INSTITUTE OF HUMAN GENETICS

# Chromatin structure and epigenetic regulation of eukaryotic gene expression

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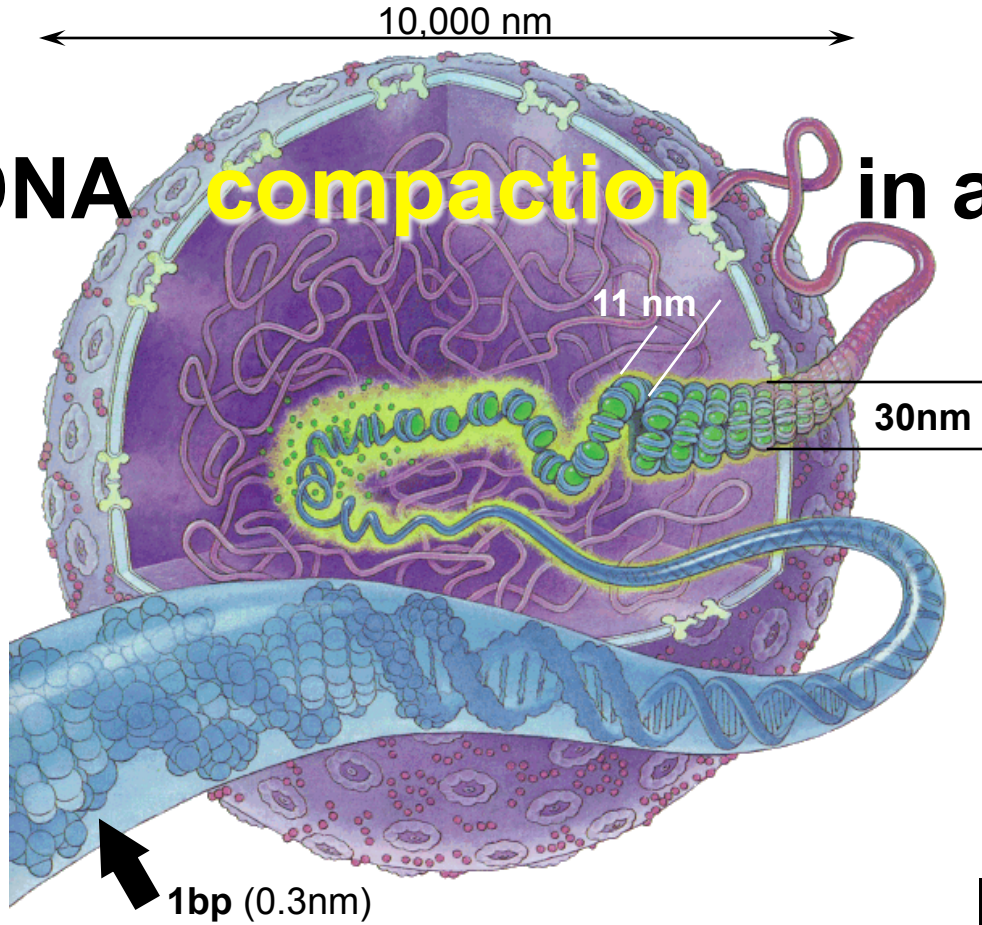
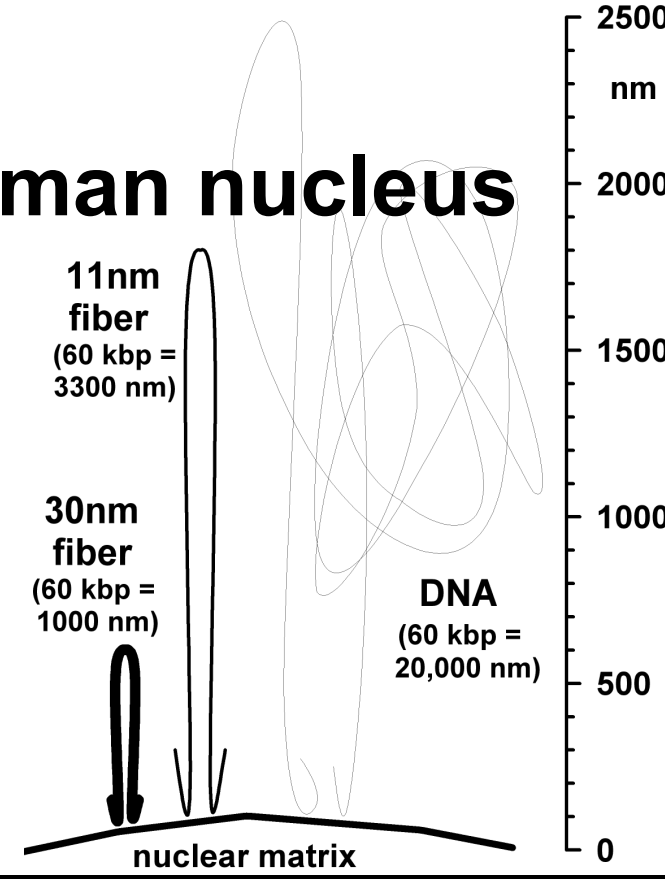
12.09.2016

# DNA compaction in a human nucleus

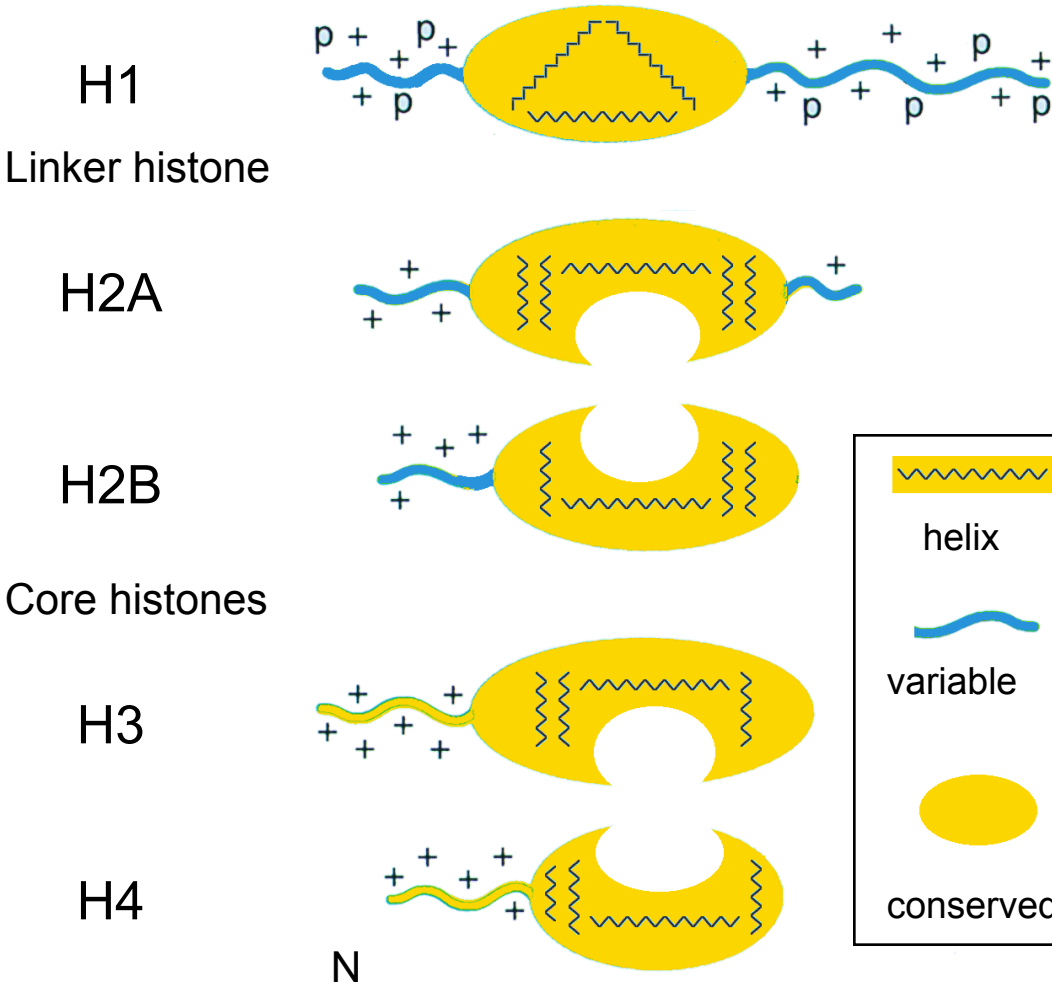



| | | | compact size | DNA | length | compaction |
|---|---|---|---|---|---|---|
| nucleus (human) | 2 x 23 = 46 chromosomes | 92 DNA molecules | 10 μm ball | 12,000 Mbp | 4 m DNA | **400,000 x** |
| mitotic chromosome | 2 chromatids, 1 μm thick | 2 DNA molecules | 10 μm long X | 2x 130 Mbp | 2x 43 mm DNA | **10,000 x** |
| DNA domain | anchored DNA loop | 1 replicon ? | 60 nm x 0.5 μm | 60 kbp | 20 μm DNA | **35 x** |
| chromatin fiber | approx. 6 nucleosomes per 'turn' of 11 nm | | 30 nm diameter | 1200 bp | 400 nm DNA | **35 x** |
| nucleosome | disk 1 ? turn of DNA (146 bp) + linker DNA | | 6 x 11 nm | 200 bp | 66 nm DNA | **6 - 11 x** |
| base pair | | | 0.33 x 1.1 nm | 1 bp | 0.33 nm DNA | **1 x** |


Compaction of DNA by histones

Compaction by chromosome scaffold / nuclear matrix



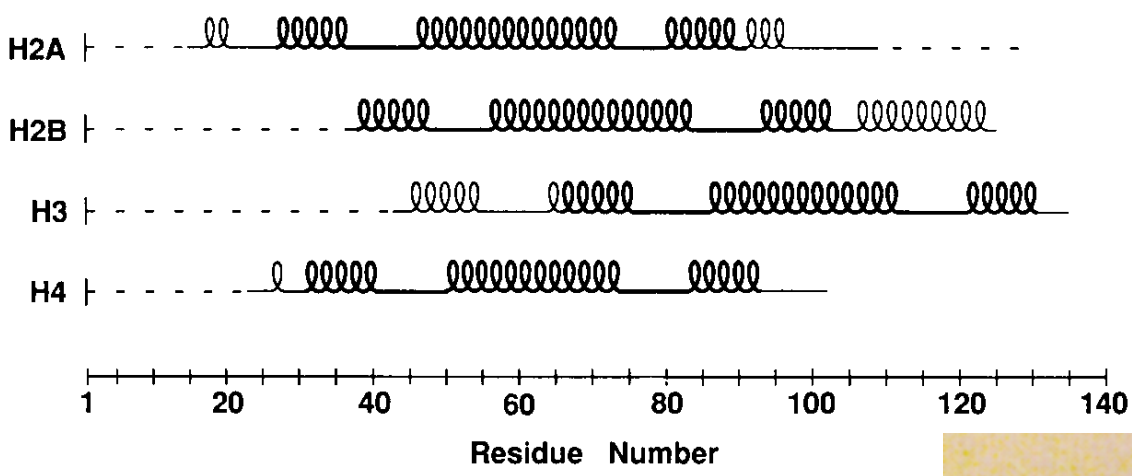

# HISTONES
are
**highly conserved, small, basic proteins**

**Histone acetylation**
is a reversible modification of lysines in the N-termini of the core histones.

**Result**:
- reduced binding to DNA
- destabilization of chromatin

| 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 |

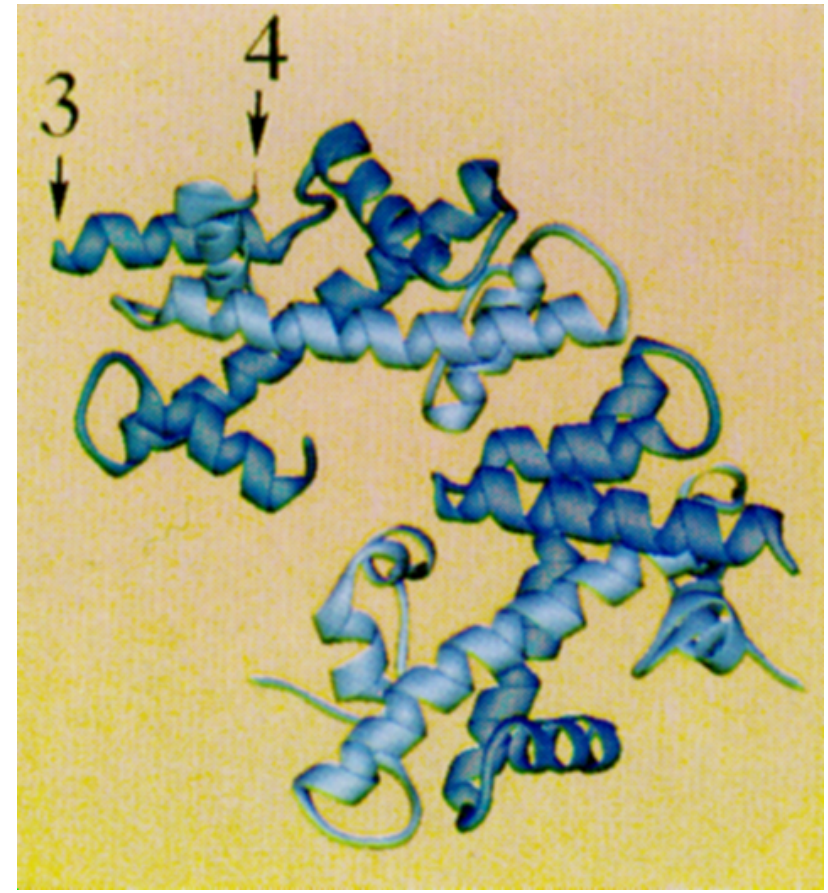
# Core Histones




# The histone-fold

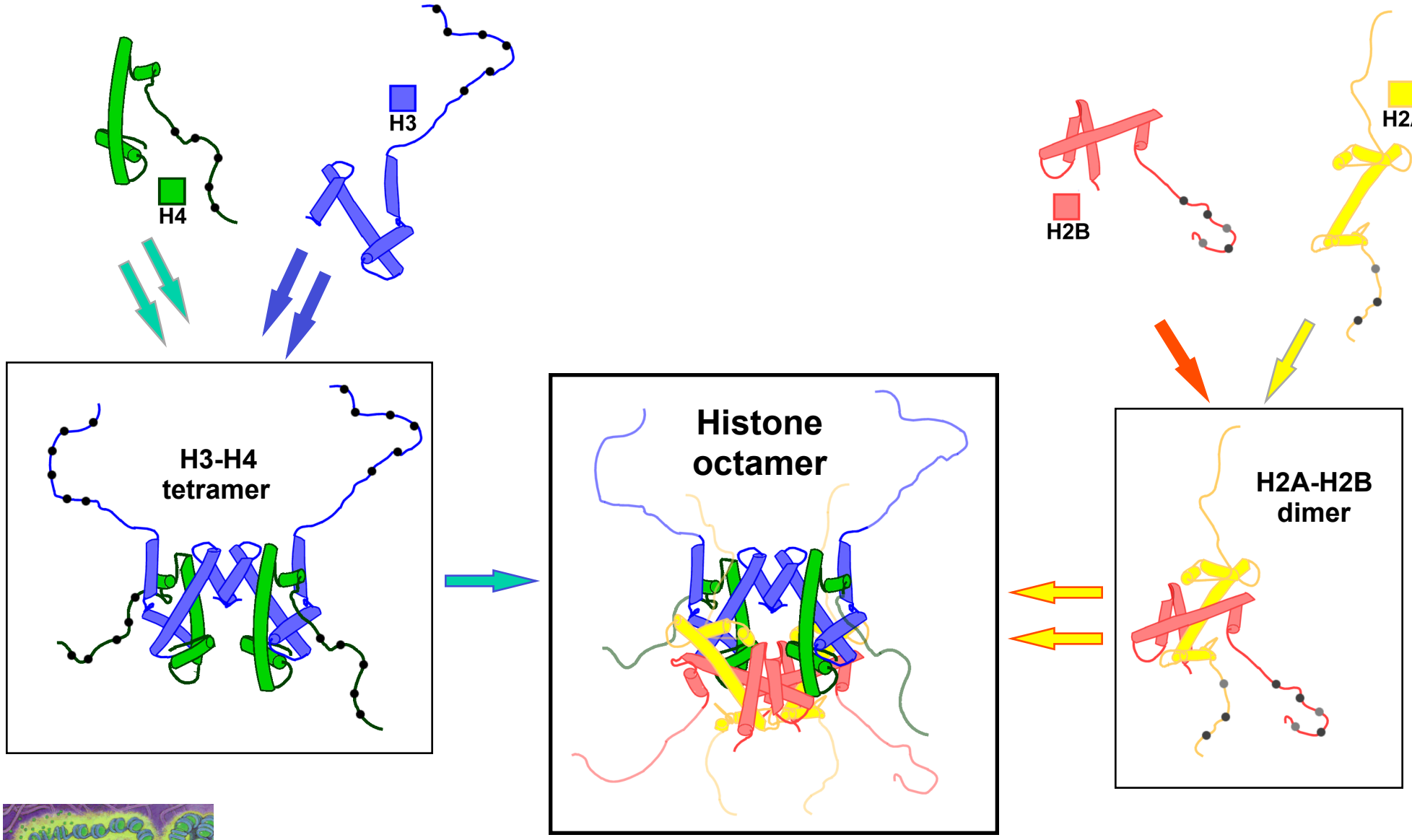
The basic structure of ALL core histones is the same:

- 1 long hydrophobic alpha-helix, bordered by
- 2 short hydrophobic alpha helices that form pairs
- H2A - H2B and H3 - H4 which interact.



