BIOL8620 Eukaryotic Genetics

Genome Epigenetics 1: Preamble- Heterochromatin

Chapter 27

Three-dimensional genome architecture: players and mechanisms

Ana Pombo[1] and Niall Dillon[2]

Abstract | The different cell types of an organism share the same DNA, but during cell differentiation their genomes undergo diverse structural and organizational changes that affect gene expression and other cellular functions. These can range from large-scale folding of whole chromosomes or of smaller genomic regions, to the re-organization of local interactions between enhancers and promoters, mediated by the binding of transcription factors and chromatin looping. The higher-order organization of chromatin is also influenced by the specificity of the contacts that it makes with nuclear structures such as the lamina. Sophisticated methods for mapping chromatin contacts are generating genome-wide data that provide deep insights into the formation of chromatin interactions, and into their roles in the organization and function of the eukaryotic cell nucleus.

Chromatin immunoprecipitation
A method in which chromatin bound by a protein is immunoprecipitated with an antibody against that protein, to allow the extraction and analysis of the bound DNA by quantitative PCR or genome-wide sequencing.

[1]*Epigenetic Regulation and Chromatin Architecture Group, Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Robert Roessle Strasse, 13125 Berlin-Buch, Germany.*
[2]*Gene Regulation and Chromatin Group, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 ONN, UK.*
e-mails: ana.pombo@mdc-berlin.de; niall.dillon@csc.mrc.ac.uk
doi:10.1038/nrm3965
Published online 11 March 2015

The 2-metre length of DNA in a mammalian cell is organized into chromosomes, which are packaged and folded through various mechanisms and occupy discrete positions in the nucleus. The multiple levels of DNA folding generate extensive contacts between different genomic regions. These contacts are influenced by the proximity of DNA sequences to one another, by the folding architecture of local and long-range chromatin contacts and by proteins that associate directly or indirectly with the DNA. Packing of chromosomes in the nucleus also brings them into contact with one another and with nuclear compartments, such as the nucleolus and the nuclear envelope. As cells progress through the cell cycle and as they differentiate into specialized cell types, their chromosomes undergo structural re-organizations that influence gene expression and cell behaviour and function, which in turn modulate the organization of contacts between chromosomal regions. Techniques such as chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) have yielded large amounts of data on transcription factor binding and post-translational histone modifications in a range of cell types. Increasingly sophisticated methods of chromatin conformation capture (3C) are also being used to map genome-wide chromatin contacts. These approaches are enabling the study of how different cellular functions are influenced by the constraints of chromatin and chromosomal organization.

Contacts between chromatin regions are increasingly thought to have important roles in gene regulation mechanisms. In particular, since the discovery of enhancers more than 30 years ago, it has become clear that this type of genetic element can regulate gene transcription over large distances. Looping out of the DNA that separates promoters and distally located enhancers was proposed as a mechanism by which factors that are bound to enhancers can directly contact their target promoters and influence the composition of transcription initiation complexes (FIG. 1a). Evidence supporting looping models was initially obtained by fluorescence *in situ* hybridization (FISH) and by measuring the effects of genomic distance on enhancer function[1,2].

In this Review, we discuss how various cellular factors are thought to contribute to the formation of higher-order chromatin structures such as active chromatin hubs (ACHs) and lamin-associated domains (LADs). We also describe the organization of the nucleus into distinct compartments and chromosome territories. We then discuss evidence that genome architecture contributes to the regulation of cellular functions such as the cell cycle, differentiation and senescence.

The organization of chromosomes

The development of the 3C[3] technique and its subsequent maturation into chromosome conformation capture on chip (4C)[4], 3C combined with high-throughput sequencing (4C-seq)[5], multiplexed 3C sequencing (3C-seq)[6], carbon-copy chromosome conformation capture (5C)[7], capture-C[8], genome conformation capture (GCC)[9], Hi-C[10], tethered conformation capture (TCC)[11] and targeted chromatin capture (T2C)[12] (BOX 1) have been critical for the rapid progress in the study of

