of specific transposable element insertion sites renders them epigenetically labile to early methyl donor nutrition. Our results show that dietary methyl supplementation of *a/a* dams with extra folic acid, vitamin B_{12}, choline, and betaine alter the phenotype of their A^{vy}/a offspring via increased CpG methylation at the A^{vy} locus and that the epigenetic metastability which confers this lability is due to the A^{vy} transposable element. These findings suggest that dietary supplementation, long presumed to be purely beneficial, may have unintended deleterious influences on the est lishment of epigenetic gene regulation in humans.

A^{vy} mice. This not only leads to a yellow coat color, it also antagonizes the melanocortin 4 receptor (MC4R) in the hypothalamus, causing the animals to become obese and develop diabetes and cancer at a high frequency. In contrast, the incidence of these diseases is markedly reduced in pseudoagouti (brown) offspring that develop when this promoter is hypermethylated



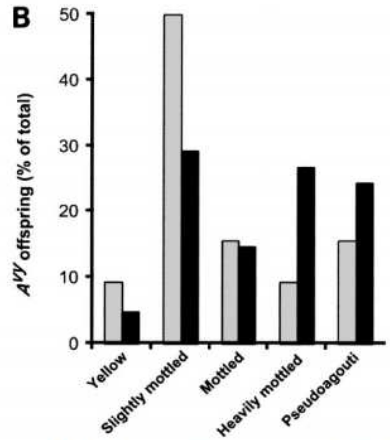


FIG. 2. Maternal dietary methyl supplementation and coat color phenotype of A^{vy}/a offspring. (A) Isogenic A^{vy}/a animals representing the five coat color classes used to classify phenotype. The A^{vy} alleles of yellow mice are hypomethylated, allowing maximal ectopic *agouti* expression. A^{vy} hypermethylation silences ectopic *agouti* expression in pseudoagouti animals (15), recapitulating the agouti phenotype. (B) Coat color distribution of all A^{vy}/a offspring born to nine unsupplemented dams (30 offspring; shaded bars) and 10 supplemented dams (39 offspring; black bars). The coat color distribution of supplemented offspring is shifted toward the pseudoagouti phenotype compared to that of unsupplemented offspring ($P = 0.008$).

PLOS | BIOLOGY

PERSPECTIVE

Transgenerational Epigenetic Contributions to Stress Responses: Fact or Fiction?

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Abstract

There has been increasing interest in the possibility that behavioral experience—in particular, exposure to stress—can be passed on to subsequent generations through heritable epigenetic modifications. The possibility remains highly controversial, however, reflecting the lack of standardized definitions of epigenetics and the limited empirical support for potential mechanisms of transgenerational epigenetic inheritance. Nonetheless, growing evidence supports a role for epigenetic regulation as a key mechanism underlying lifelong regulation of gene expression that mediates stress vulnerability. This Perspective provides an overview of the multiple meanings of the term epigenetic, discusses the challenges of studying epigenetic contributions to stress susceptibility—and the experimental evidence for and against the existence of such mechanisms—and outlines steps required for future investigations.

Cell

Volume 161, Issue 6, 4 June 2015, Pages 1248–1251

Preview

Forget the Parents: Epigenetic Reprogramming in Human Germ Cells

Ferdinand von Meyenn[1], Wolf Reik[1, 2, 3]

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doi:10.1016/j.cell.2015.05.039

Refers To

Sofia Gkountela, Kelvin X. Zhang, Tiasha A. Shafiq, Wen-Wei Liao, Joseph Hargan-Calvopiña, Pao-Yang Chen, Amander T. Clark

DNA Demethylation Dynamics in the Human Prenatal Germline

Cell, Volume 161, Issue 6, 4 June 2015, Pages 1425-1436

PDF (4472 K) | Supplementary content

Fan Guo, Liying Yan, Hongshan Guo, Lin Li, Boqiang Hu, Yangyu Zhao, Jun Yong, Yuqiong Hu, Xiaoye Wang, Yuan Wei, Wei Wang, Rong Li, Jie Yan, Xu Zhi, Yan Zhang, Hongyan Jin, Wenxin Zhang, Yu Hou, Ping Zhu, Jingyun Li, Ling Zhang, *et al.*