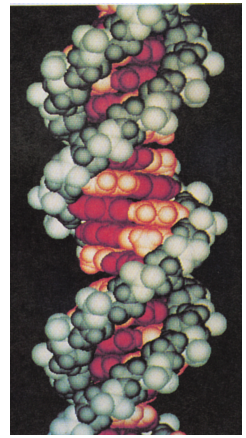

**References:** *Moudrianakis et al. PNAS 88, 10138 (1991); PNAS 90, 10489 (1993); PNAS 92, 11170 (1995)*

# Histone octamer assembly

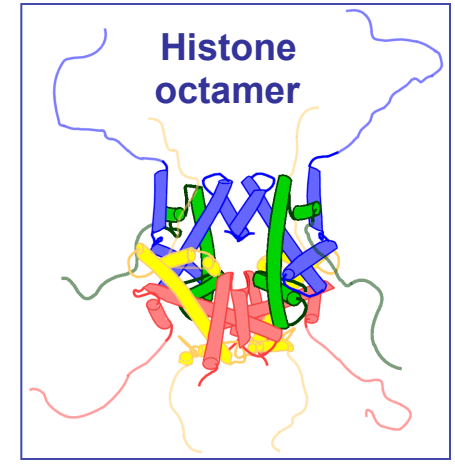


# Nucleosome assembly

**145 bp of DNA**

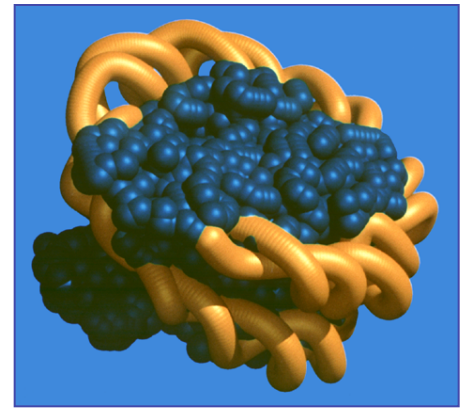


+



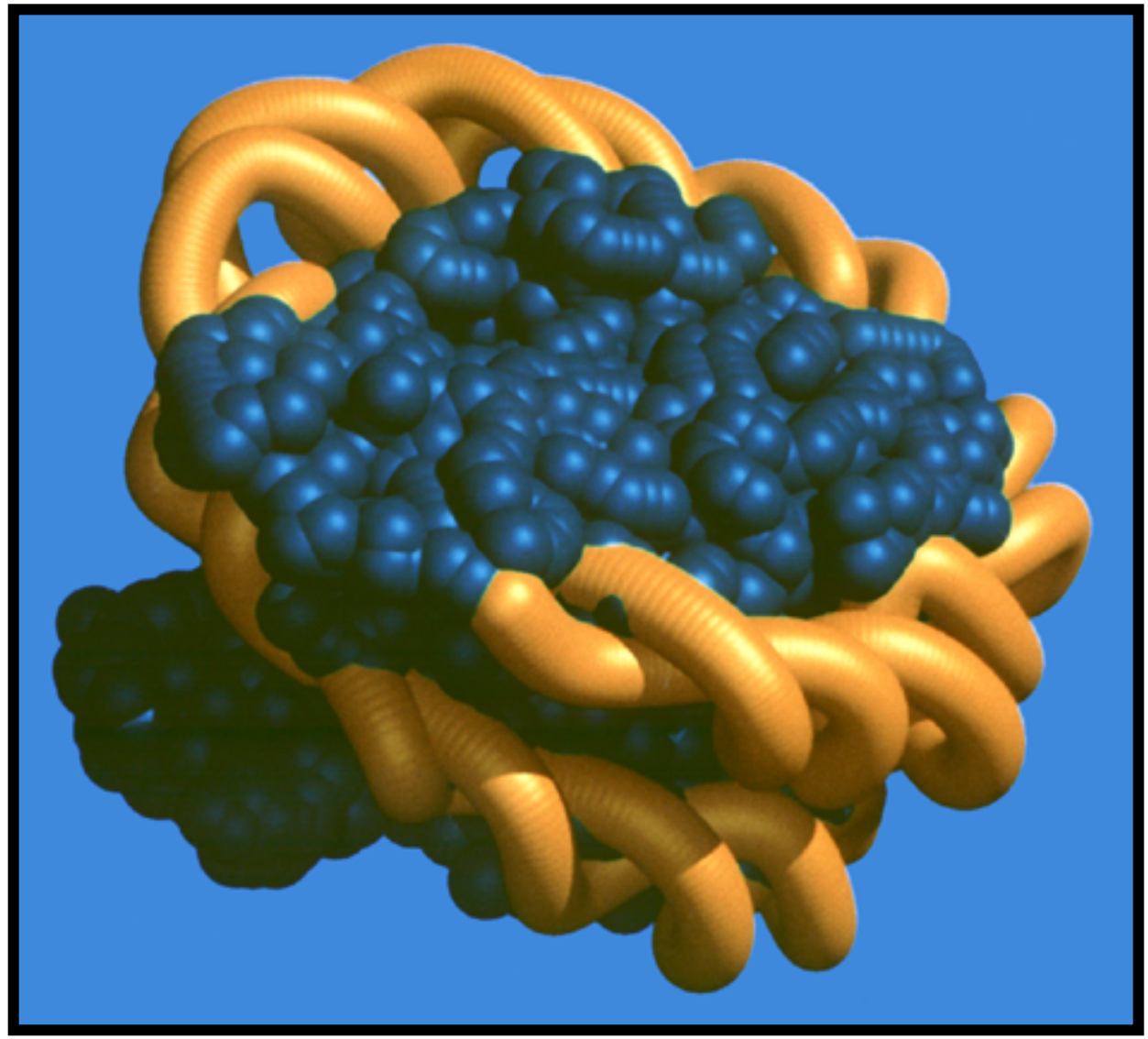

**An octamer (8 units) of histones:**
4 core histones
**H2A**, **H2B**, **H3**, **H4**
(X2)

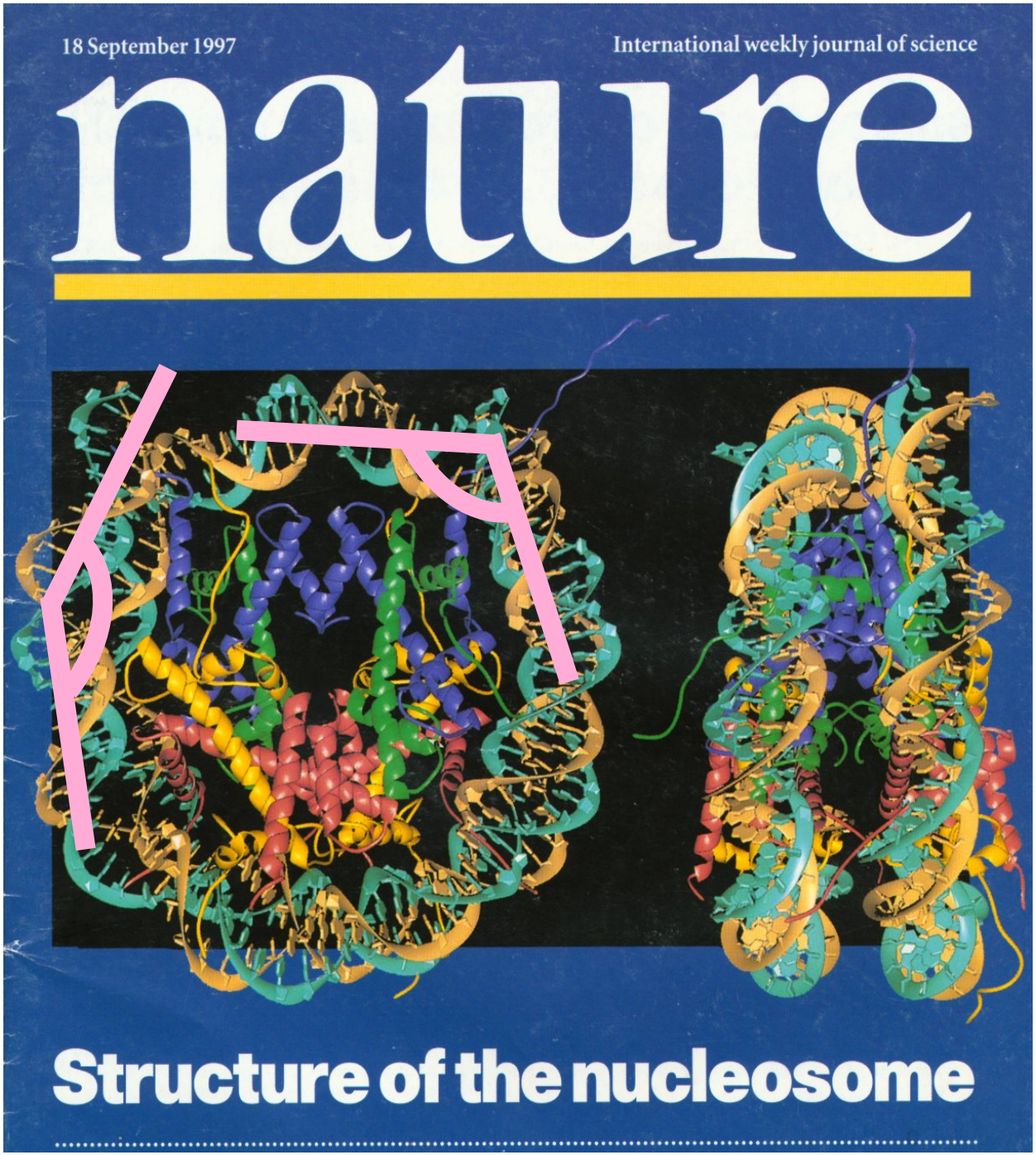
**Nucleosome**



--> The histone octamer organizes **145 bp of DNA in 1 3/4 helical turn of DNA**:
48 nm of DNA packaged in a disc of 6 x 11nm

# The Nucleosome as the fundamental chromatin unit






# Nucleosome features

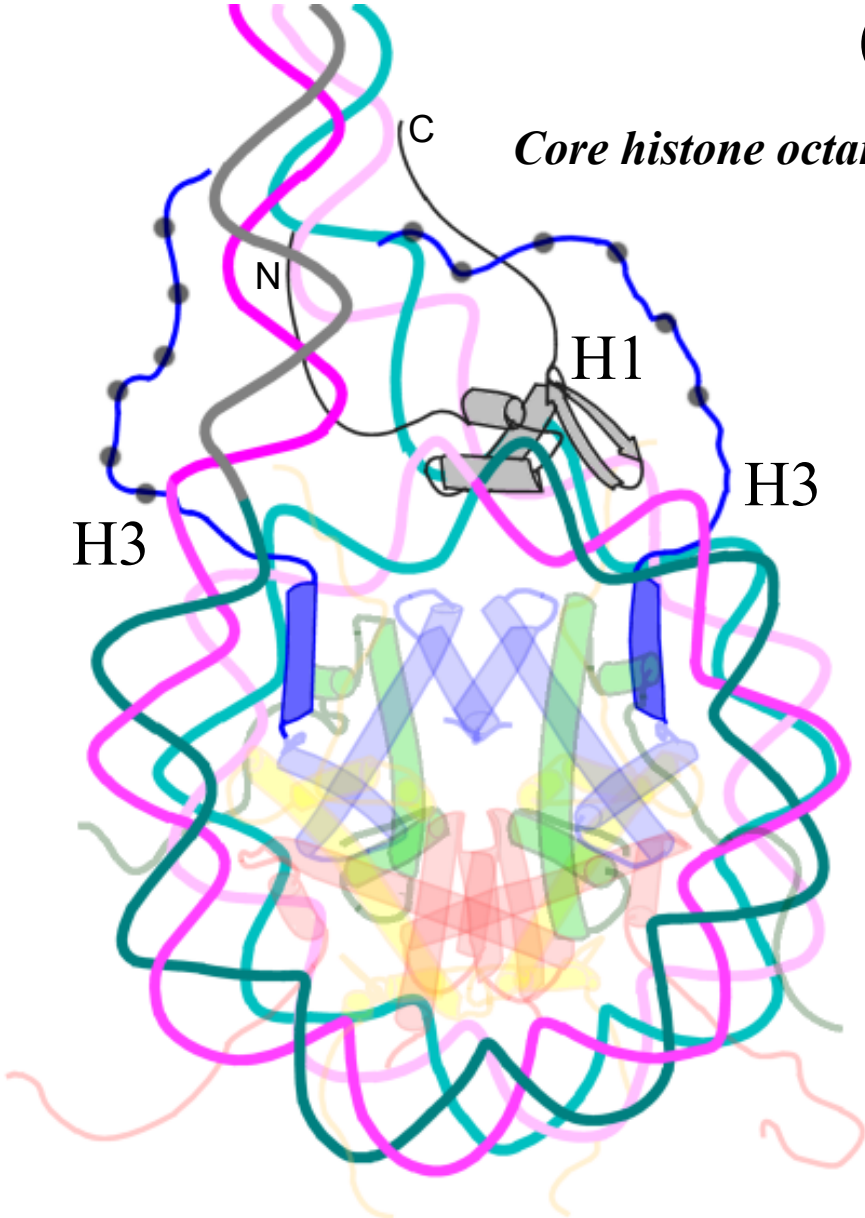
- 146-149 bp DNA in a 1.65 turns of a flat, left-handed superhelix
- one pseudo twofold axis centered at the "dyad" (reference: 0 helical turns)
- one base-pair precisely at the dyad
- sharp bends at ± 1.5 and ± 4-5 turns
- Histone-fold domains organize 121 bp of DNA. The DNA is bound at 10 bp intervals through many contacts, including penetration of arginines at all 14 minor grooves facing the protein core
- The grooves from neighboring DNA turns line up; forming channels
- H3 and H2B N-termini exit one of these channels every 20bp.
- The H4 tail establishes contacts with the next core particle.

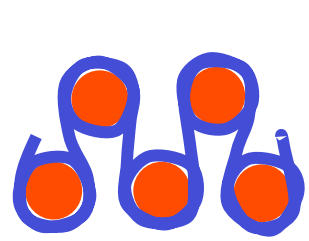
H2A H2B H3 H4

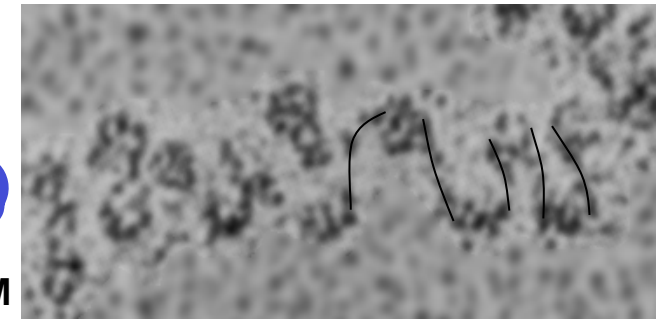
*Luger, Mader, Richmond, Sargent & Richmond*

# Chromatosome

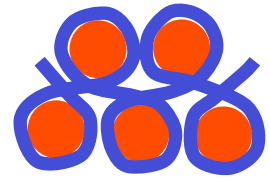
***Core histone octamer + 1 Linker Histone + 2 full turns of DNA (168 bp)***

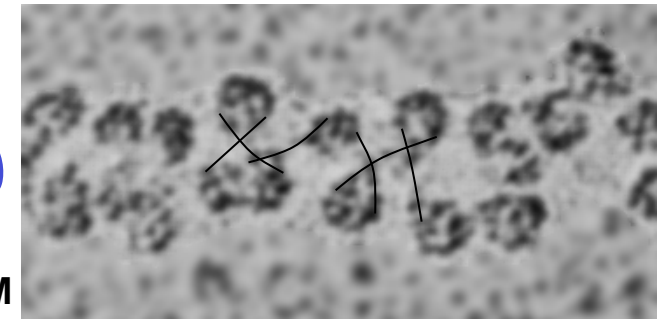




1 mM





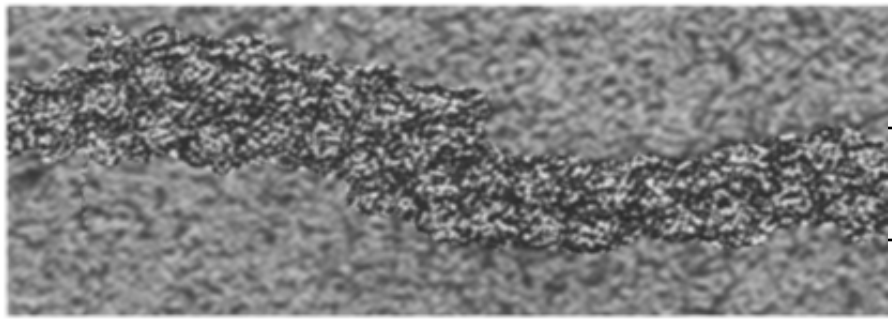
5 mM

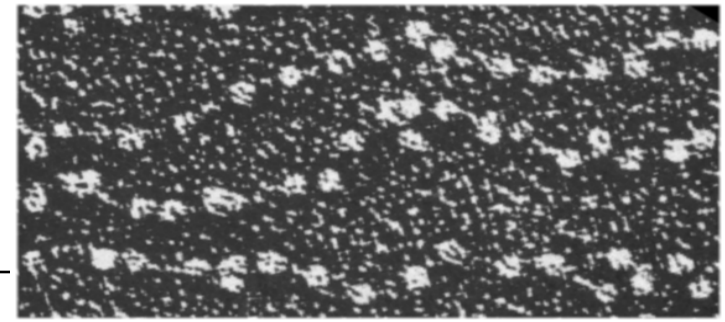
**Linker Histone and histone termini control linker DNA entry/exit of chromatosome in chromatin fiber.**


*Zhou, Gerchman, Ramakrishnan, Travers, Muyldermans* **Nature 395**, 402 (**1998**)

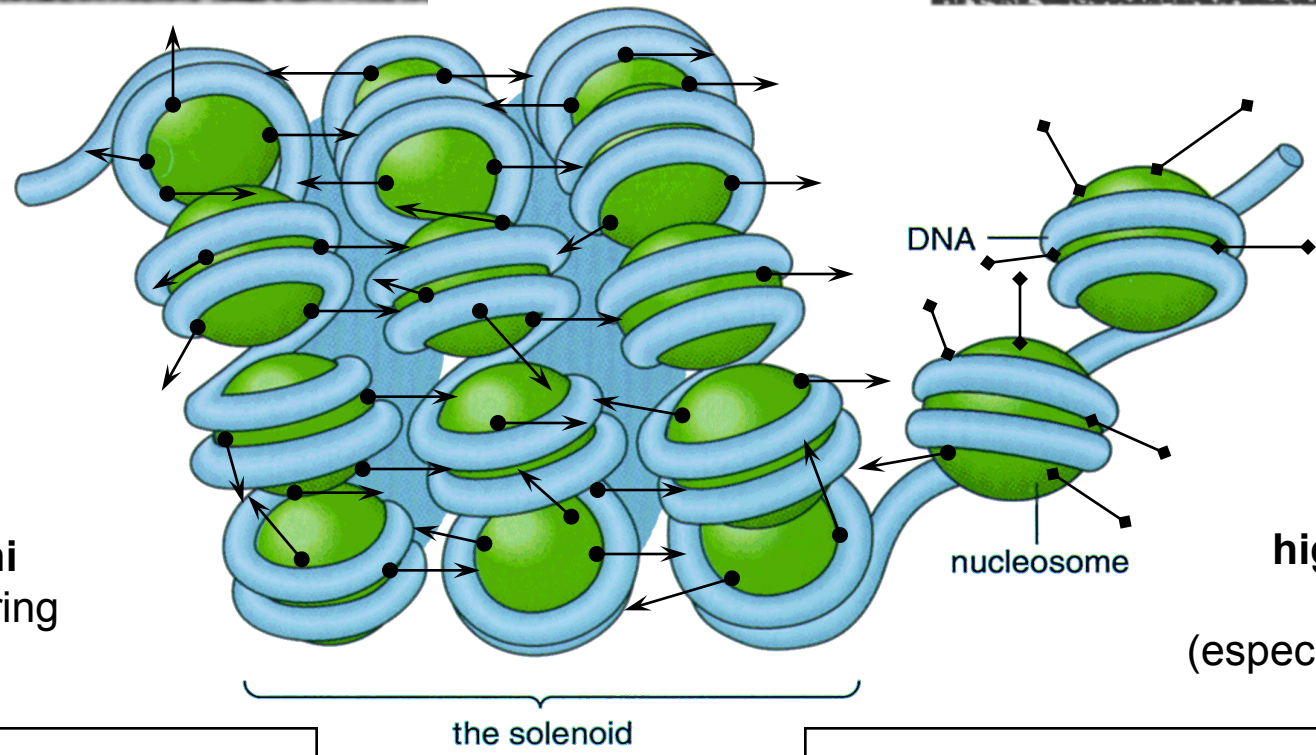
*An, Leuba, van Holde, Zlatanova* **PNAS 95**, 3396 (**1998**)