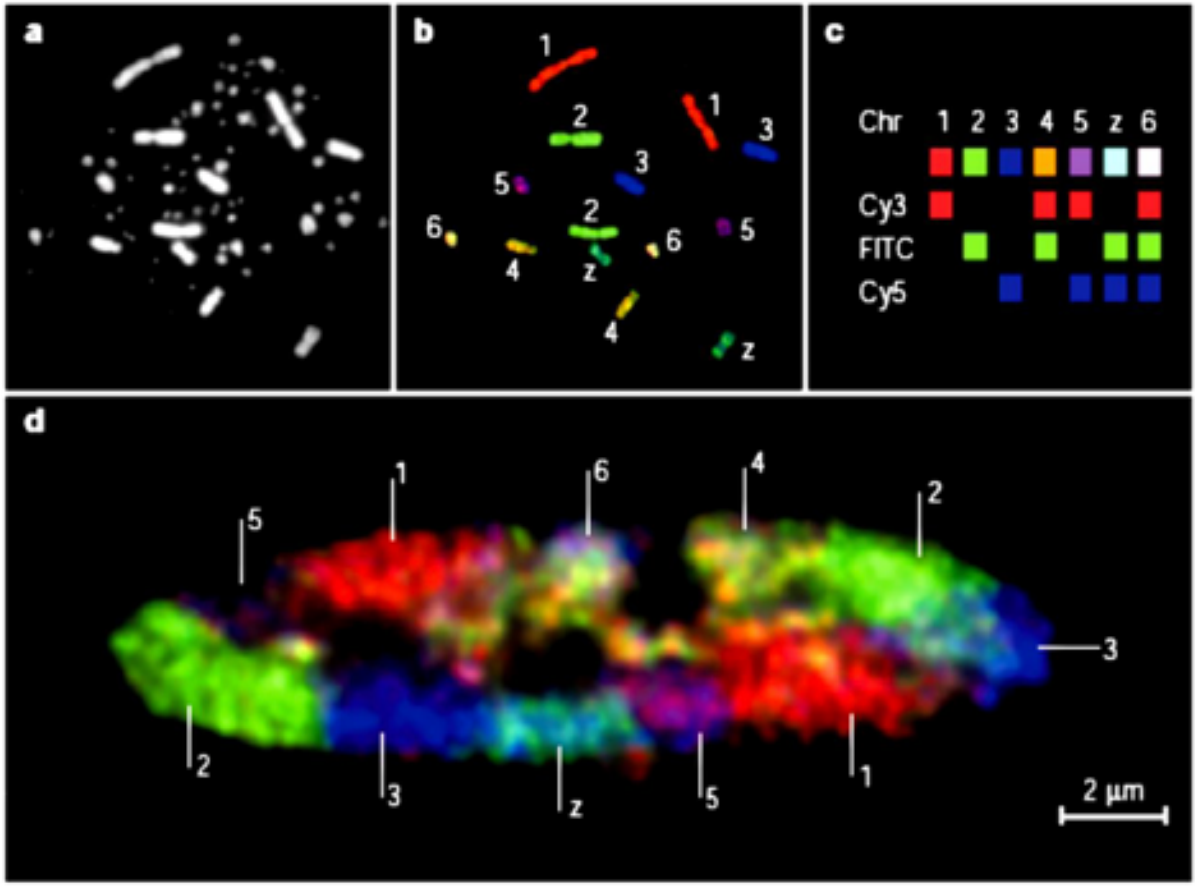
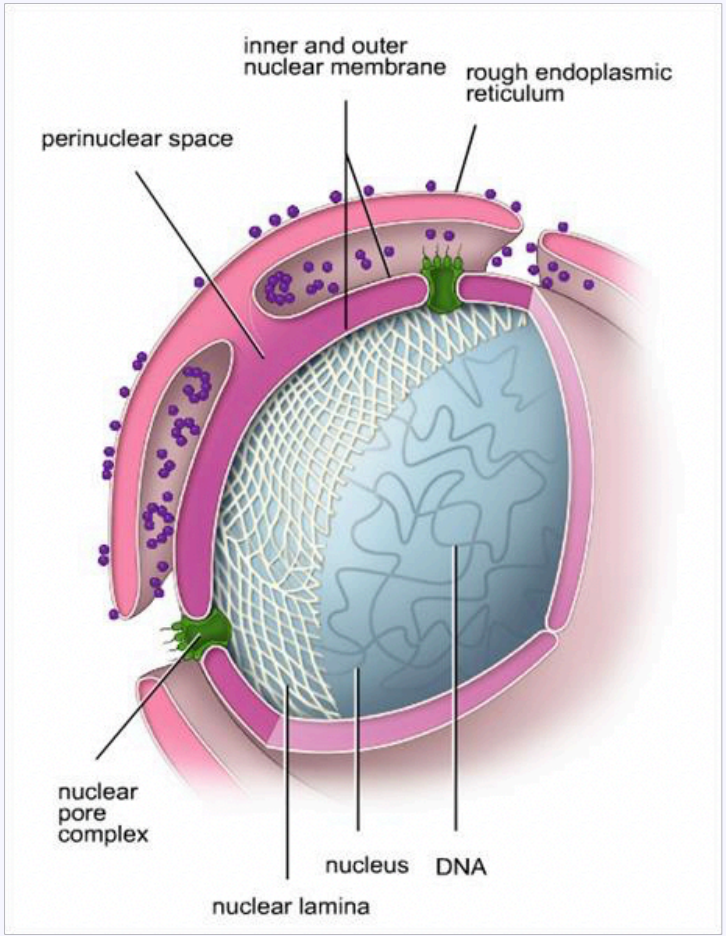
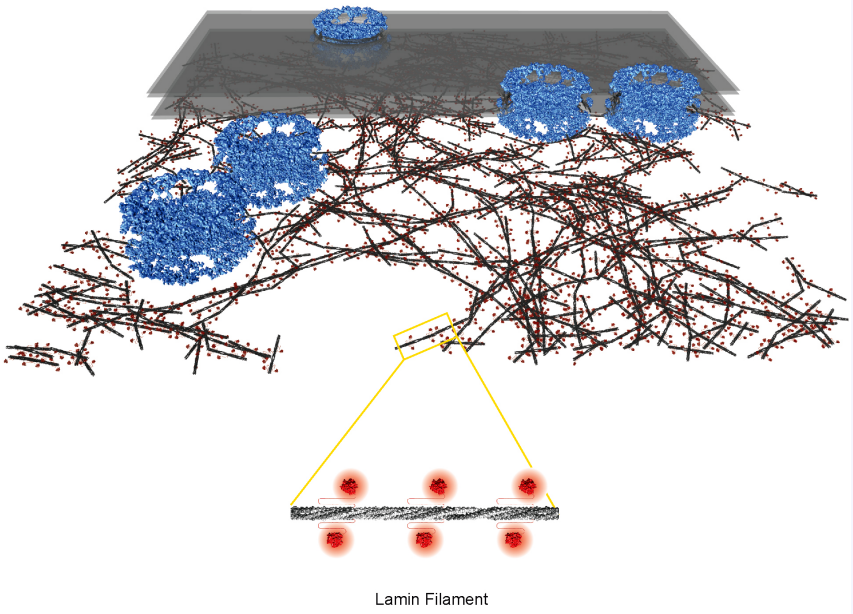


Figure 2 | **Chromosome territories in the chicken. a** | 4,6-diamidino-2-phenylindole (DAPI)-stained, diploid, chicken metaphase spread with macro- and microchromosomes. **b** | The same metaphase spread after multicolour fluorescence *in situ* hybridization with pseudocoloured chromosomes. Chicken chromosome paint probes (image courtesy of Johannes Wienberg) were labelled by a combinatorial scheme with oestradiol (1, 4, 5, 6), digoxigenin (2, 4, 6, Z) and biotin (3, 5, 6, Z). **c** | Oestradiol- and digoxigenin-labelled probes were detected using secondary antibodies labelled with Cy3 and fluorescein isothiocyanate (FITC); biotinylated probes were detected with Cy5-conjugated streptavidin. **d** | Mid-plane light optical section through a chicken fibroblast nucleus shows mutually exclusive chromosome territories (CTs) with homologous chromosomes seen in separate locations. (Note that only one of the two CTs for each of 4 and 6 is displayed in this section.) (Image courtesy of F. Habermann.)