The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells

Cell, Volume 161, Issue 6, 4 June 2015, Pages 1437-1452

PDF (3316 K) | Supplementary content

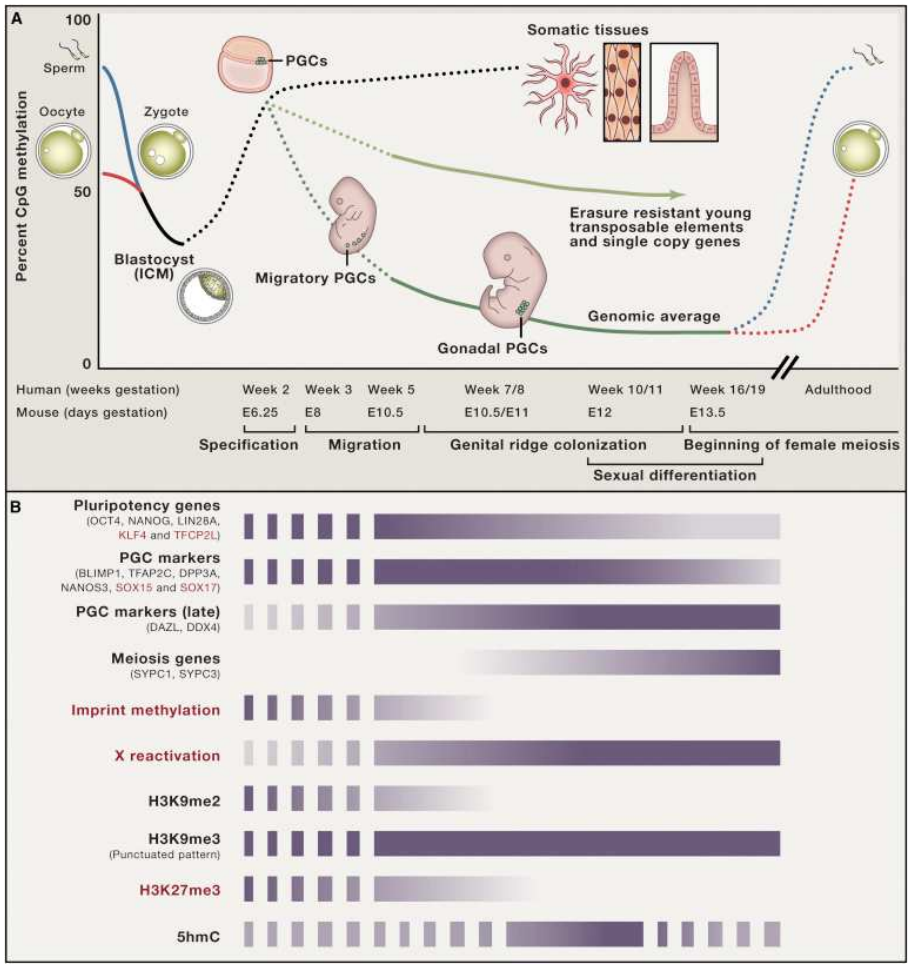
Walfred W.C. Tang, Sabine Dietmann, Naoko Irie, Harry G. Leitch, Vasileios I. Floros, Charles R. Bradshaw, Jamie A. Hackett, Patrick F. Chinnery, M. Azim Surani

A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development

Cell, Volume 161, Issue 6, 4 June 2015, Pages 1453-1467

PDF (11830 K) | Supplementary content

Epigenetic reprogramming in the germline resets genomic potential and erases epigenetic memory. Three studies by Gkountela et al., Guo et al., and Tang et al. analyze the transcriptional and epigenetic landscape of human primordial germ cells, revealing a unique transcriptional network and progressive and conserved global erasure of DNA methylation.



Epigenetic Reprogramming in Human Primordial Germ Cells

(A) After fertilization, the paternal (blue) and maternal (red) genomes undergo global demethylation, resetting the human epigenome for naive pluripotency at the blastocyst stage. Following a yet-uncharacterized phase of de novo methylation in the epiblast, human PGCs are specified in the posterior epiblast (week 2), from where they migrate through the hindgut to the developing genital ridges. During this migratory phase (weeks 3–5), human PGCs must undergo a first wave of global DNA demethylation, including significant loss of methylation at imprint control regions. The methylomes and transcriptomes from human PGCs between 5.5 and 19 weeks of age have now been analyzed. During this phase, DNA methylation is further erased genome wide, restoring germline potency, whereas only a small number of evolutionarily young transposable elements and single copy genes are not completely demethylated and could be potential sites of transgenerational epigenetic inheritance.

(B) The chart summarizes the main transcriptional and epigenetic characteristics of human germ cell development and also highlights key differences between human and mouse PGCs (marked in red).

THE HISTONE SONG

(Sulla musica dei "Flintstones")

Histones!
Meet the Histones!
They're a basic protein family.
There are
Just four subtypes.
They're conserved evolutionarily.

Two each
Of each histone
Do their thing.
Forming
Just like beads upon a string.

H1
Binds the complex.
And it inhibits new transcription
And the creation
Of new mRNA.