# Chromatin fibers





**30 nm chromatin fiber**




**11 nm (beads)**

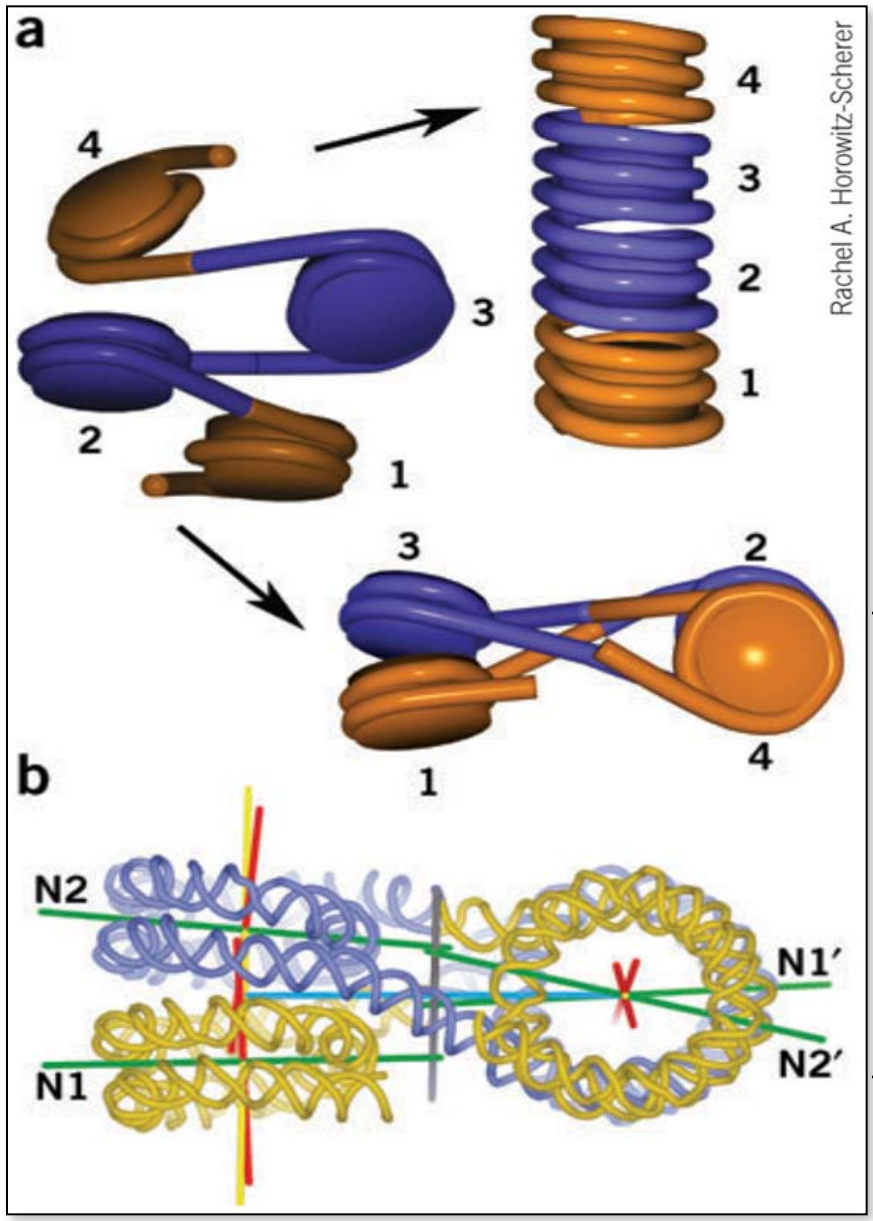
**(+) charged N termini**
(bind DNA on neigboring nucleosomes)

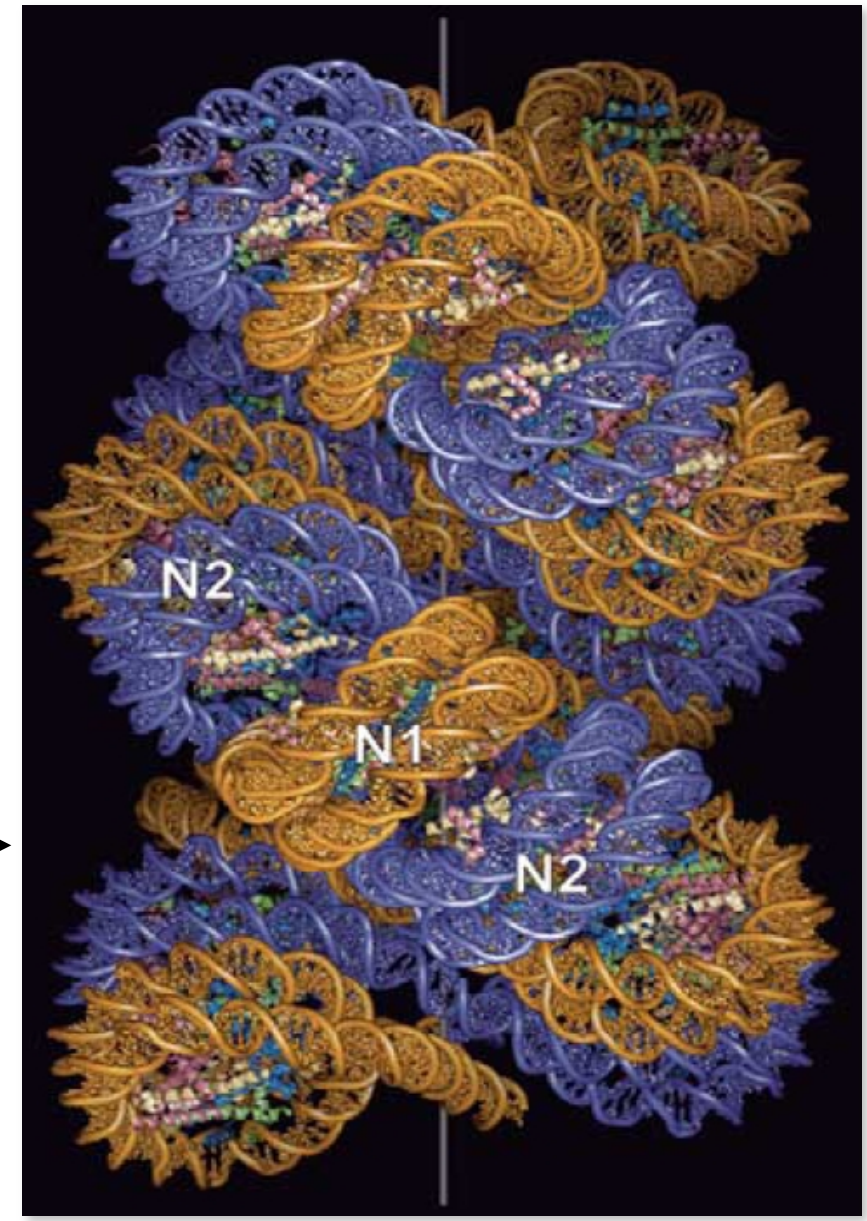
**highly acetylated core histones**
(especially H3 and H4)

- **HIGH level of histone H1**
- **NO gene transcription**

- **Reduced level of histone H1**
- **Gene transcription possible**

# Alternative chromatin fiber models




*Schalch, Duda, Sargent & Richmond, Nature 2005*

Zigzag fiber

# Molecular basis of epigenetics

- Chromatin regulation via post-translational modifications of histones and the action of chromatin proteins such as HP1, Polycomb et Trithorax

- Histone variants

- DNA methylation

- Noncoding RNAs

- Links between DNA methylation, histone modifications and the function of ncRNAs

extrait du film

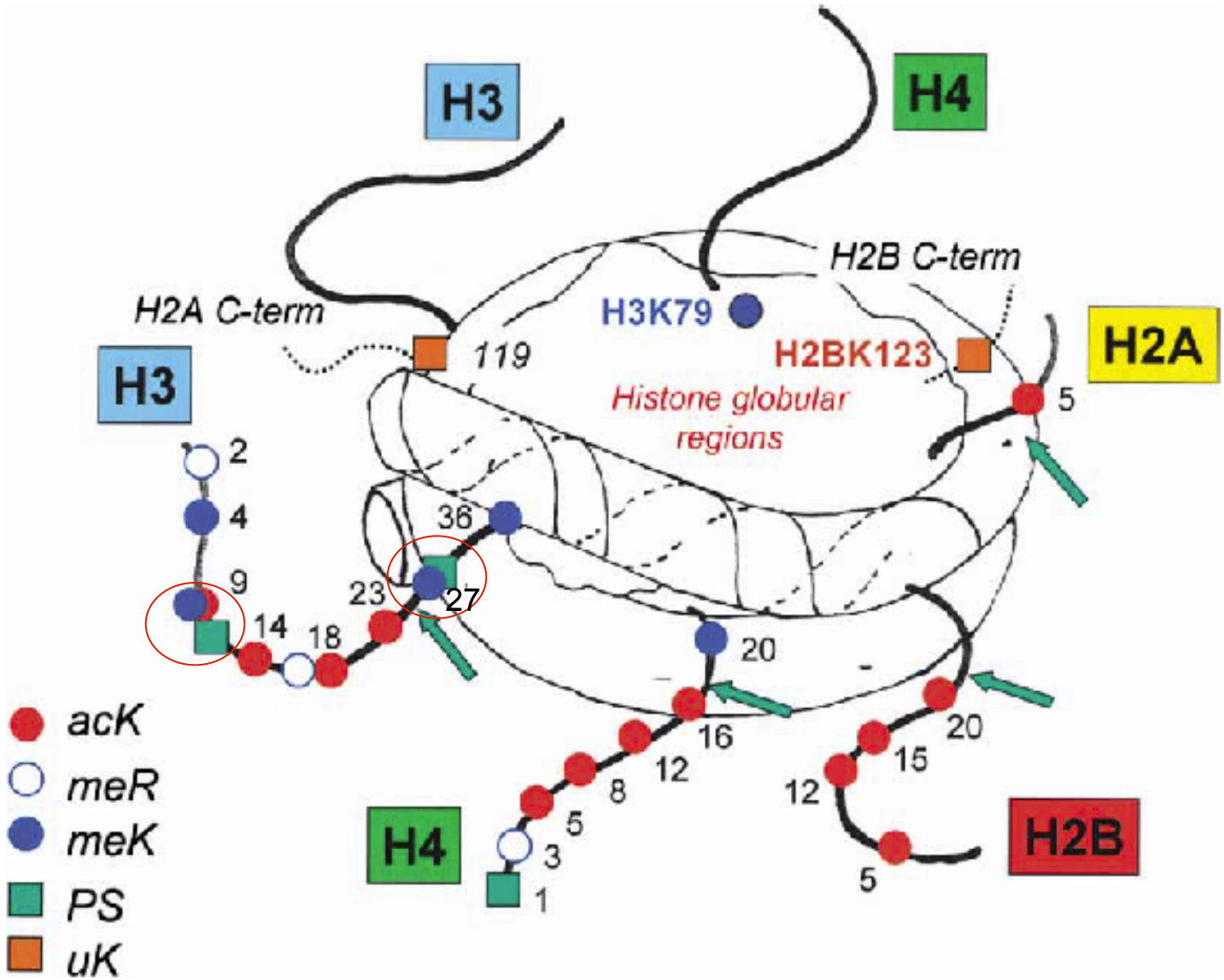
La vie cachée de nos gènes

de Hervé Nisic

Production HELIOX

# Post translational histone modifications

# Histone modifications




Cell (2002) 111, 285-91

# Various PTMs

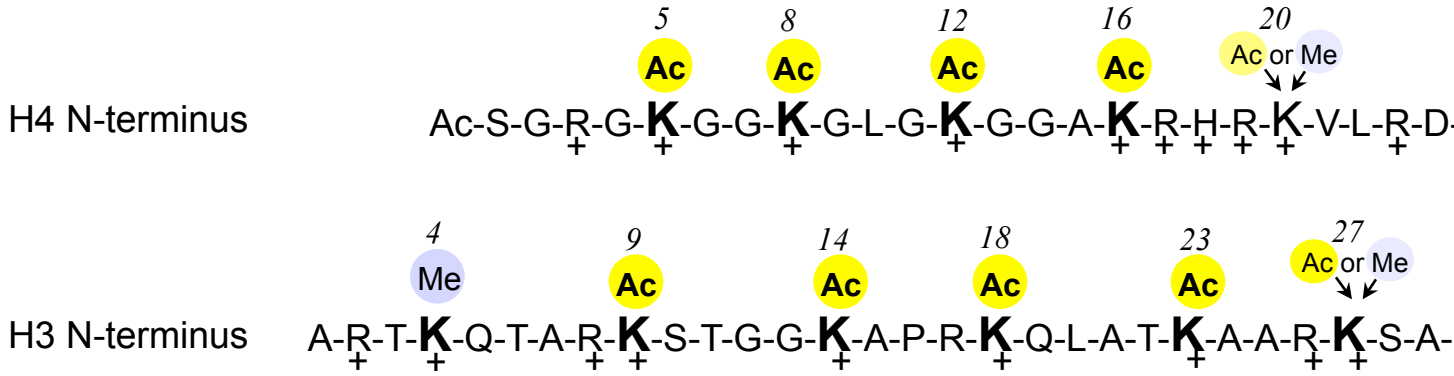
**Table 1.** Histone modification types and the interacting domains that "read" them

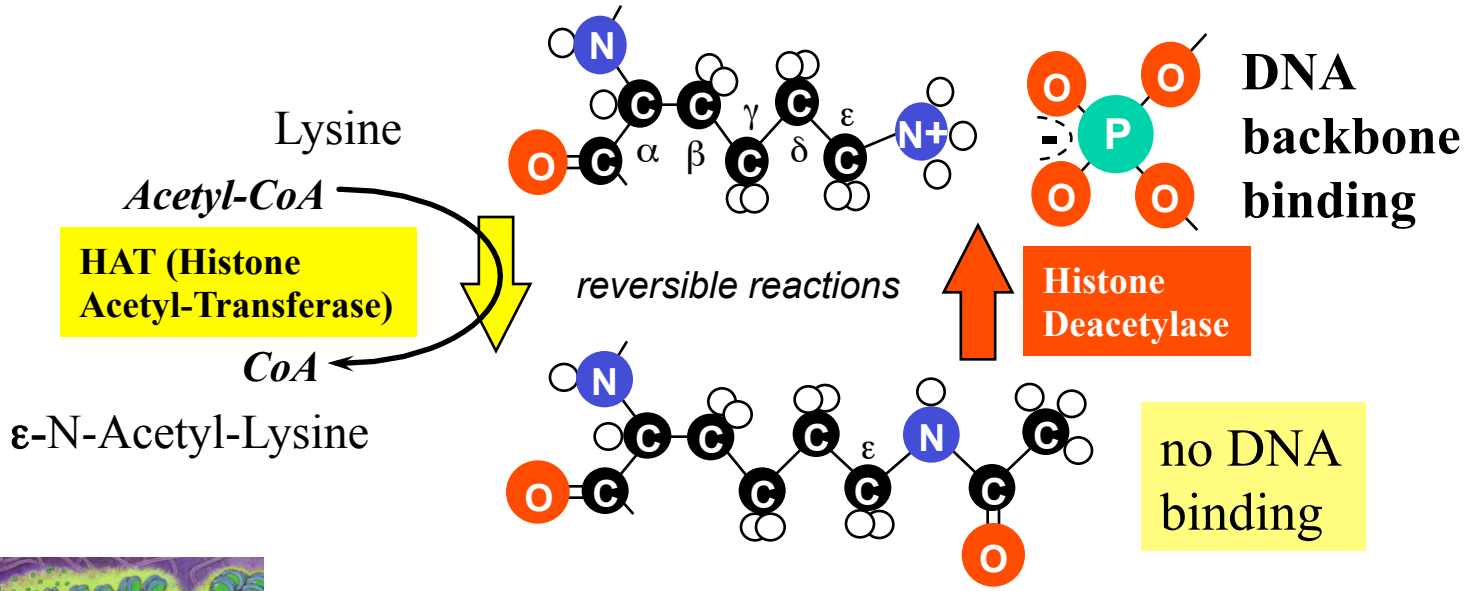
| Modification types | Residue(s) modified | Reader domain(s) |
|---|---|---|
| Unmodified lysine | Lysine | PHD |
| Acetylation | Lysine | Bromo |
| Methylation | Lysine/arginine | Ankyrin, Chromo, HEAT, MBT, PHD, Tudor, PWWP, WD40 |
| Phosphorylation | Serine/threonine | 14-3-3, BIR, BRCT |
| Ubiquitylation | Lysine | ? |
| Sumoylation | Lysine | ? |
| ADP-ribosylation | Lysine | ? |
| Citrullination | Arginine | ? |
| Butyrylation | Lysine | ? |
| Propionylation | Lysine | ? |
| Glycosylation | Serine/threonine | ? |

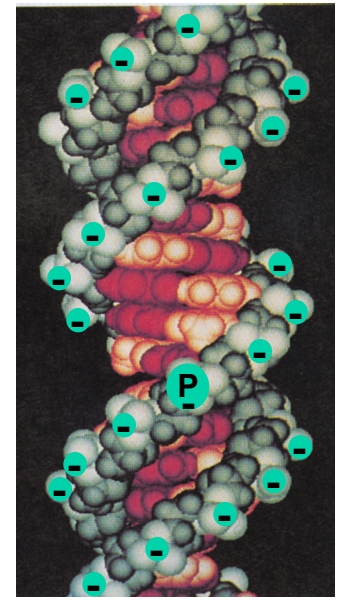
Review from Gardner, Allis and Strahl et al, *J.Mol.Biol. 2011*