Cremer and Cremer,
Nature Reviews in Genetics

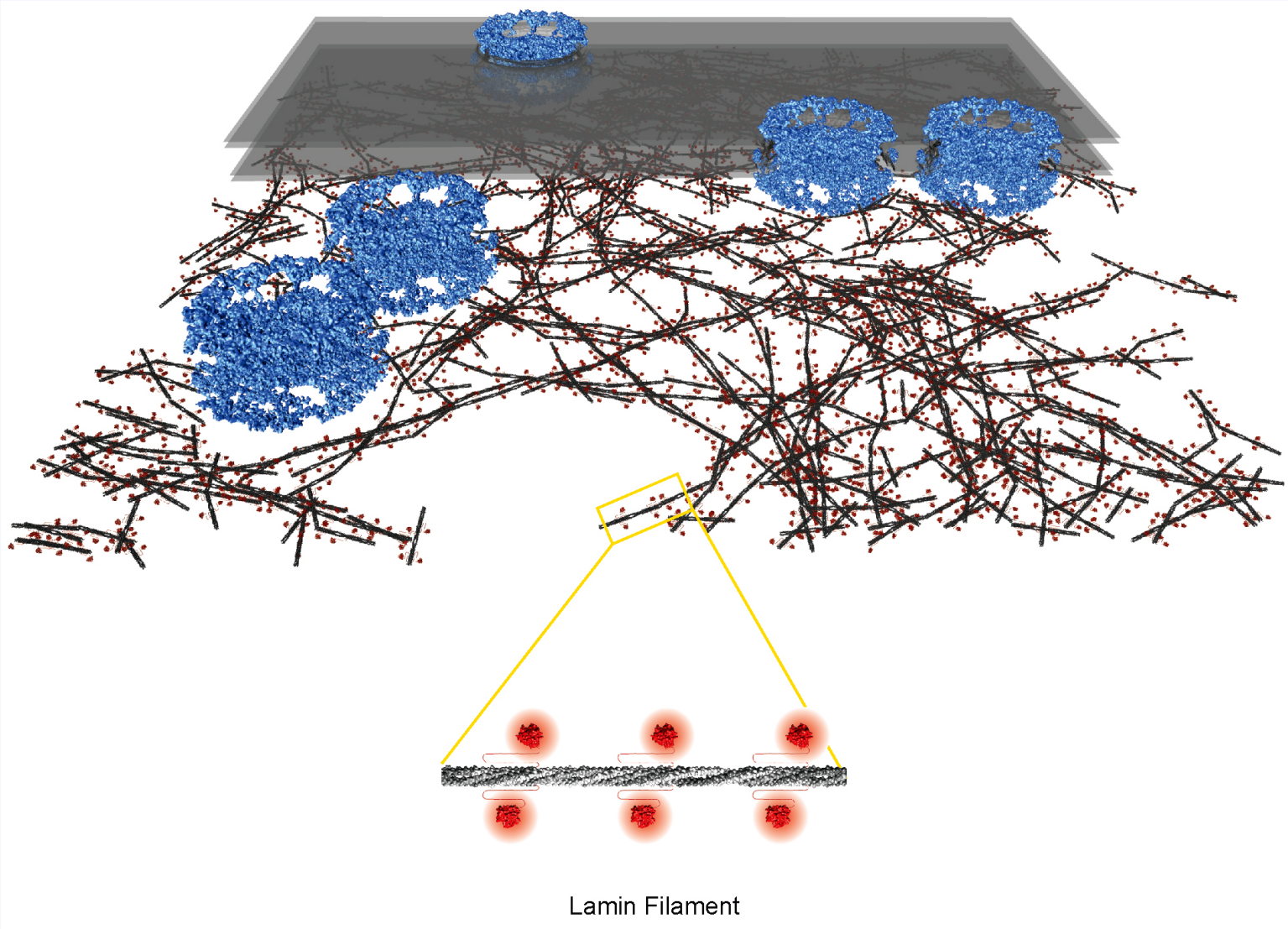
292 | APRIL 2001 | VOLUME 2





The **nuclear lamina** is a dense (~30 to 100 nm thick) fibrillar network inside the nucleus of most cells. It is composed of intermediate filaments and membrane associated proteins.

Besides providing mechanical support, the nuclear lamina regulates important cellular events such as DNA replication and cell division. Additionally, it participates in chromatin organization and it anchors the nuclear pore complexes embedded in the nuclear envelope.



The 3D view of the **nuclear lamina** shows a section of the architecture of the delicate meshwork made of lamin filaments (**filament rod in dark grey** and its globular domains in red) beneath the cell nuclear membrane (transparent grey) and the nuclear pore complexes (blue). (Image: Yagmur Turgay, UZH). Diseases such as muscular dystrophy and premature aging, caused by mutations in the lamin gene, the major constituent of the lamina, can now be studied more effectively.

Comparison Chart

BASIS FOR COMPARISON	HETEROCHROMATIN	EUCHROMATIN
Meaning	The tightly packed form of DNA in the chromosome is called as heterochromatin.	The loosely packed form of DNA in the chromosome is called as euchromatin.
DNA density	High DNA density.	Low DNA density.
Kind of stain	Stained dark.	Lightly stained.
Where they are present	These are found at the periphery of the nucleus in eukaryotic cells only.	These are found in the inner body of the nucleus of prokaryotic as well as in eukaryotic cells.
Transcriptional activity	They show little or no transcriptional activity.	They actively participate in the process of transcription.
Other features	They are compactly coiled.	They are loosely coiled.
	They are late replicative.	They are early replicative.
	Regions of heterochromatin are sticky.	Regions of euchromatin are non-sticky.
	Genetically inactive.	Genetically active.
	Phenotype remains unchanged of an organism.	Variation may be seen, due to the affect in DNA during the genetic process.
	It permits the gene expression regulation and also maintains the structural integrity of the cell.	It results in genetic variations and permits the genetic transcription.

Heterochromatin differs from euchromatin in that **heterochromatin** is effectively inert; remains condensed during interphase; is transcriptionally repressed; replicates late in S phase and may be localized to the centromere or nuclear periphery

Heterochromatin is not restricted to regions of chromosome defined by **pre-designated sequence(s)**; genes that are moved within or near heterochromatic regions can become inactivated as a result of their new location.

Chromatin inactivation (or **heterochromatin formation**) occurs by the addition of proteins to the nucleosomal fiber.

May be due to:

Chromatin condensation -making it inaccessible to transcriptional apparatus

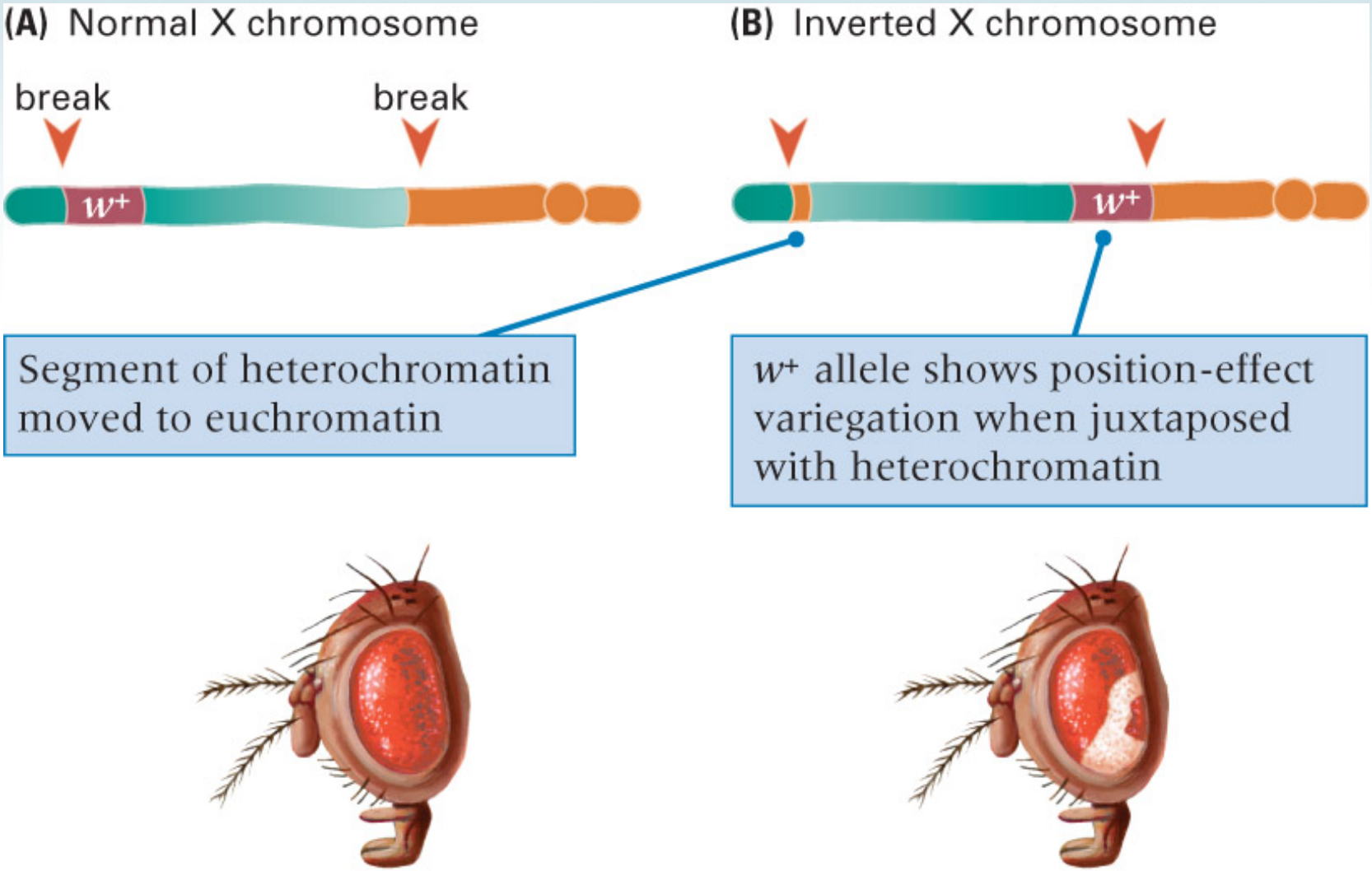
Proteins that accumulate and inhibit accessibility to the regulatory sequences

Proteins that directly inhibit transcription

Two well characterized systems: **HP1** in mammals and **SIR** complexes in yeast.

- **Constitutive heterochromatin** contains specific sequences that have no coding function. Typically these include satellite DNAs, and are often found at the centromeres. These regions are invariably heterochromatic because of their intrinsic nature.

- **Facultative heterochromatin** takes the form of entire chromosomes that are inactive in one cell lineage, although they can be expressed in other lineages. The example *par excellence* is the mammalian X chromosome. The inactive X chromosome is perpetuated in a heterochromatic state, while the active X chromosome is part of the euchromatin. So *identical DNA sequences are involved in both states.* Once the inactive state has been established, it is inherited by descendant cells. This is an example of epigenetic inheritance, because it does not depend on the DNA sequence.



Position-effect variegation (PEV)

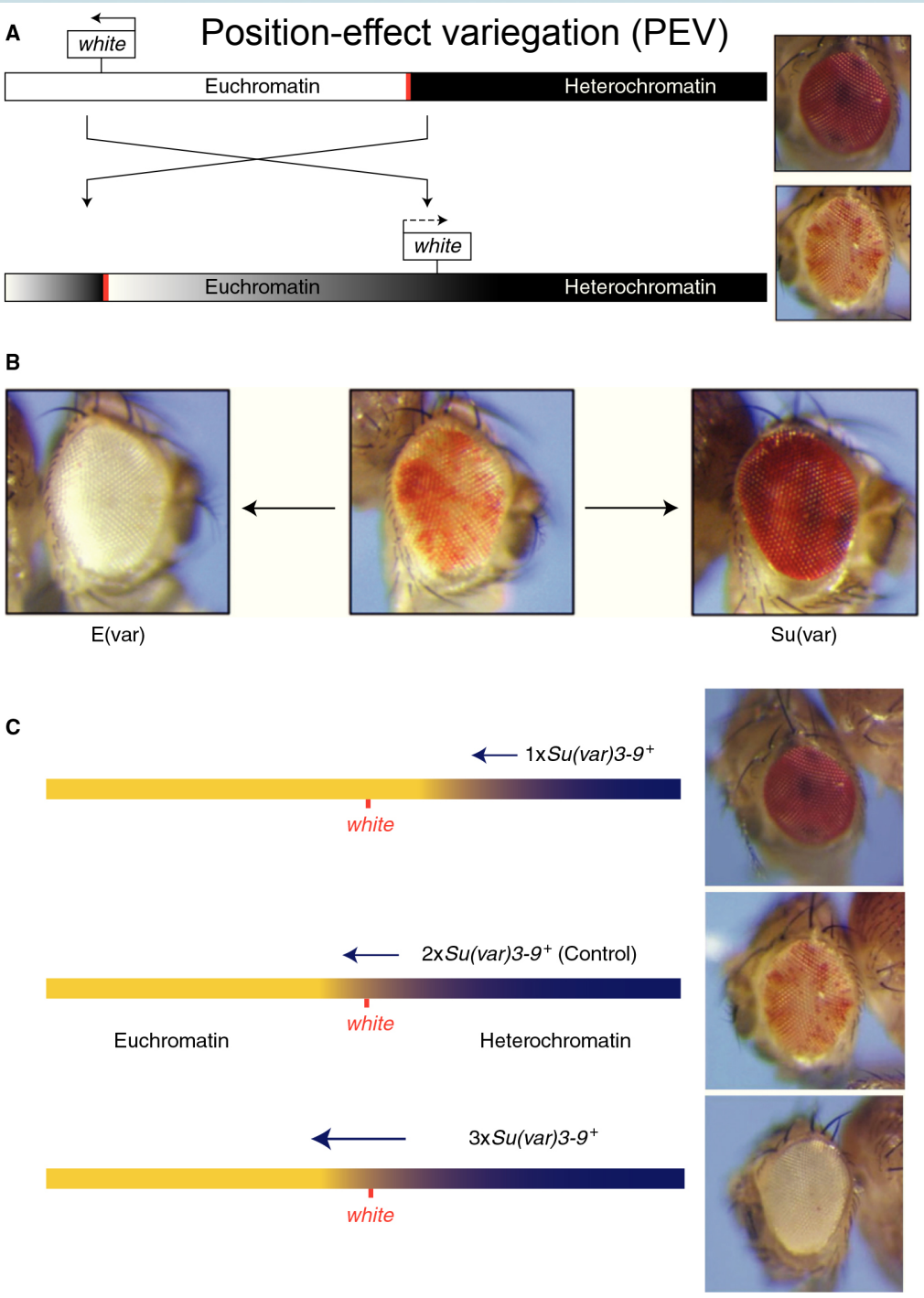


Figure 1. A schematic illustration of *white* variegation in the X chromosome inversion *In(1)w*^{m4}.