# Acetylation of conserved lysines

**The N-termini of histones H4 and H3, and their acetylation patterns, are <u>absolutely</u> conserved.**



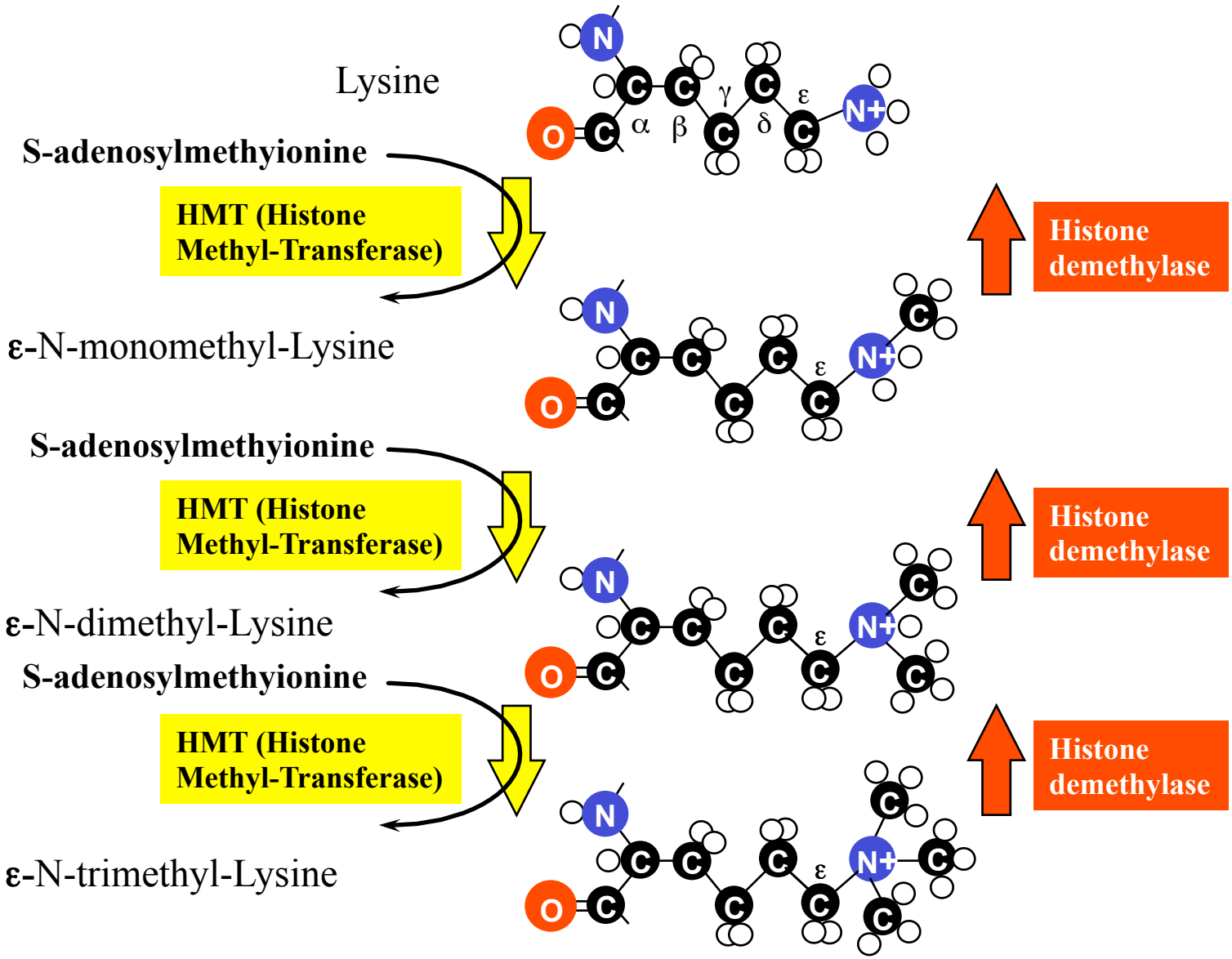
# Histone acetylation versus deacetylation

Histone acetylation → Decondensed chromatin: Active chromatin state

Histone deacetylation → Condensed chromatin: repressed transcriptional state

# Histone Methylation / Demethylation

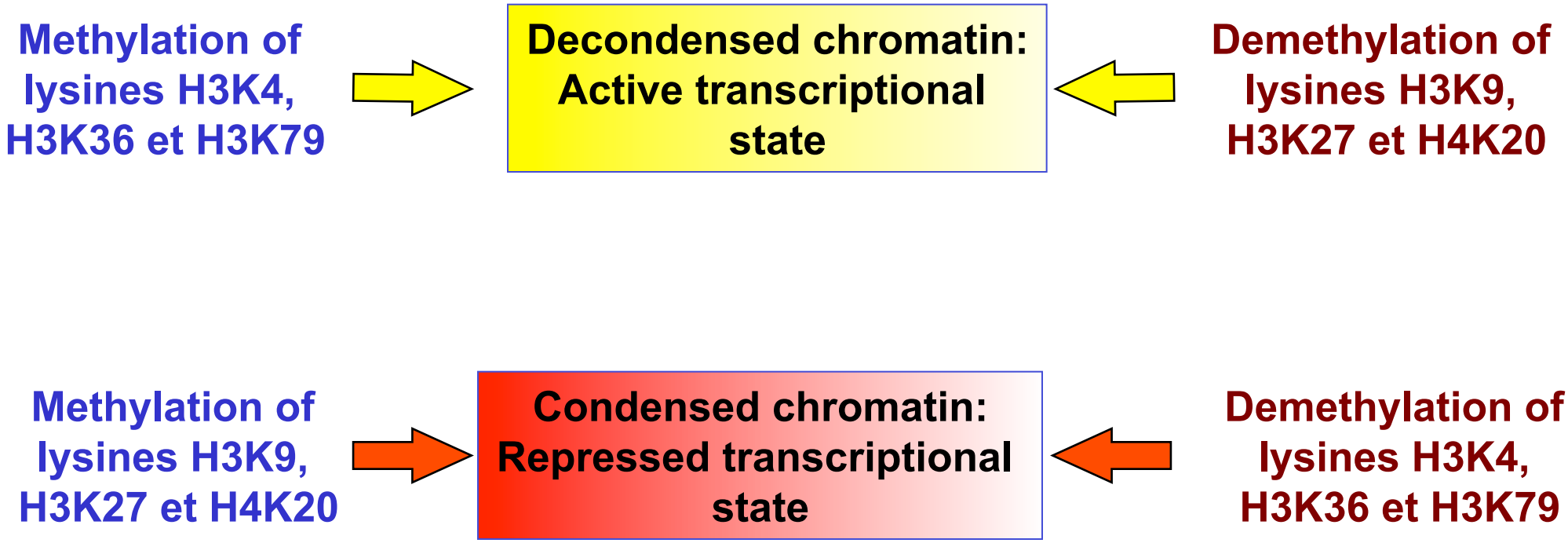
**Histones can be methylated at lysines or arginines. Example: H3 K4 methylation**

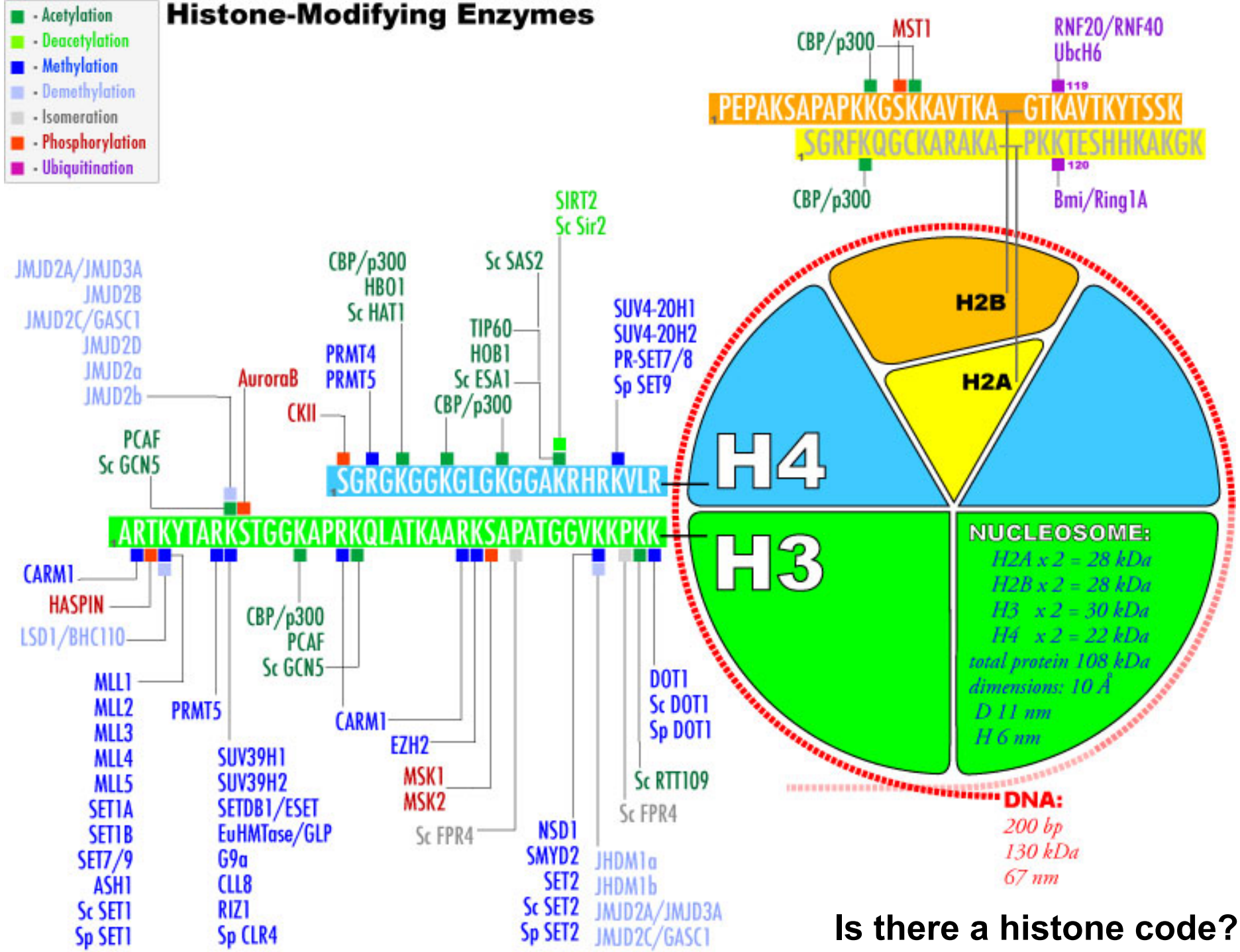



**DNA backbone binding may not be strongly affected, but specific proteins may recognize these modifications**

**Histone demethylases found for K4, K9, K27 and K36**

# Histone methylation versus demethylation



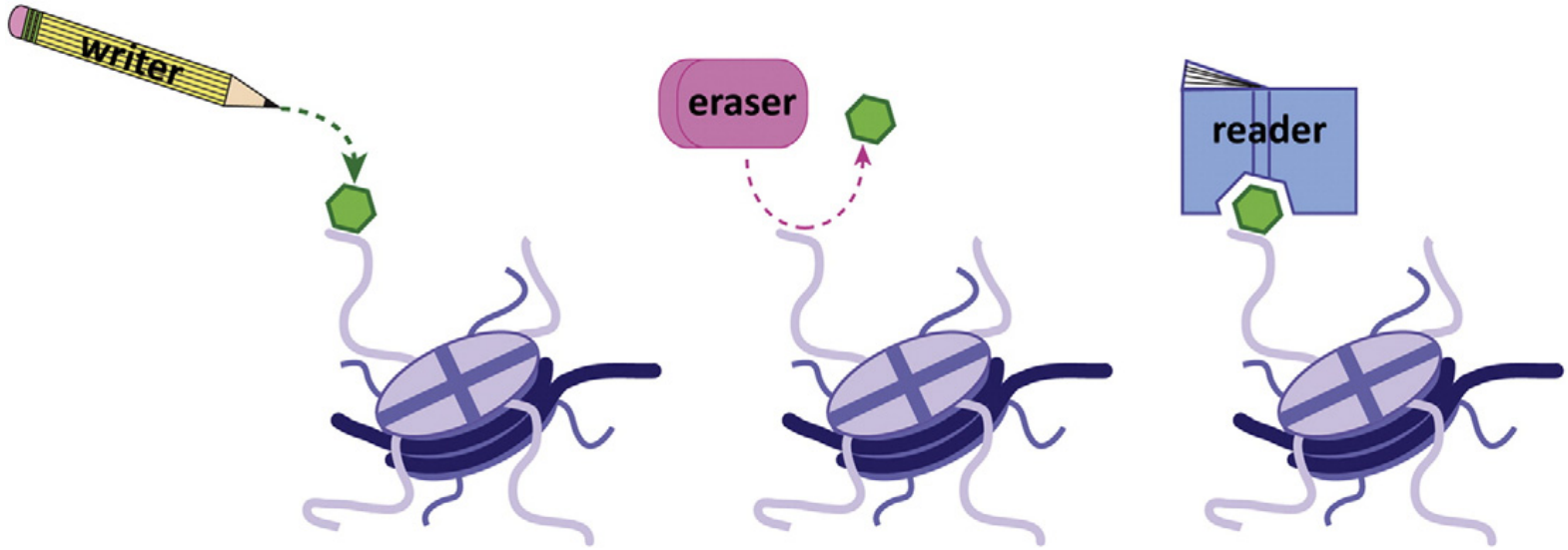



**Is there a histone code?**

**Histone modifications can be recognized by chromatin proteins in order to elicit specific functions:**

**The concept of « writer », « reader » and «  eraser »**

# « writers » and « readers »



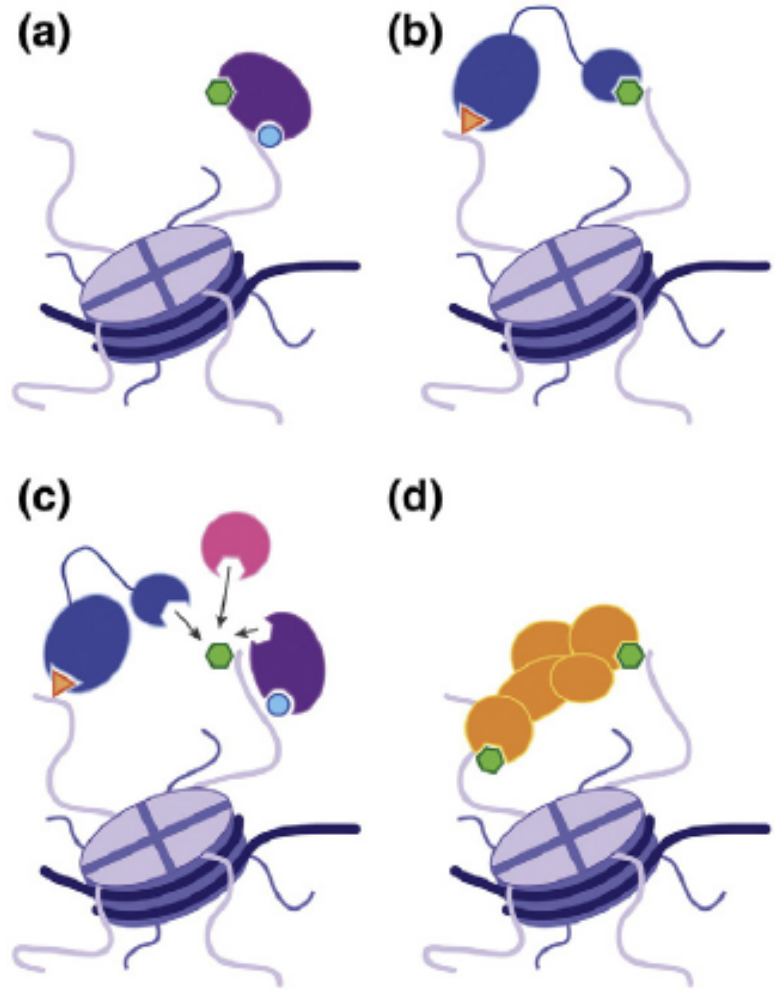

**Writer:**
**HAT: Histone acetyl-transferases**
**HMT: Histone methyl-transferases**

**Reader:**
**Specific factors**
**or macromolecular complexes**

**Eraser:**
**HDAC: Histone deacetylases**
**KDM: Histone demethylases**

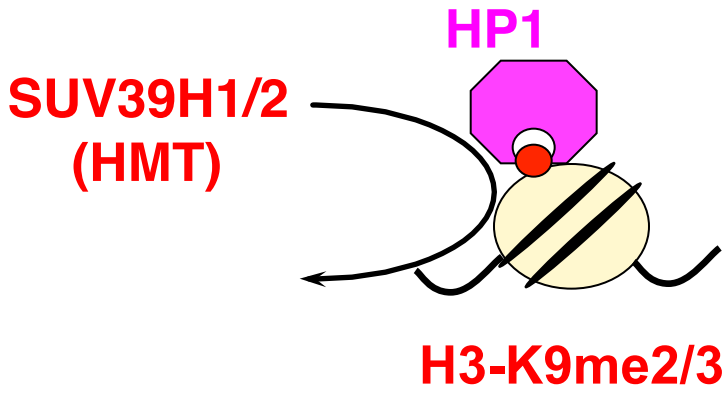
Review from Gardner, Allis and Strahl et al, *J.Mol.Biol. 2011*

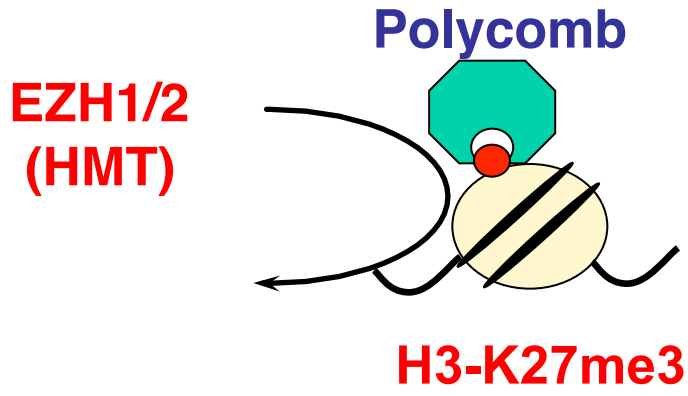
# Targeting specific multiprotein chromatin complexes via histone modifications

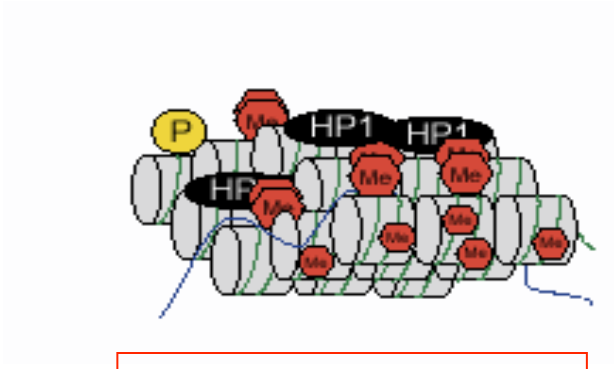



Review from Gardner, Allis and Strahl et al, *J.Mol.Biol. 2011*

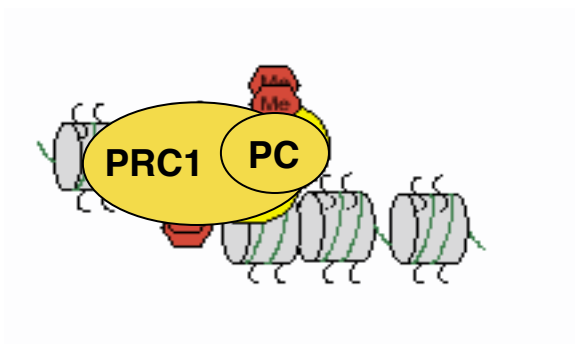
# H3-K9me and H3-K27me recognition by the chromodomain proteins HP1 and Polycomb



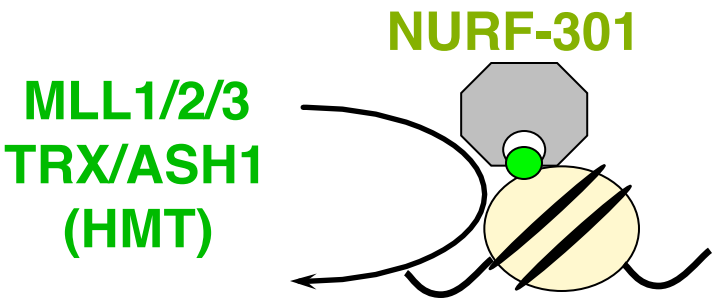



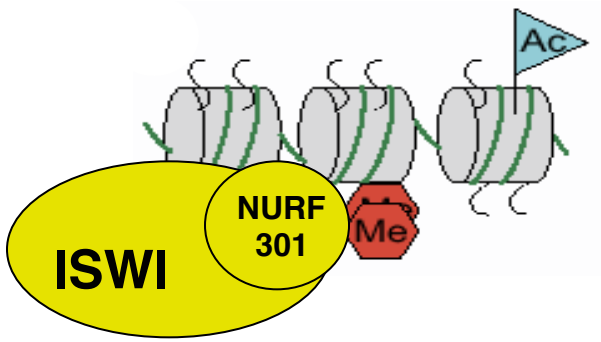

Constitutive Heterochromatin




Polycomb silencing (Facultative Heterochromatin)

# H3-K4me recognition by the PHD domain protein NURF-301



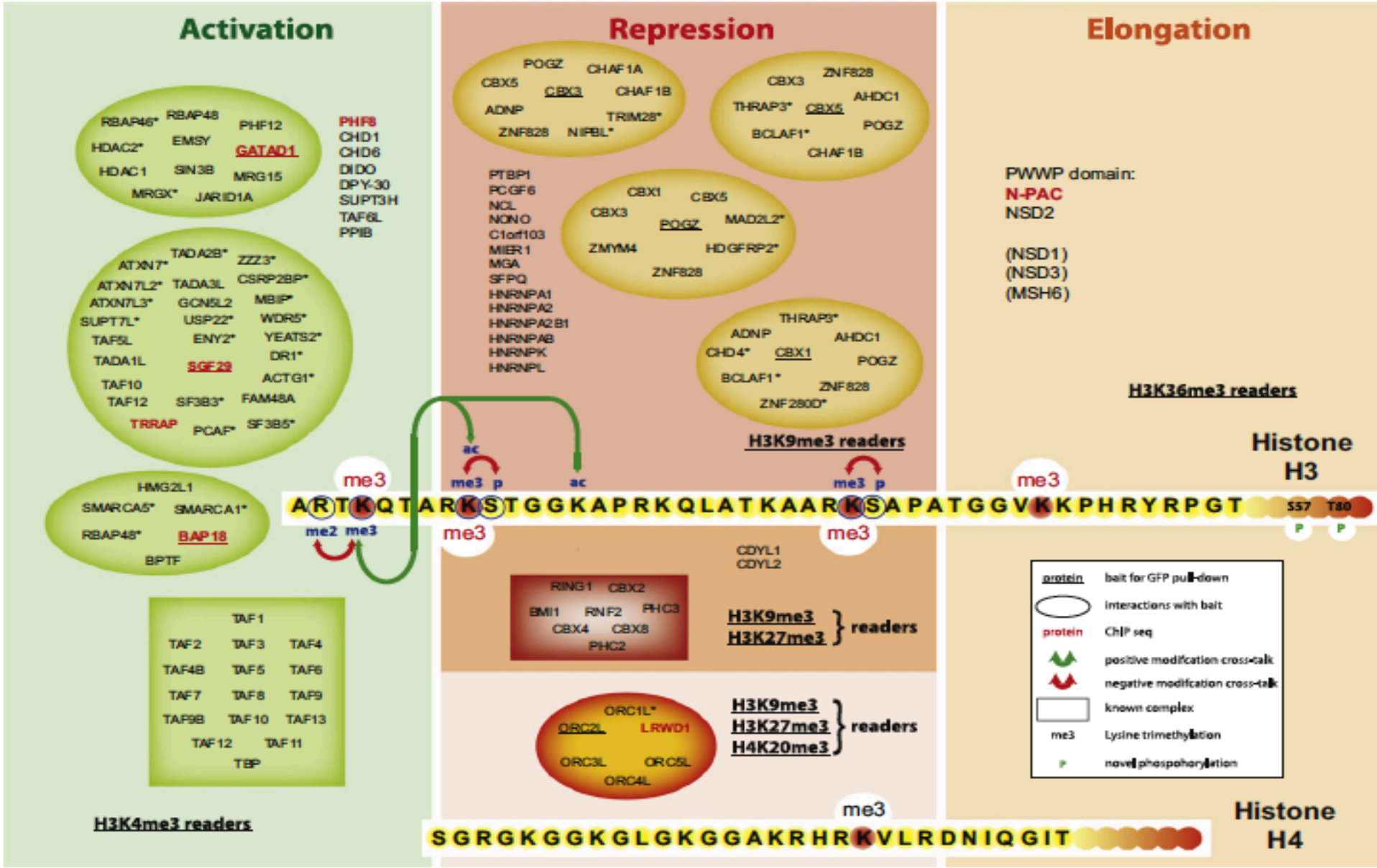




**Trithorax activation**

**ISWI-dependent Chromatin remodeling leading to Chromatin opening and activation**

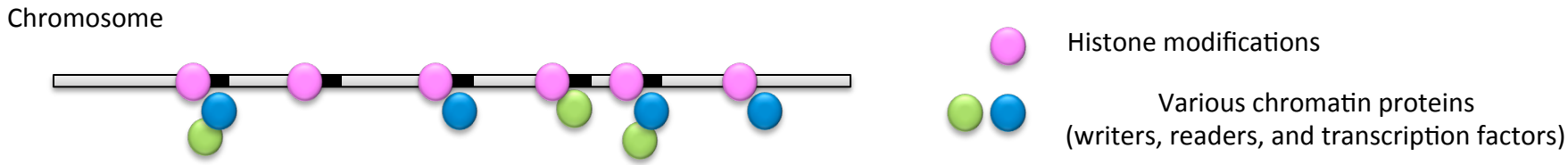
Wysocka et al., *Nature*, May 2006
Li et al., *Nature*, May 2006

# Histone Marks and Their Readers

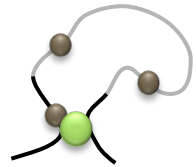



Readers identified by SILAC (Stable Isotope Labeling by Amino acids in Cell culture). Vermeulen et al., *Cell 2010*

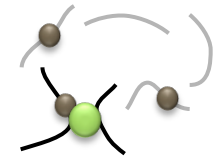
# Chromatin Immunoprecipitation (ChIP)



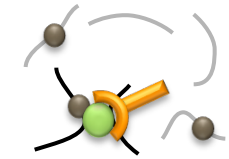

**Principle**



1- Cross-link protein-DNA interactions with formaldehyde

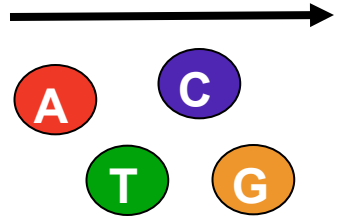


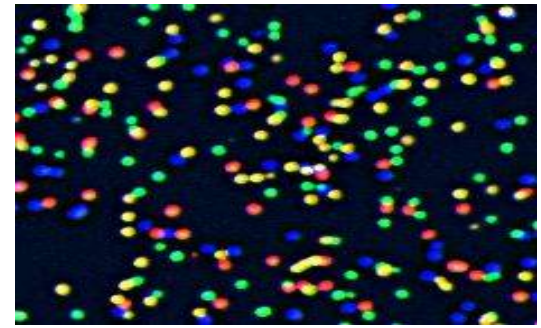
2- chromatin fragmentation by sonication



3- immunoprecipitate using a specific antibody against **protein** or **histone modification** of interest

→ **DNA amplification** → Ligation of Illumina linkers → Formation of DNA clusters in a flow cell

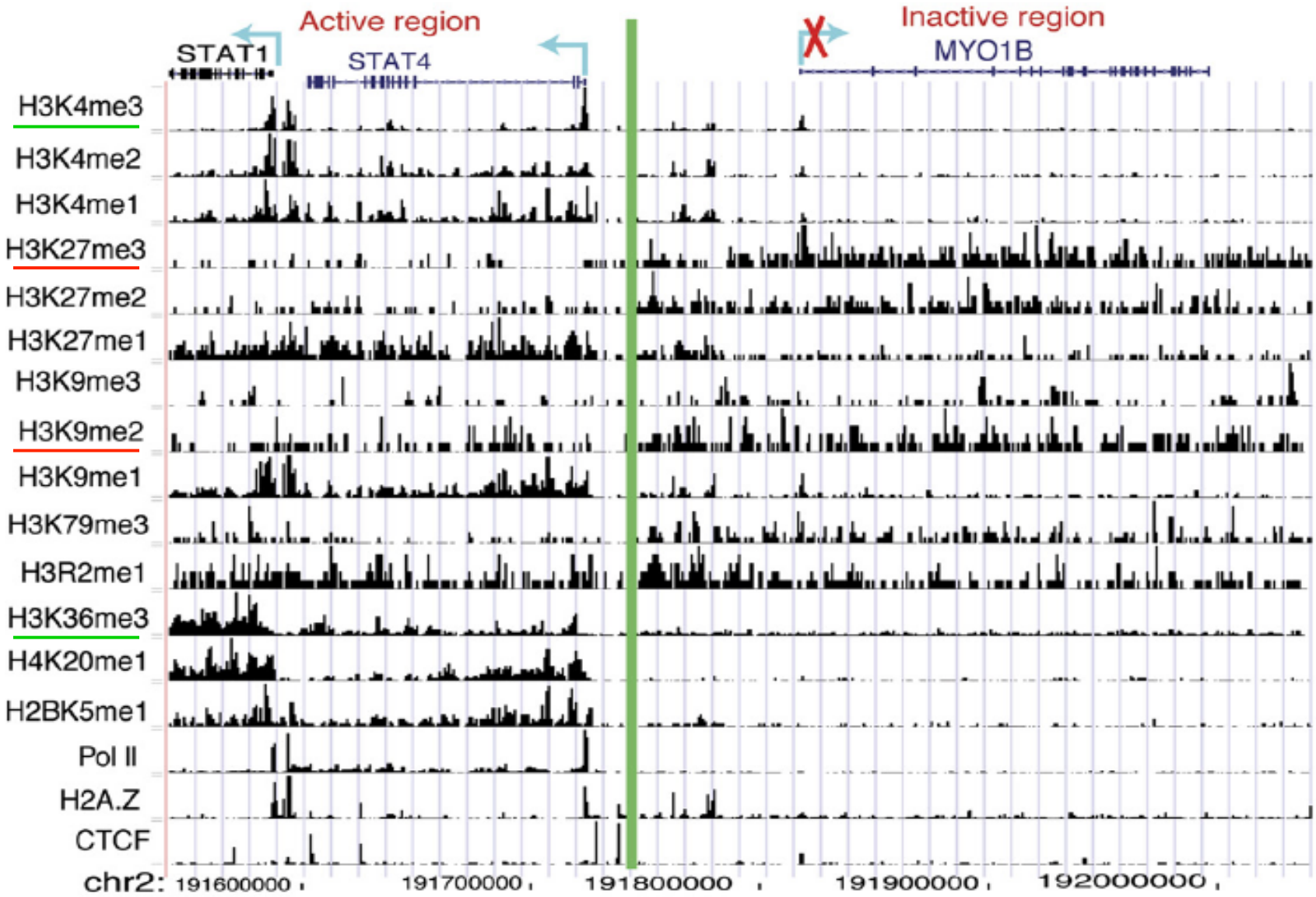


Sequence
one base at a time

**High-throughput sequencing: ChIP-seq**

**High-resolution profiling of Histone Methylations in the Human Genome**
**Data from Barski *et al.*, *Cell 2007***



# Same, but in *Drosophila* Sg4 cells – *Abd-B* active

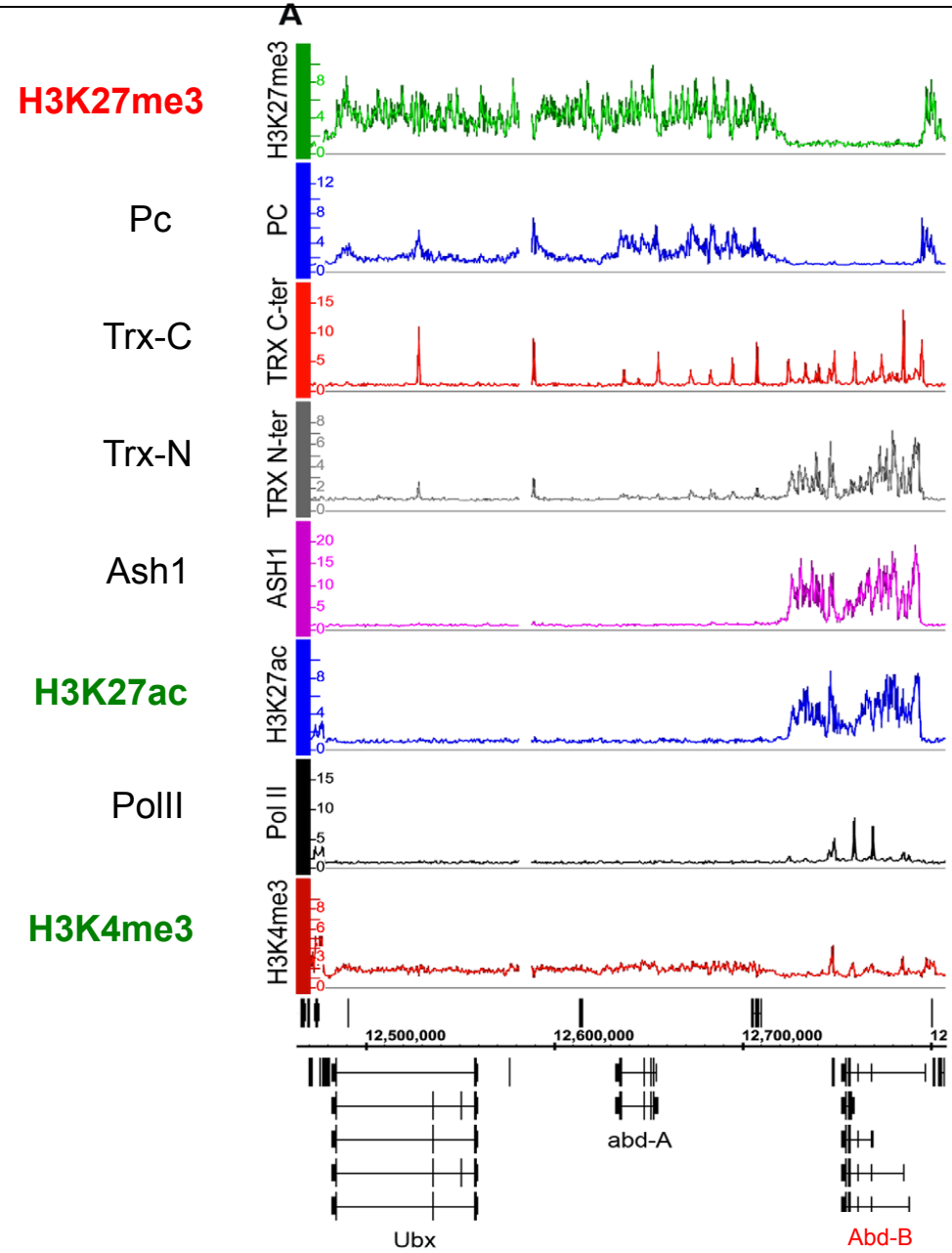
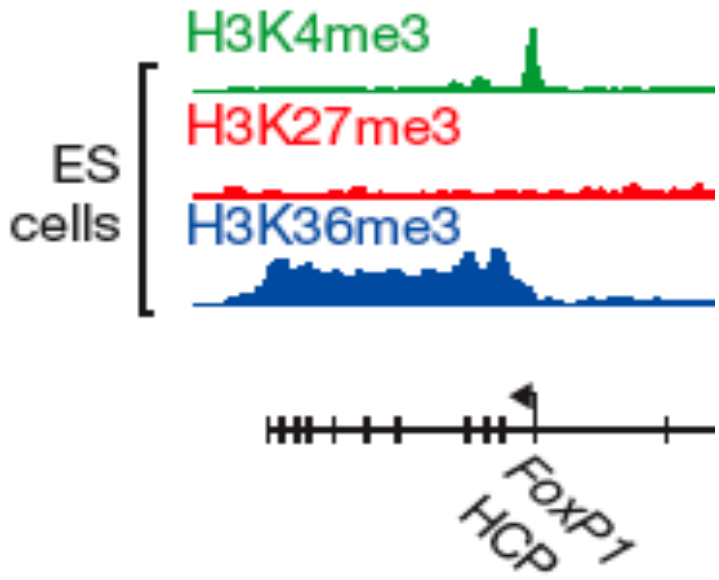