(A) Rearrangement attributable to an X-ray-induced inversion places the *white* locus, normally located in the distal euchromatin (white bar) of the X chromosome (see *top* line), ~25 kb from a breakpoint in the pericentric heterochromatin (black bar; *bottom* rearranged line). Spreading of heterochromatin packaging into the euchromatic domain results in silencing (causing a white eye in this case); loss of silencing in some cells during differentiation results in a variegating phenotype (*bottom* line, *right*).

(B) (*B*) Given a variegating phenotype, screens for second site mutations can recover suppressors (*Su(var)*s) and enhancers (*E(var)*s) as described in the text.

(C) (*C*) Some *Su(var)* loci (e.g., *Su(var)3-9*, shown here) show an antipodal dosage-dependent effect, and are consequently thought to be structural proteins of heterochromatin. The presence of only one copy of the modifier gene results in less heterochromatin formation, and more expression from the reporter gene (suppression of PEV, *top* fly eye); conversely, the presence of three copies of such a modifier gene will drive more extensive heterochromatin formation, resulting in an enhancement of reporter gene silencing (enhancement of PEV, *bottom* fly eye).

Epigentics, Second Edition 2015 Cold Spring Harbor La. Press

Heterochromatin differs from euchromatin in that **heterochromatin** is effectively inert; remains condensed during interphase; is transcriptionally repressed; replicates late in S phase and may be localized to the centromere or nuclear periphery

Heterochromatin is not restricted by **pre-designated sequence(s)**; genes that are moved within or near heterochromatic regions can become inactivated as a result of their new location.

Chromatin inactivation (or **heterochromatin formation**) occurs by the addition of proteins to the nucleosomal fiber.

May be due to:

Chromatin condensation -making it inaccessible to transcriptional apparatus

Proteins that accumulate and inhibit accessibility to the regulatory sequences

Proteins that directly inhibit transcription

Two well characterized systems: **HP1** in mammals and **SIR** complexes in yeast.

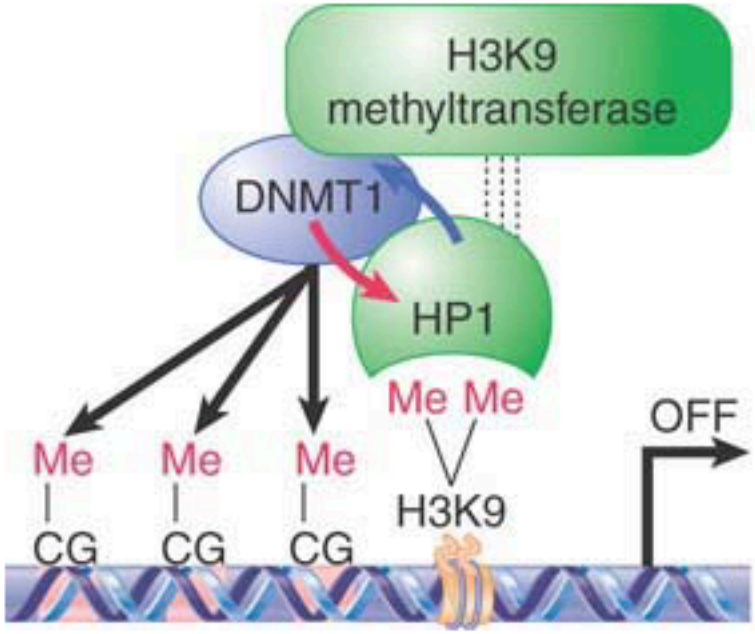


FIGURE 27.12 Mammalian HP1 is recruited to regions where lysine 9 of histone H3 (H3K9) has been methylated by a histone methyltransferase. HP1 then binds to Dnmt1 and potentiates its DNA methyltransferase activity (blue arrow), thereby enhancing cytosine methylation (meCG) on nearby DNA. Dnmt1 may, in turn, assist HP1 loading onto chromatin (red arrow). Furthermore, association of Dnmt1 with the histone methyltransferase could allow a positive feedback loop to stabilize inactive chromatin.

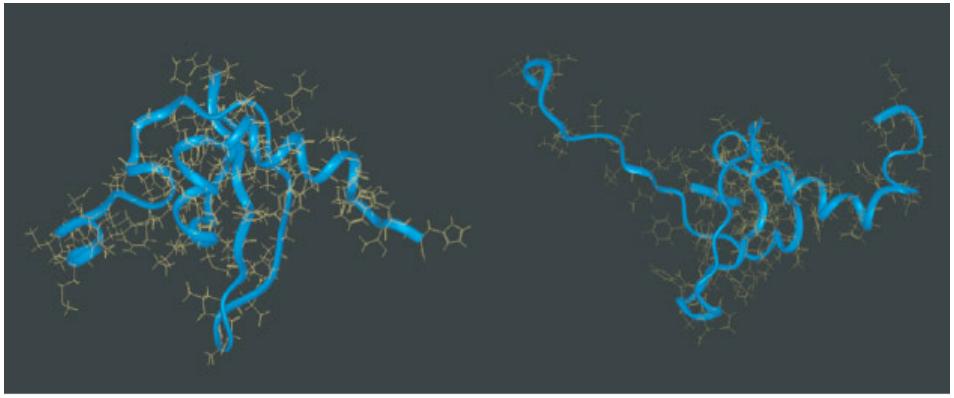
Histone	Site	Modification	Function
H3	K-4	Methylation	Transcription activation
H3	K-9	Methylation	Chromatin condensation
	K-9	Methylation	Required for DNA methylation
	K-9	Acetylation	Transcription activation
H3	S-10	Phosphorylation	Transcription activation
H3	K-14	Acetylation	Prevents methylation at Lys-9
H3	K-79	Methylation	Telomeric silencing
H4	R-3	Methylation	
H4	K-5	Acetylation	Nucleosome assembly
H4	K-12	Acetylation	Nucleosome assembly
H4	K-16	Acetylation	Nucleosome assembly
	K-16	Acetylation	Fly X activation

Most modified sites in histones have a single, specific type of modification, but some sites can have more than one type of modification

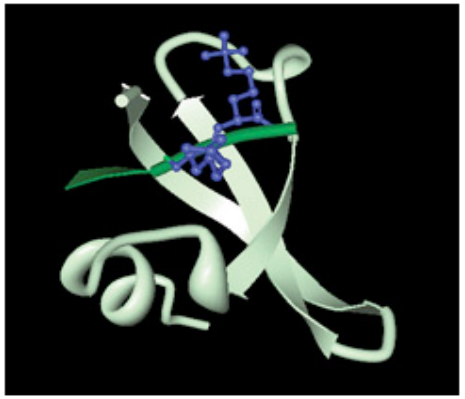
Heterochromatin Depends on Interactions with Histones

- HP1 is the key protein in forming mammalian heterochromatin, and acts by binding to methylated histone H3.