Figure 1 from

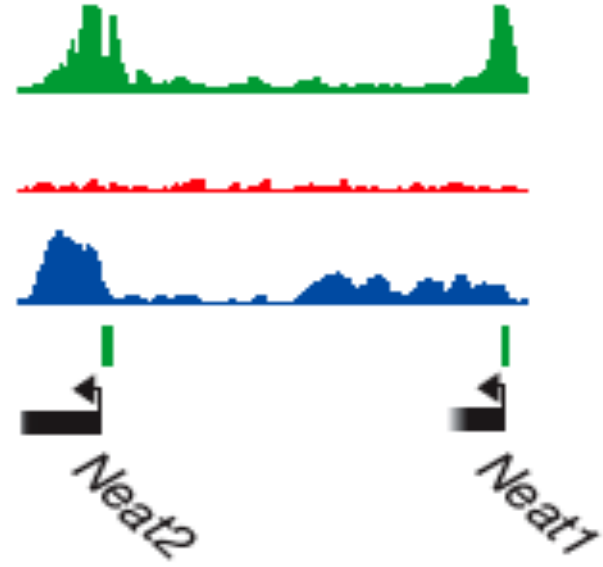
Schwartz et al, *PlosGenetics 2010*

# H3K4me3 and H3K36me3 annotate genes and non-coding RNA

The *FoxP1* gene



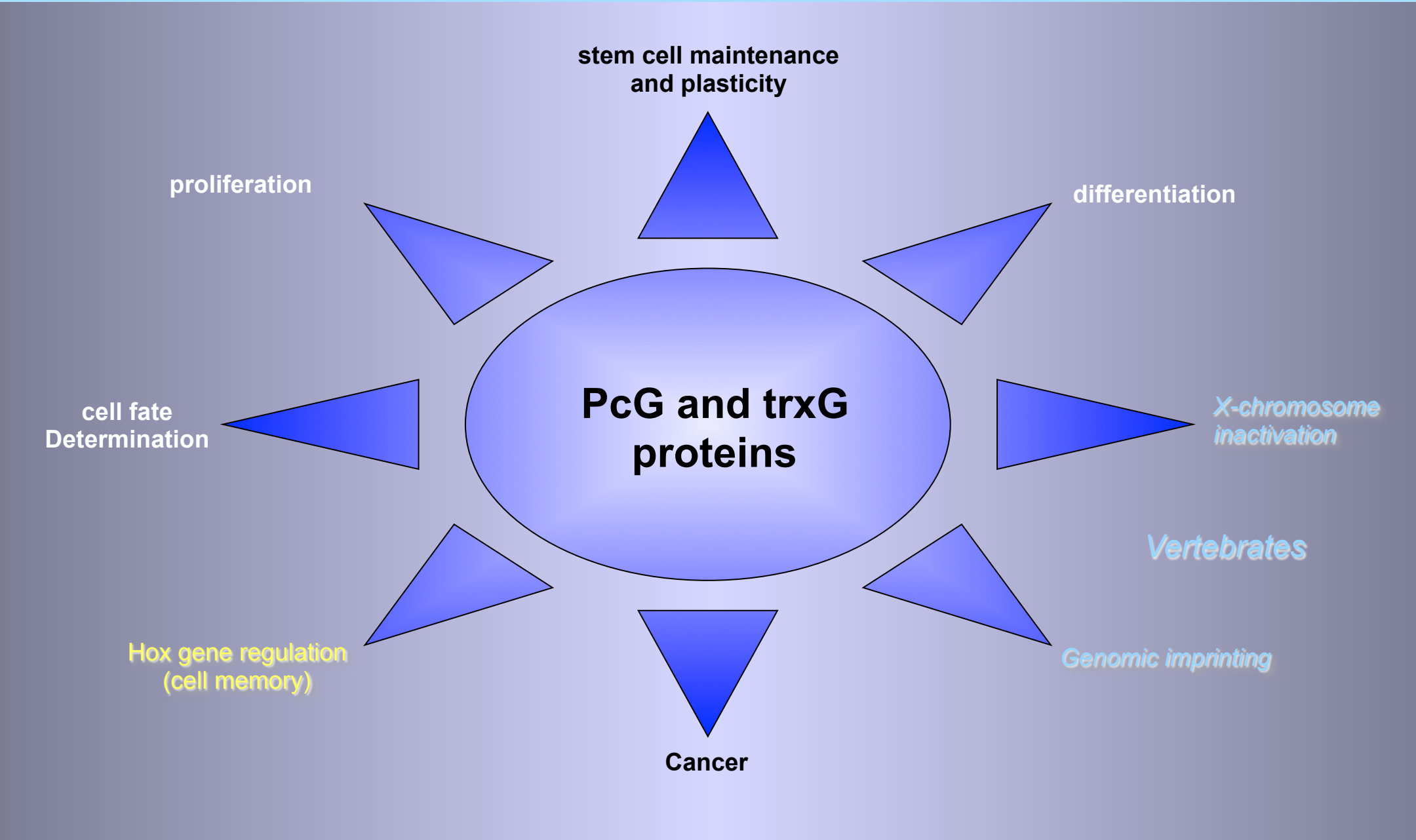

The *Neat* non-coding RNA



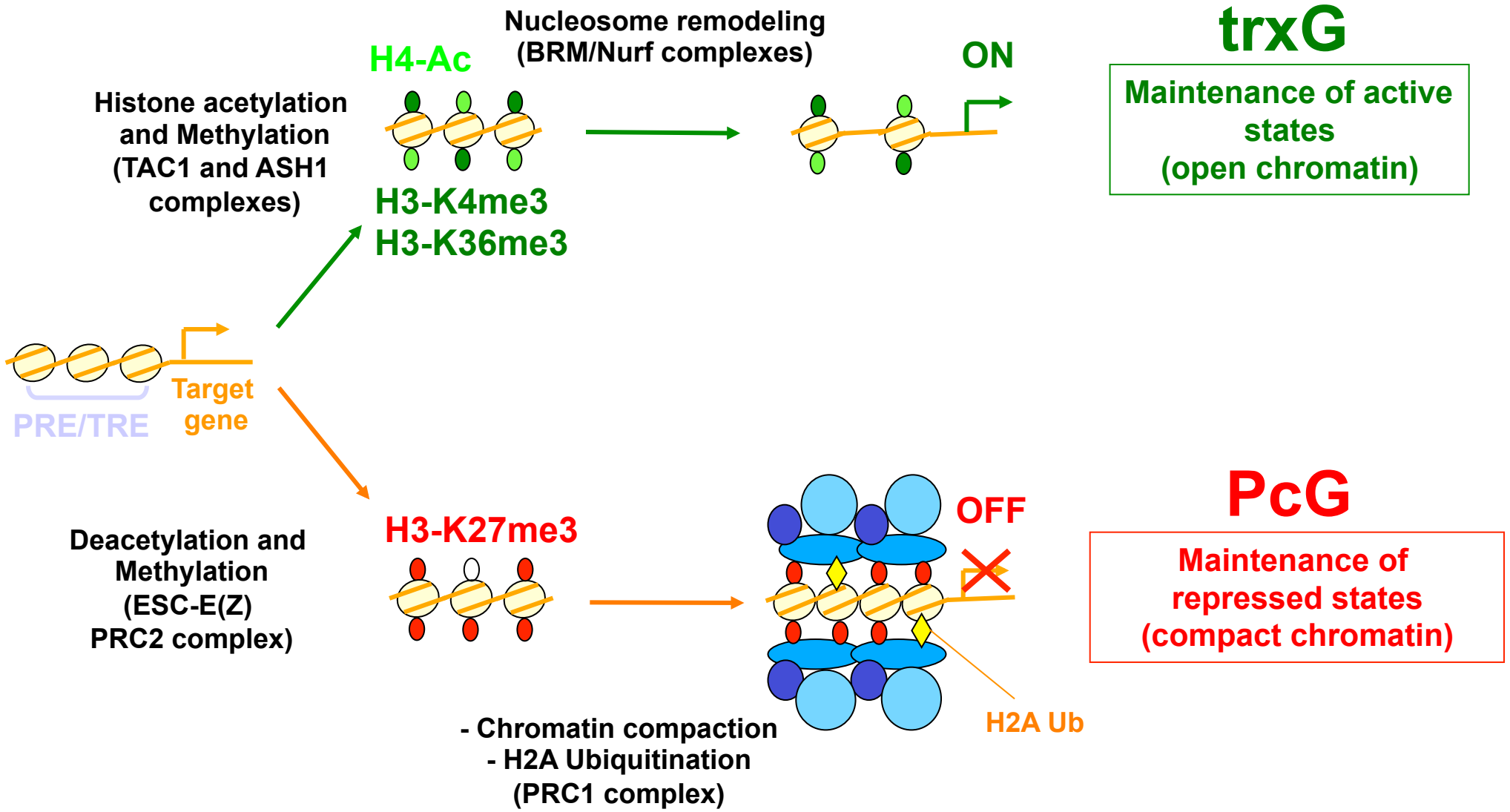

Data (ChIP-seq) from Mikkelsen *et al*., Nature 2007

# Polycomb and Trithorax proteins

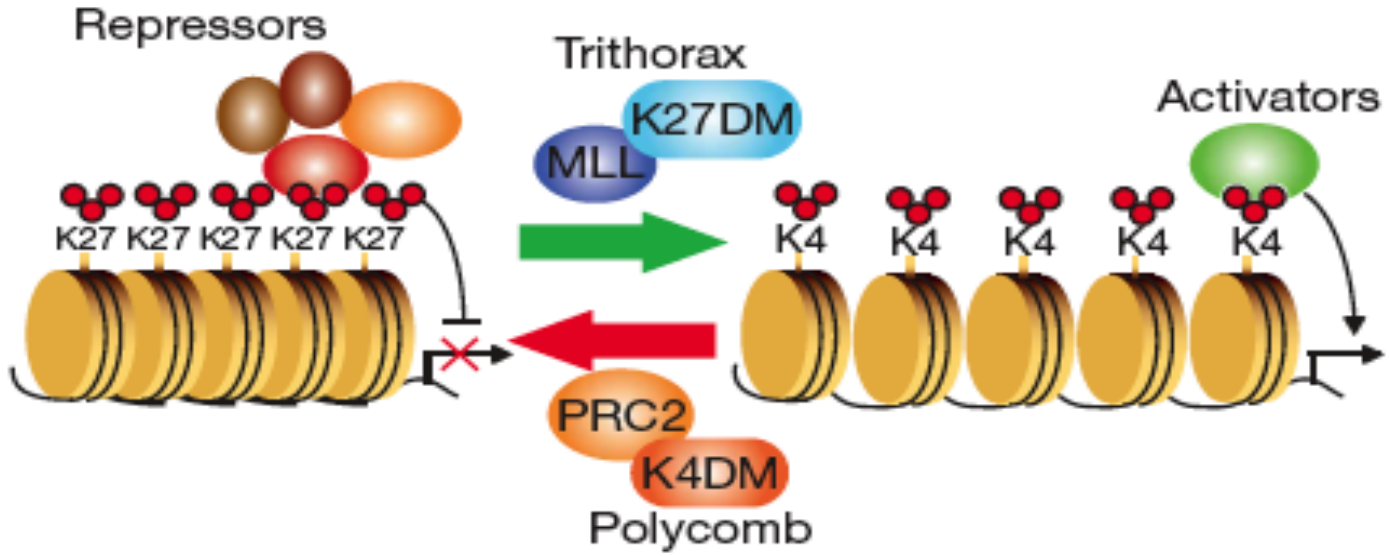
# PcG and trxG proteins regulate cell memory and dynamic patterns of gene expression



# Opposing functions of PcG (Polycomb group) and trxG (trithorax group) complexes on chromatin

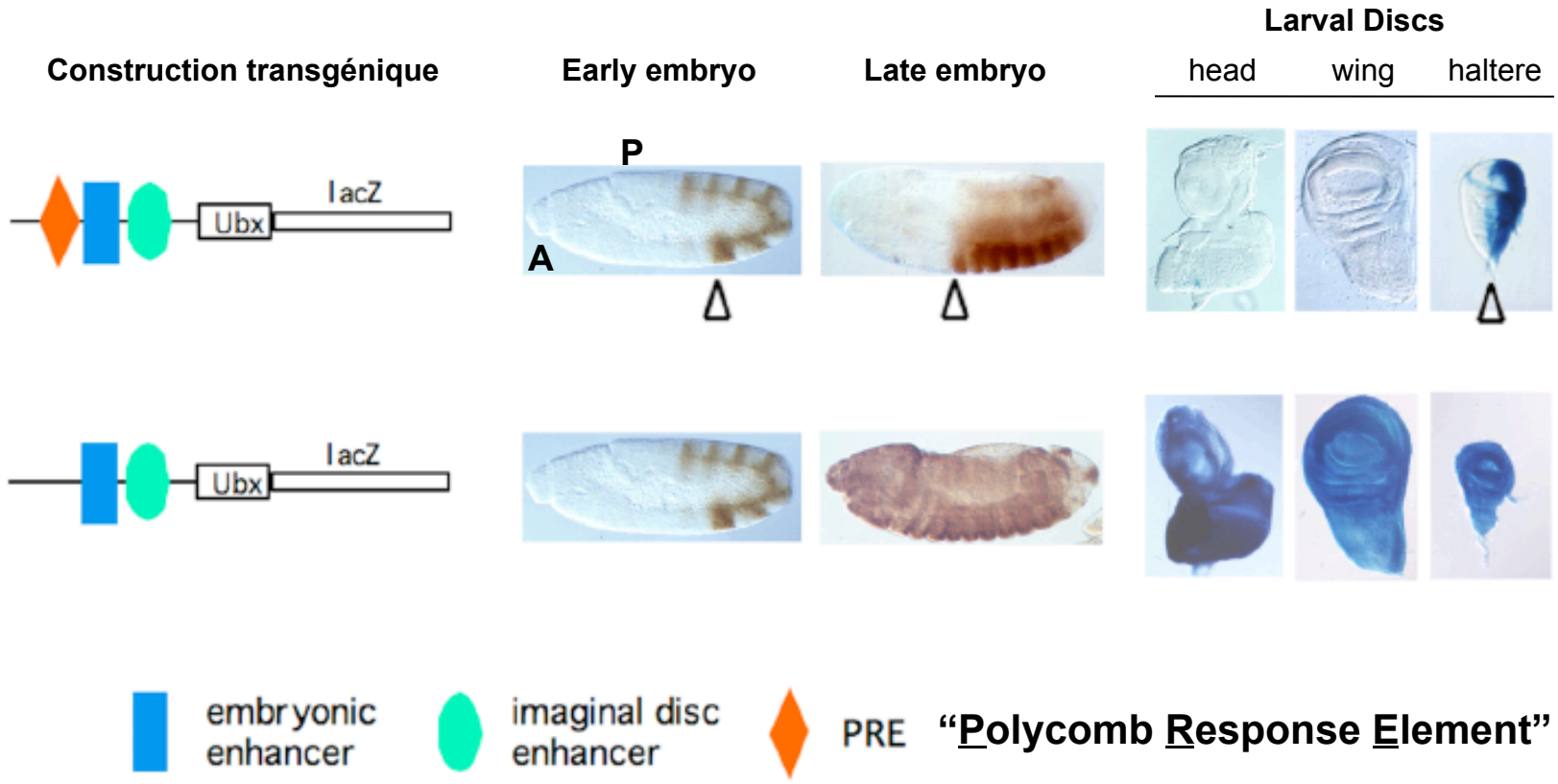


# Methylase *vs.* Demethylase
# Polycomb *vs.* Trithorax
# H3-K27me3 *vs.* H3-K4me3
# « The Ying and the Yang » on chromatin



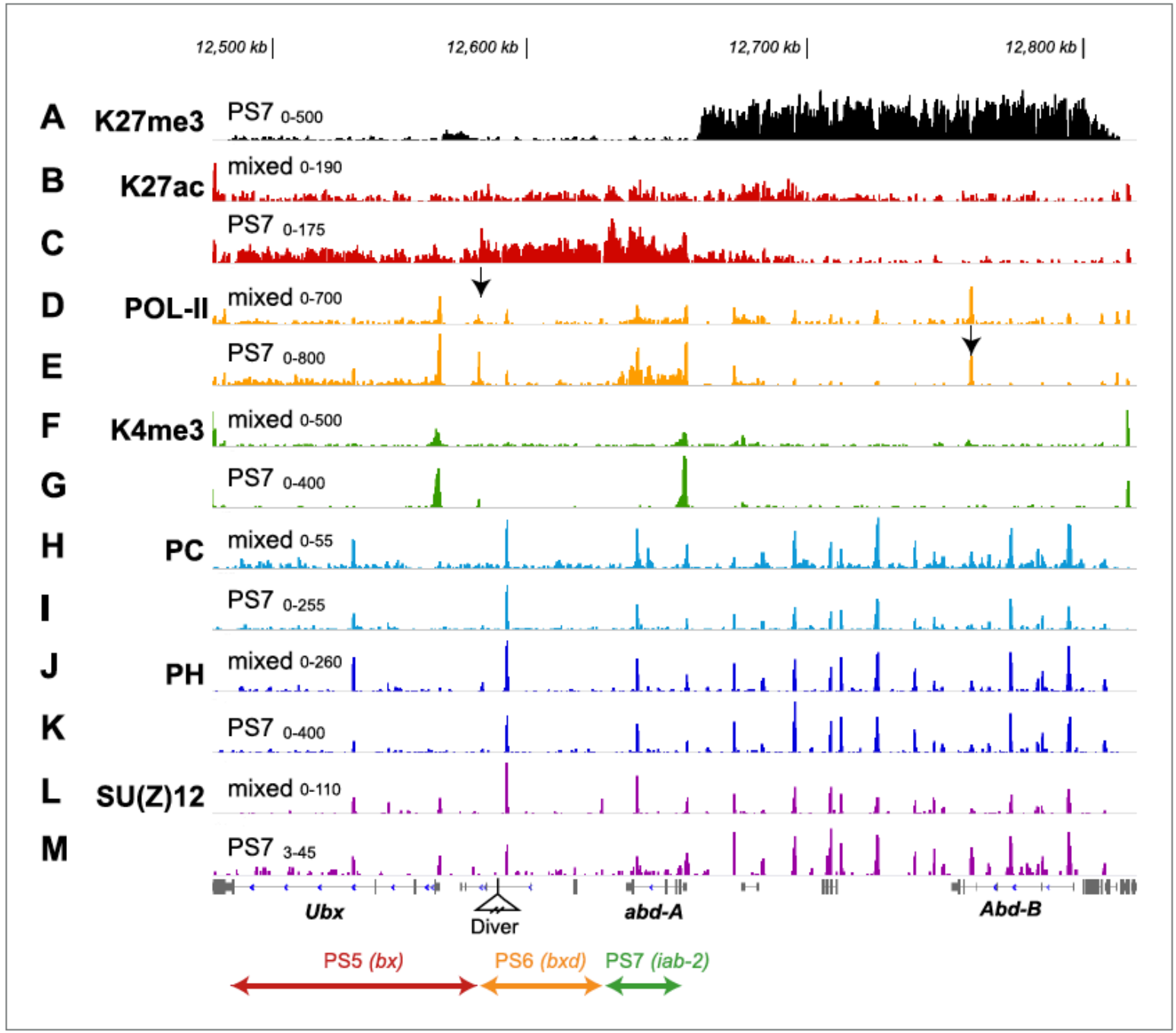

From Agger et al., *Nature*, August 2007

# Illustration of the epigenetic memory by the PcG proteins for the regulation of the Hox *Ubx* gene in *Drosophila*



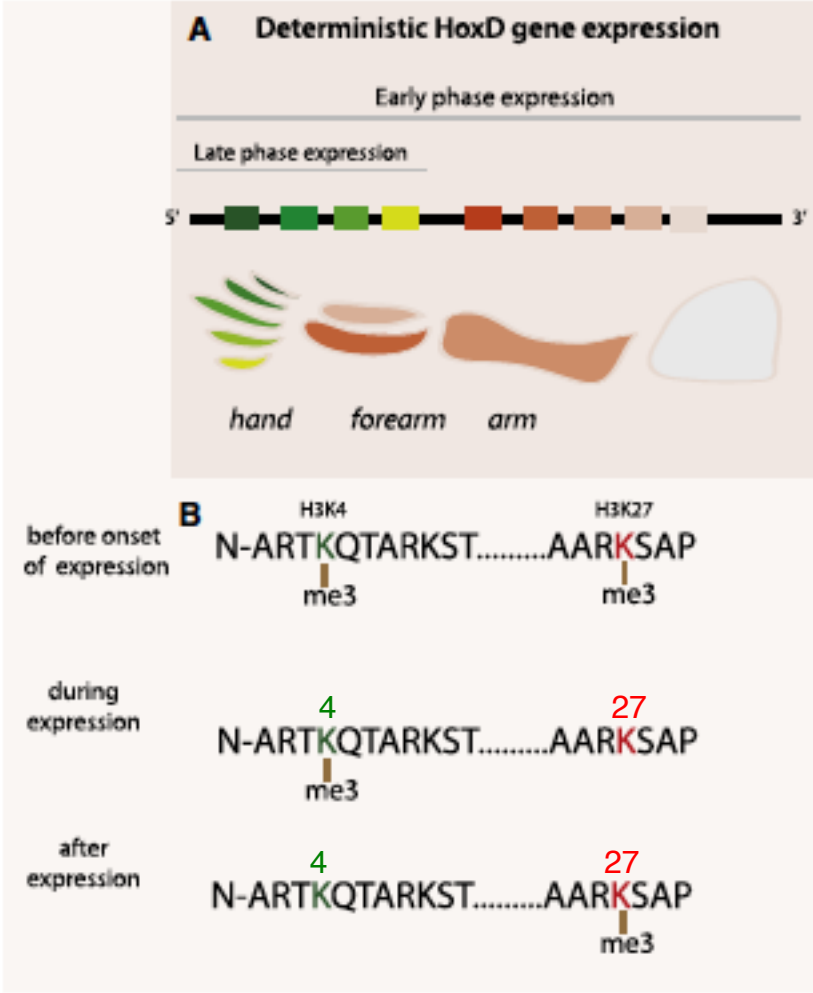

**Legend:** The correct expression pattern is created in the embryo and epigenetically maintained throughout development when all three elements are combined: the embryonic enhancer sets the pattern, the PRE maintains the repressed or non-repressed state and the imaginal disc now remains active only posterior to parasegment 6, not in the head or wings.