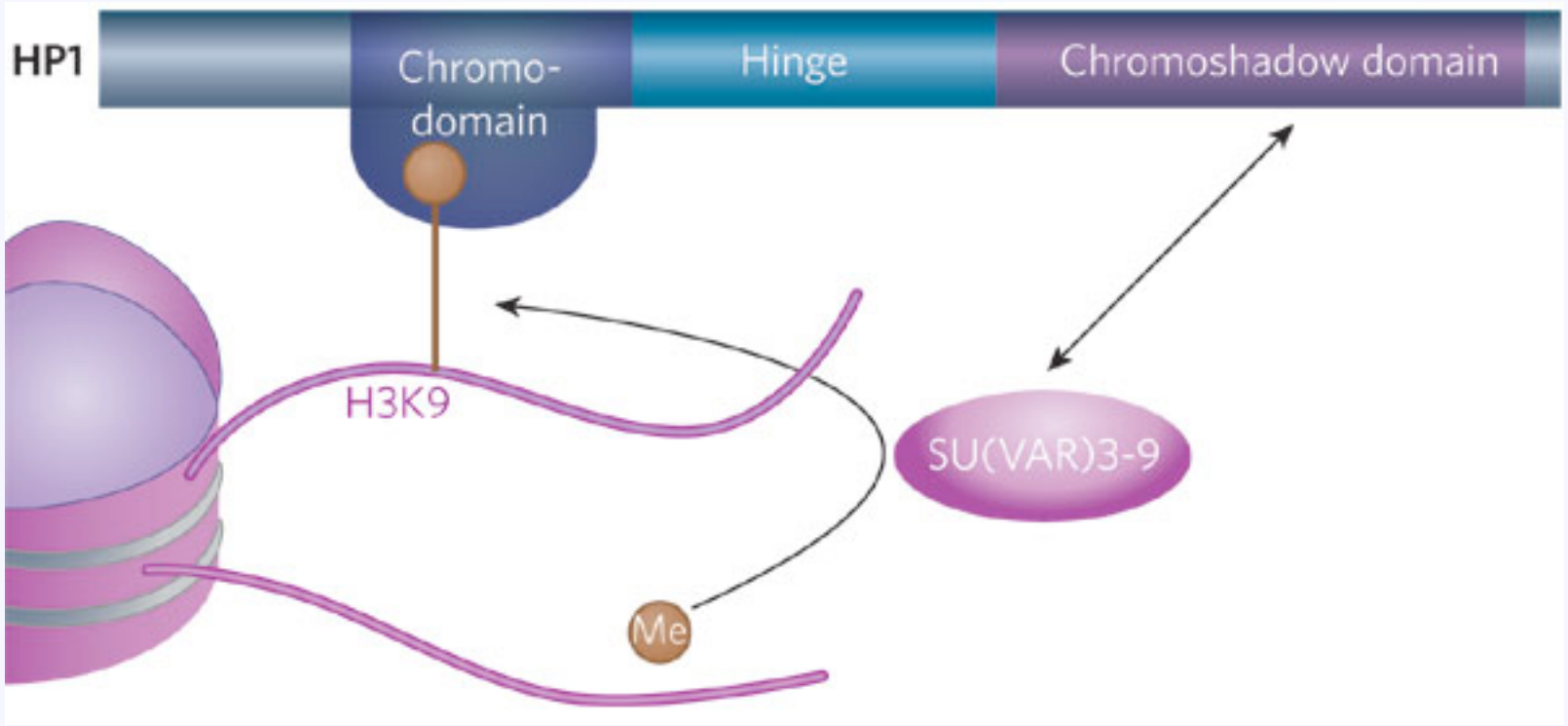
HP1 contains a chromodomain and a **chromoshadow** domain



HP1 contains a chromodomain and a **chromoshadow** domain



HP1 contains a chromodomain and a **chromoshadow** domain



HP1 interacts with **H3K9me2** and **H3K9me3** through its chromodomain, and with SU(VAR)3-9 through its chromoshadow domain. By interacting with both the modified histone and the enzyme responsible for the histone modification, **HP1** provides a foundation for heterochromatin spreading and epigenetic inheritance. (Figure adapted, with permission, from ref. 10.)

Transcription and RNA interference in the formation of heterochromatin
Shiv I. S. Grewal & Sarah C. R. Elgin
Nature **447**, 399-406(24 May 2007)
doi:10.1038/nature05914

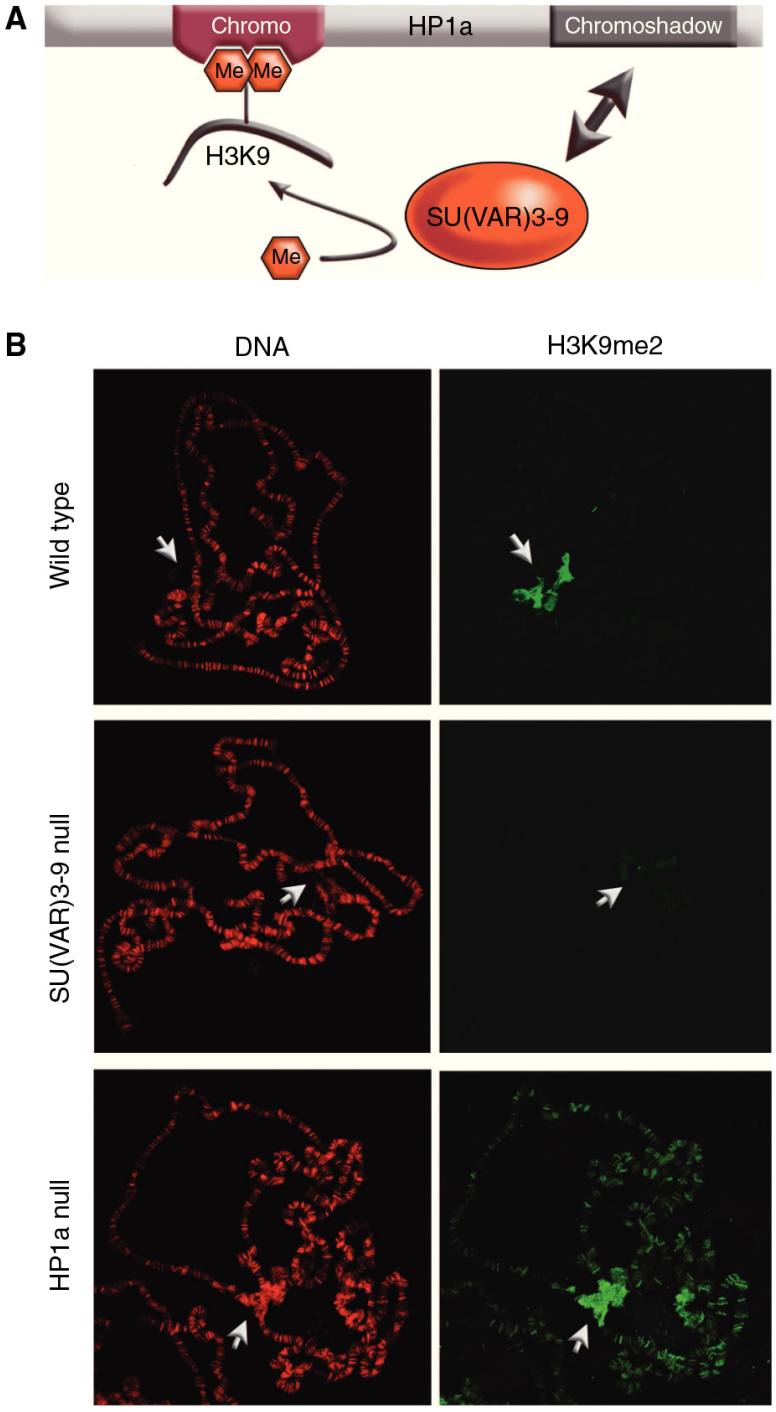


Figure 4. Interaction of SU(VAR)3-9 and **HP1a** in setting the distribution pattern of **H3K9** methylation.

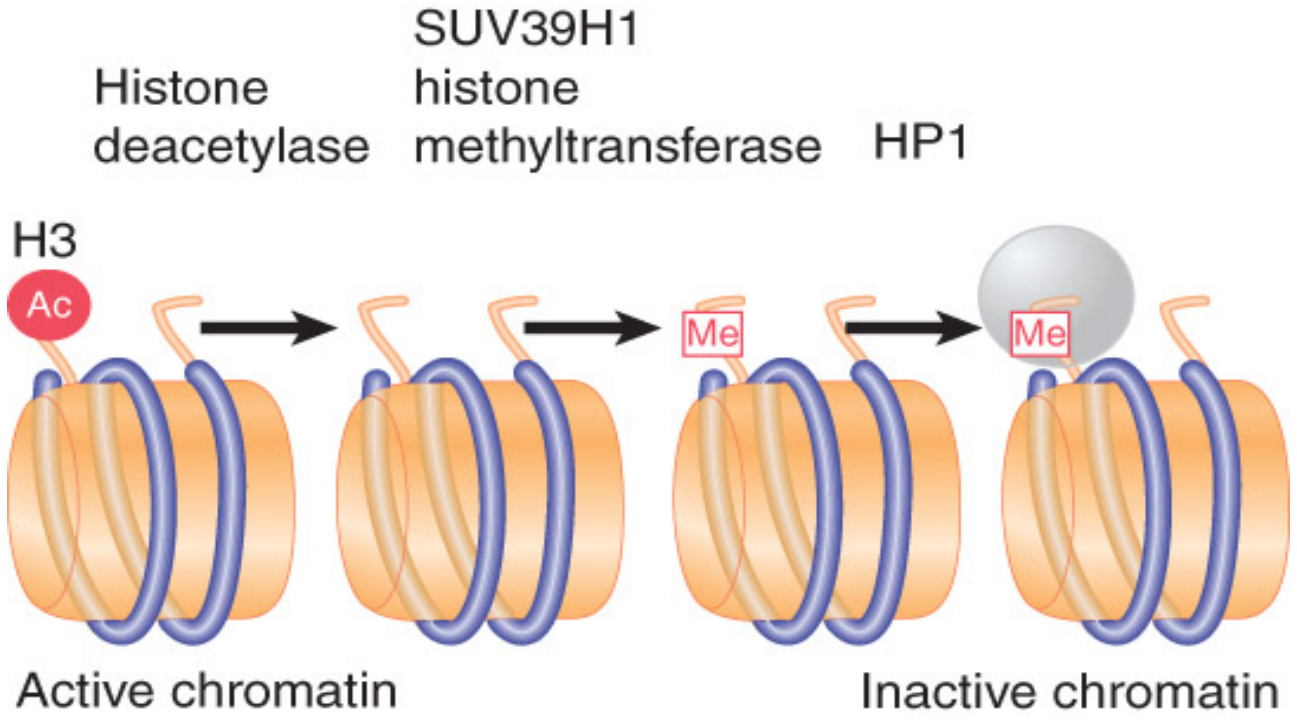
(A) HP1a interacts with **H3K9me2/3** through its chromodomain, and with SU(VAR)3-9 through its chromoshadow domain. By recognizing both the histone modification and the enzyme responsible for that modification, **HP1a** provides a mechanism for heterochromatin spreading and epigenetic inheritance.