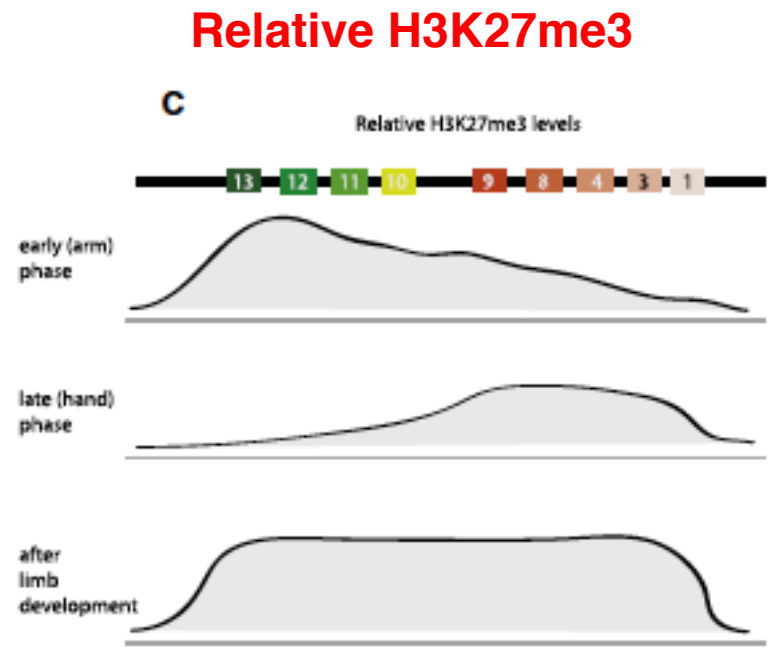
Illustration from a Review of Schwartz and Pirrotta




Data (ChIP-seq in Drosophila embryos) from Bowman *et al*., eLIFE 2014

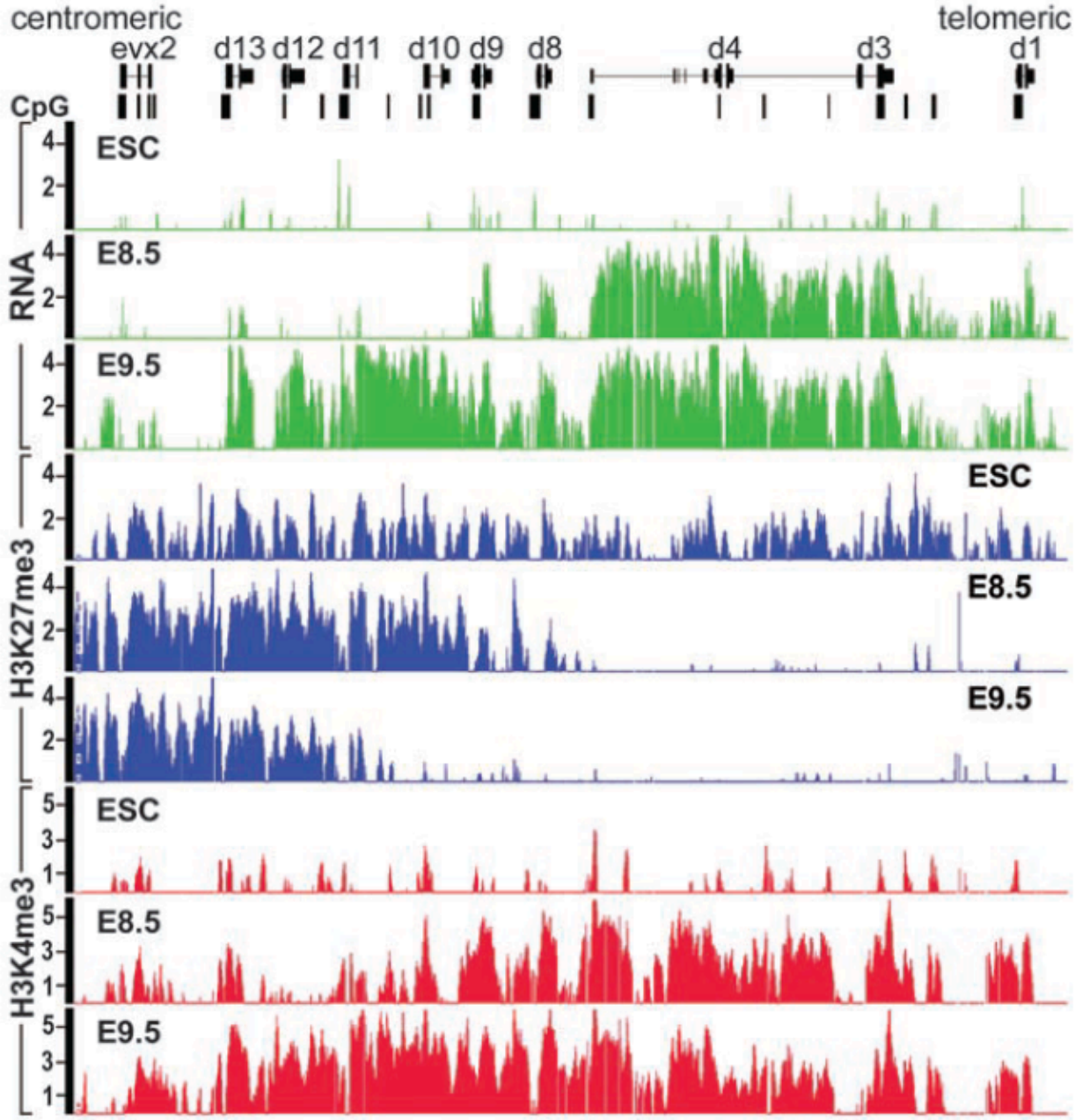
# Hox gene regulation in mouse development




From a Review of Lyons and Lomvardas, *BBA 2014*

# Chromatin marks during temporal collinearity at the HoxD cluster




Data (ChIP-chip in mouse embryos) from Soshnikova and Duboule, Science 2009

# Histone variants

# General remarks

- The incorporation of **histone variants** is an alternative way to mark chromatin. **H2A** and **H3** variants are known since decades and they have been shown to regulate and to maintain differential chromatin states.

- H2A, H2B, H3 and H4 core histones are coded by gene cluster sont codées par des gènes en clusters fortement exprimé lors that are strongly expressed during the S phase of DNA replication, whereas **histone variants** are coded by separate genes, which are expressed **throughout the cell cycle**.

Therefore, histone variants can be incorporated in chromatin all the time.

# Notable examples of histone variants:

- **CENP-A:** H3-like protein found at **centromeres** in mammalian species.
It is highly conserved throughout evolution and generally called CenH3

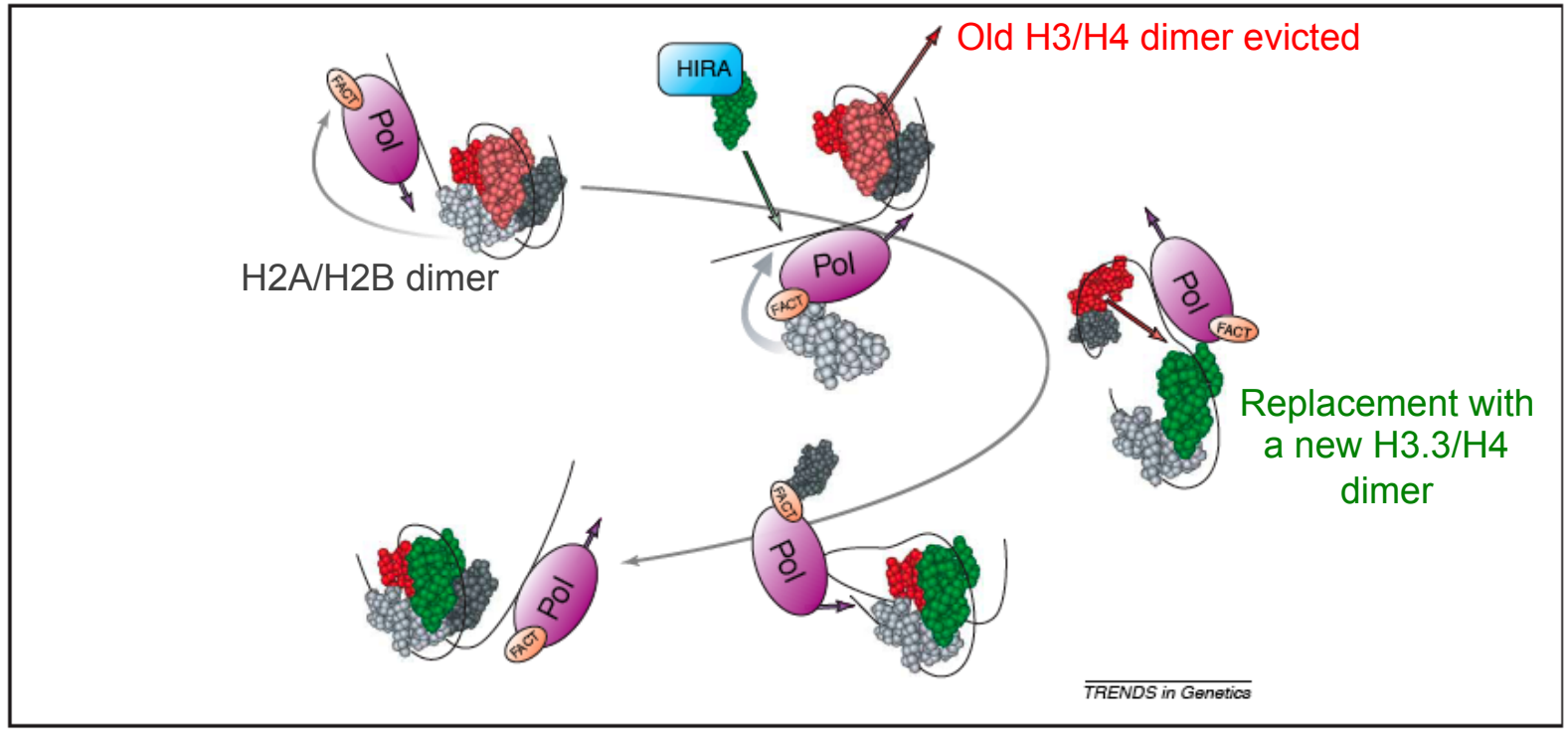
- **H3.3:** differs by just 4 amino acids from canonical H3.
Outside S-phase, H3.3-H4 dimers may replace H3-H4 dimers during gene activation
(« **replacement variant** »)
Moreover, H3.3 is enriched 2 to5 fold in activating post translational modifications (K9, K14, K18 and K23 acetylation, K4 and K79 methylation)
H3.3 is an important mark of **active chromatin states**

- **H2A.Z:** nucleosomes containing H2A.Z are biochemically less stable than those with H2A. This variant is thus associated to **active chromatin**

- **MacroH2A:** specific mark of **X chromosome inactivation** in mammals

**See for reviews: 1. Zink and Hake**: « Histone variants, nuclear function and disease », *Curr Opin Genet Dev 2016 37, 82-9* **and *2.* Harr, Gonzalez-Sandoval, and Gasser**: « Histones and histone modifications in perinuclear chromatin anchoring: from yeast to man ». *EMBO Reports* 2016, 17, 139-55.

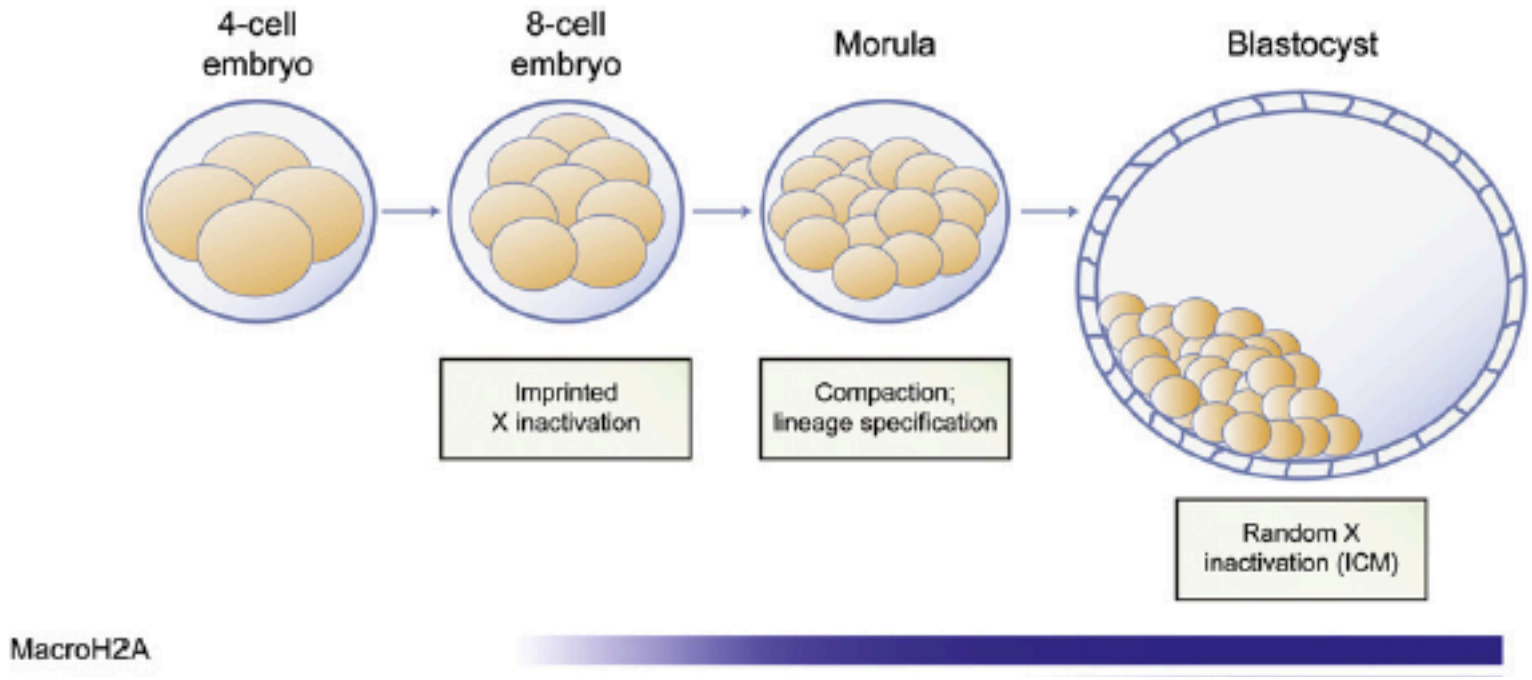
# H3.3 Variant and transcriptional activation




- Lead to the inheritance of an active chromatin state.

Review by Henikoff, Furuyama and Ahmad: « Histone variants, nucleosome, assembly and epigenetic inheritance », *TRENDS in Genetics* 2004, 20:320–326

# MacroH2A variant and X chromosome inactivation



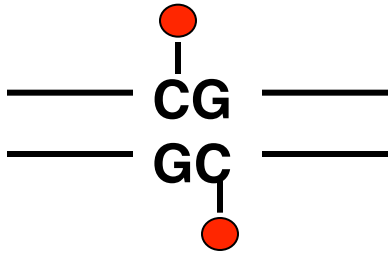

Review by Banaszynski, Allis and Lewis: « Histone variants in Metazoan Development, Developmental Cell, 2010, 19:662–674

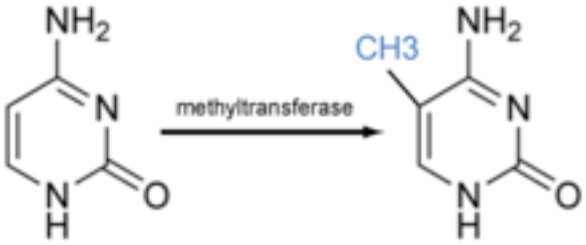
# DNA methylation

➢ **DNA methylation**: DNA modification which is not a mutation (it does not modify the base pairing code)

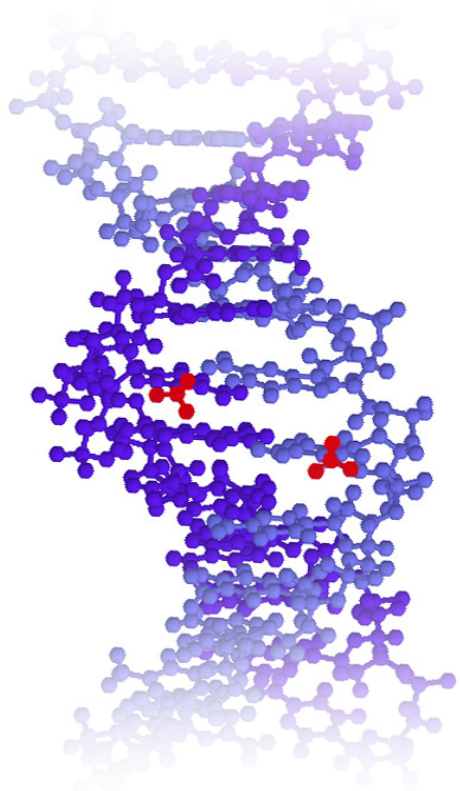
➢ In **mammals**, the most frequent DNA methylation is observed at cytosines to produce 5-methylcytosine

- **DNA methylation is generally observed in CG dinucleotides and is symmetric:**




- **CH3 groups are exposed in the large groove**



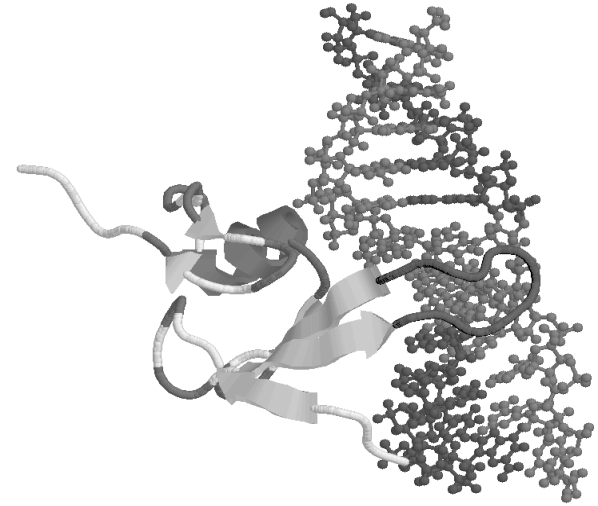
- **NOTE: recently 6mA DNA methylation has been observed in multiple species. Its biological significance is currently under investigation…**

**- CpGs, the methylation targets,** are most concentrated in **gene promoters** (CpG islands), and on **repetitive elements**

The enzymes for DNA methylation are called **DNA methyltransferases** (DNMTs. In human: DNMT1 is a maintenance methylase, whereas DNMT3a and DNMT3b, can methylate DNA de novo)

- Specific transcriptional repressors can « read » the methyl mark and bind 5mCpG

**MeCP2**
**MBD1**
**MBD2**



# Epigenetic regulation involving DNA methylation

- Some genes inactivated by methylation during development (IL4, but this is not a major mechanism)

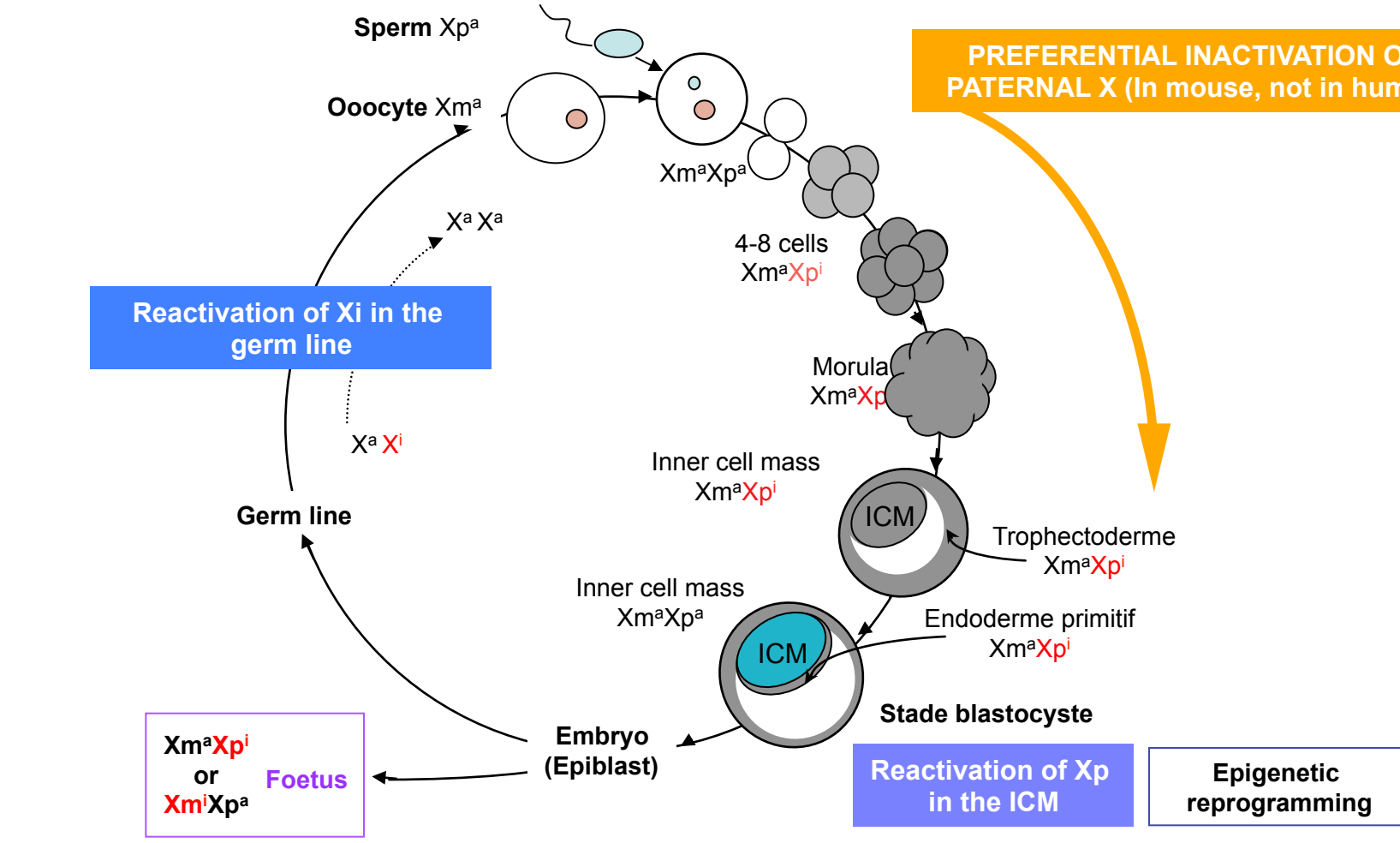
- **X-chromosome inactivation** in mammals

- **Polycomb-dependent silencing** in mammals

- **Genomic imprinting**

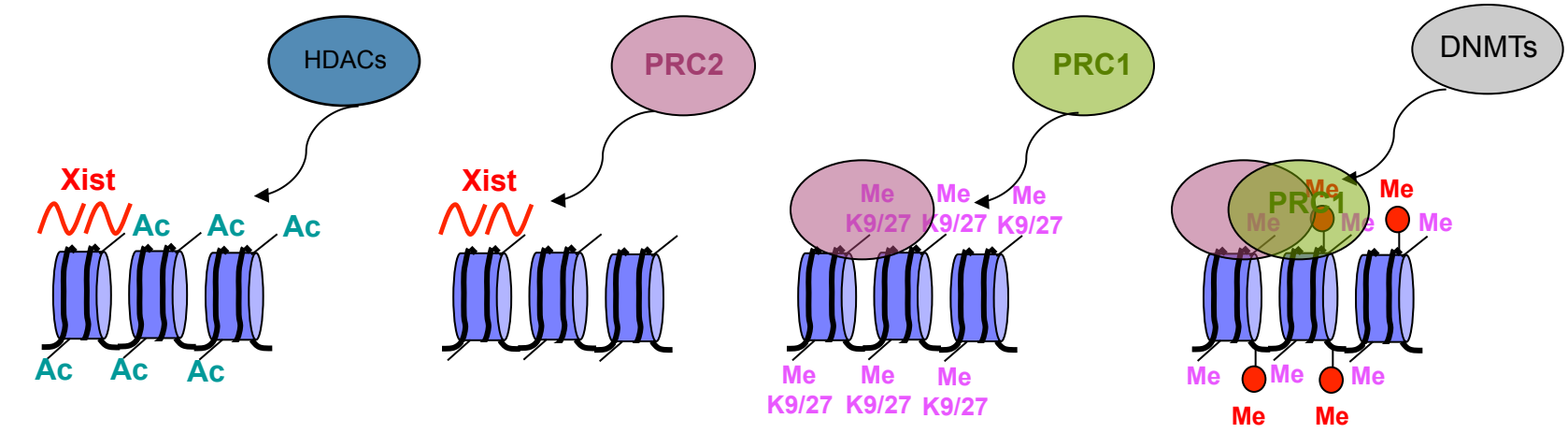
- DNA methylation is frequently involved in the **malignant transformation**

# X chromosome inactivation




Chaumeil and Heard, *Biofutur, avril 2004*

# Molecular mechanisms of X chromosome inactivation




Active gene

**Reversible Xist-dependent gene repression**

- Establishment of histone modifications -

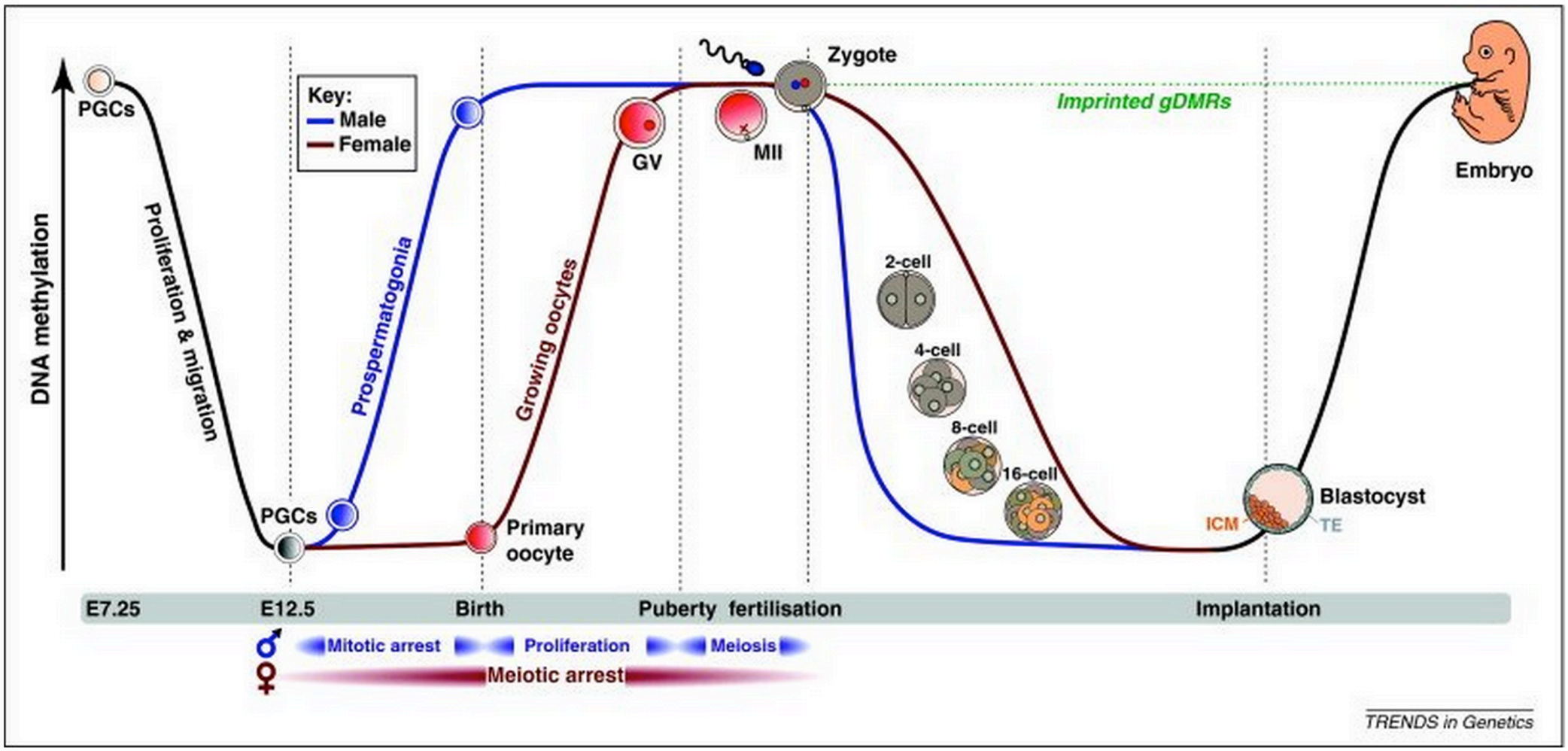
**Stable and irreversible X Inactivation (Xist independent)**

- DNA methylation, histone modification and histone variant deposition lock the inactive state -

| | |
|---|---|
| Me K9/27 | Methylation of lysines 9 and 27 of histone H3 |
| Ac | Acetylation of histones H3 et H4 |
| | DNA methylation |
| | Chromatin = ADN + nucleosomes |

**Adapted from Chaumeil and Heard, *Biofutur, avril 2004***

# Like histone modifications, DNA methylation is reversible




Sébastien A. Smallwood, Gavin Kelsey, **De novo DNA methylation: a germ cell perspective,** TIG, Volume 28, Issue 1, 2012, 33–42, http://dx.doi.org/10.1016/j.tig.2011.09.004

# Like histone modifications, DNA methylation is reversible

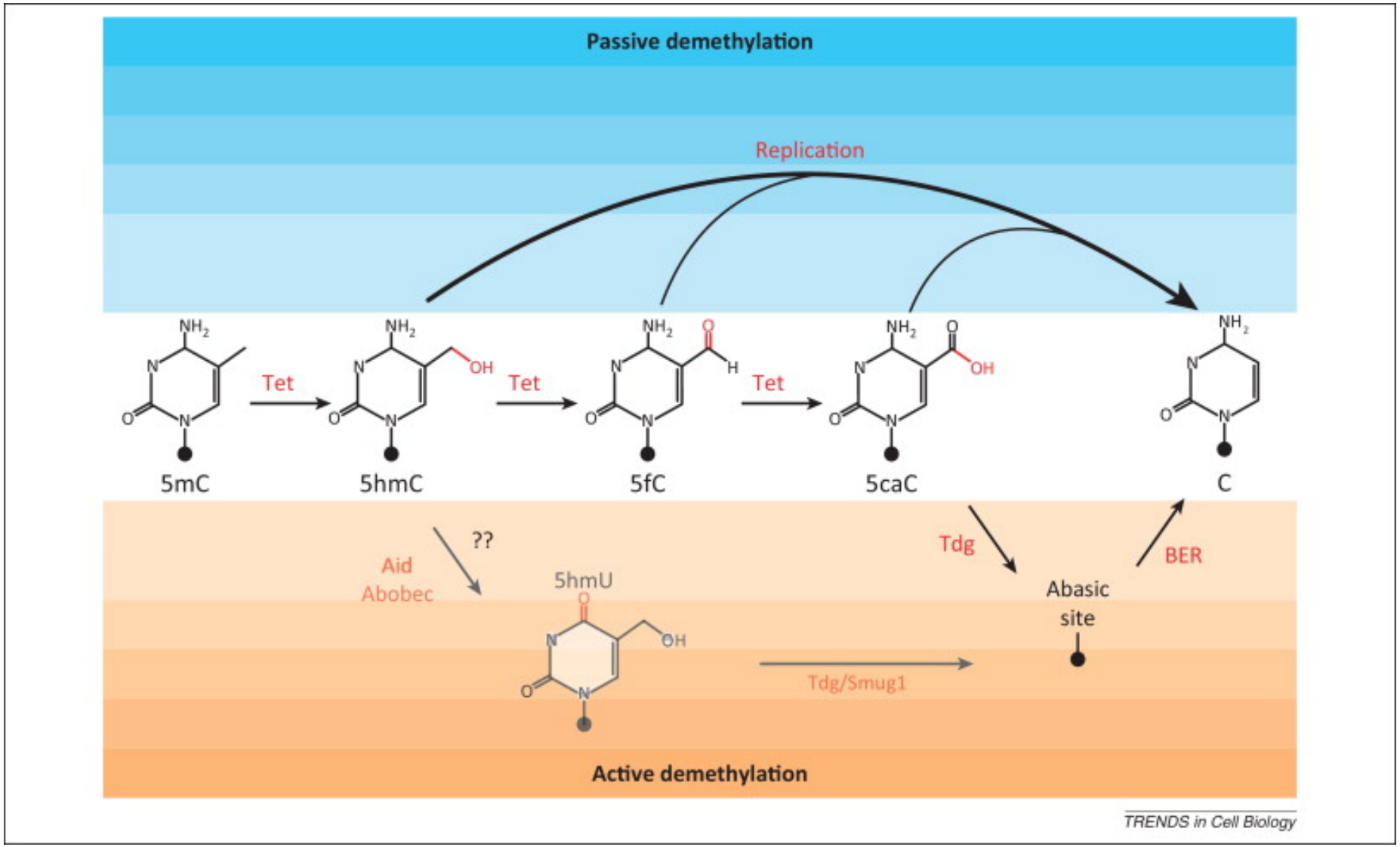



Figure 4. Routes for ten–eleven translocation (Tet)-mediated DNA demethylation. Methylated cytosine (5mC) can be converted into 5-hydroxymethylcytosine (5hmC) then to 5-formylcytosine (5fC), then 5-carboxylcytosine (5caC). Alternatively, Aid/Apobec deaminases can transform 5hmC into 5-hydroxymethylUracyl (5hmU). 5caC and 5hmU can be removed by the DNA glycosylase Tdg and then by the base excision repair to finally restor a C instead of 5mC

Francesco M. Piccolo, Amanda G. Fisher, **Getting rid of DNA methylation**, Trends Cell Biol., Volume 24, Issue 2, 2014, 136–143

# Roles of DNA methylation regulation

- The discovery of molecular pathways capable of erasing DNA methylation is recent

- genome-wide detection of hmC is difficult because the technique most widely used, bisulfite sequencing, detects 5mC as well as its oxydized forms

- nevertheless, it is clear that **DNA methylation regulation is important in normal physiology**

- Furthermore, DNA methylation plays an important role in cancer, where it is frequently altered. Furthermore Dnmts and Tet genes are frequently mutated or misexpressed in **cancer**

# DNA methylation and cancer

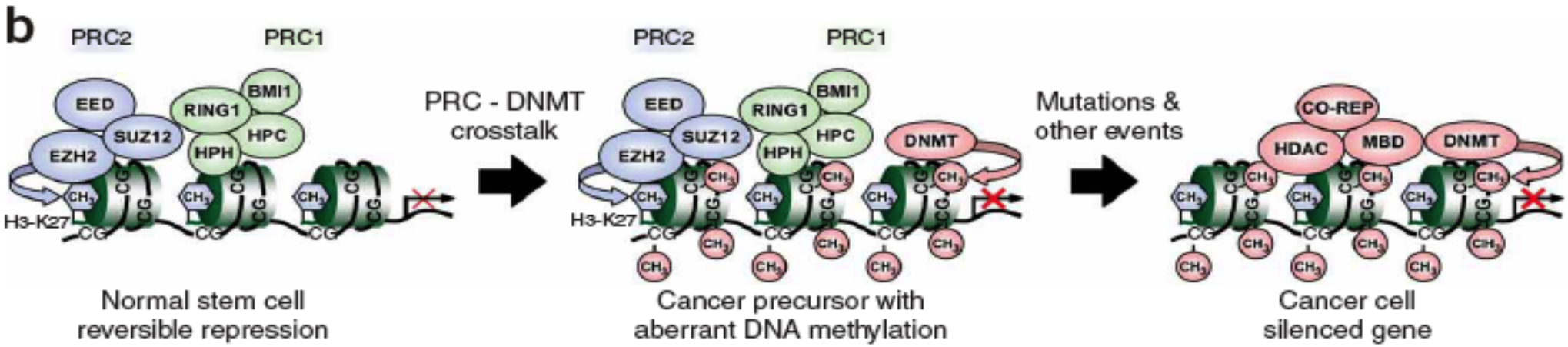
✓ Several diseases have genetic determinants, which may be modified by epigenetic processes, such as DNA methylation

✓DNA methylation can be inhibited. In general, epigenetic phenomena are good targets for therapy because they are reversible, whereas DNA mutation can generally not be reversed

✓ In most cancer cells, a fraction of the **CpG islands** is **hypermethylated** whereas the rest of the genome is globally **hypomethylated**

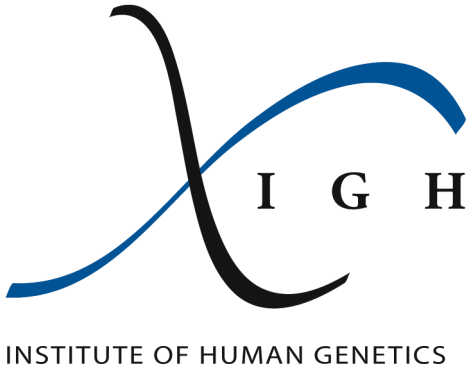
This implies that DNA methylation profiles of specific genes can be diagnostic at least for certain cancers

# Methylations, Polycomb and cancer ....




- Stem cell Polycomb group targets are up to 12-fold more likely to have cancer-specific promoter DNA hypermethylation than non-targets.
- Reversible gene repression is replaced by permanent silencing, locking the cell into a perpetual state of selfrenewal and thereby predisposing to subsequent malignant transformation.

**From Epigenetic stem cell signature in cancer**, *Nature Genetics* 2006
Martin Widschwendter, Heidi Fiegl, Daniel Egle, Elisabeth Mueller-Holzner, Gilbert Spizzo, Christian Marth, Daniel J Weisenberger4, Mihaela Campan4, Joanne Young5, Ian Jacobs & Peter W Laird




# For more info...


http://www.igh.cnrs.fr/equip/cavalli

EpigeneSys network of excellence: http://www.epigenesys.eu

Labex EpiGenMed: http://epigenmed.fr

Chromatin movie: http://www.dailymotion.pl/tag/nisic

Website to download this course:
http://www.igh.cnrs.fr/equip/cavalli/link.PolycombTeaching.html

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