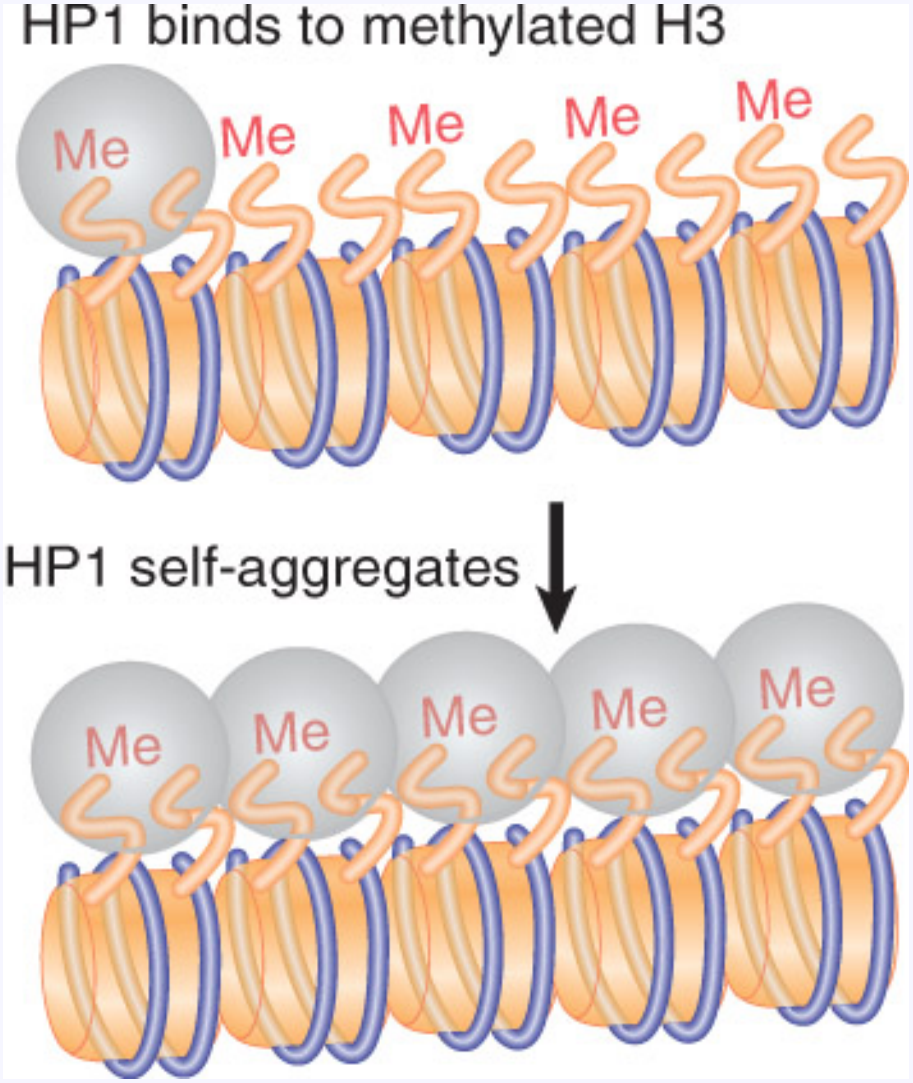
(C) (*B*) SU(VAR)3-9 is responsible for much of the dimethylation of **H3K9** (**H3K9me2**); loss of the enzyme results in loss of this modification in the pericentric heterochromatin, as shown by loss of antibody staining of the polytene chromosomes (compare *middle* panel with *top* panel). Loss of HP1a results in a loss of targeting of SU(VAR)3-9; high levels of **H3K9me** are consequently now seen throughout the chromosome arms (*bottom* panel).

Epigenetics, Second Edition 2015 Cold Spring Harbor La. Press

Heterochromatin Depends on Interactions with Histones

- HP1:
  - is the key protein in forming mammalian heterochromatin
  - acts by binding to **methylated histone H3**



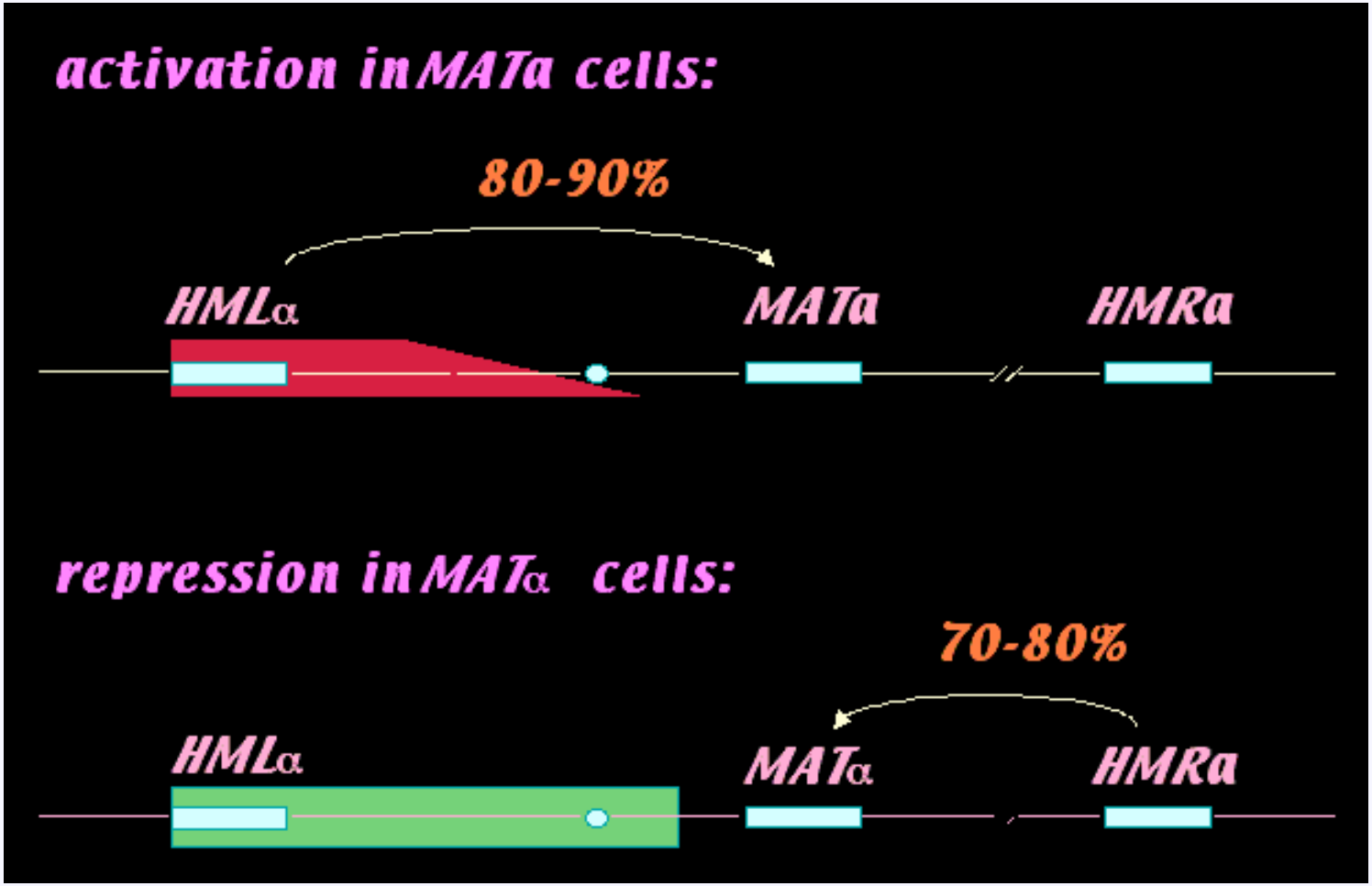


HP1 then self-aggregates

Heterochromatin Depends on Interactions with Histones

- **Telomeric silencing** in yeast is analogous to **PEV in *D. melanogaster*** eye colour.
- **Rap1** initiates formation of heterochromatin in yeast by binding to specific target sequences in DNA.
- The targets of **Rap1** include **telomeric repeats** and silencers at ***HML*** and ***HMR***.

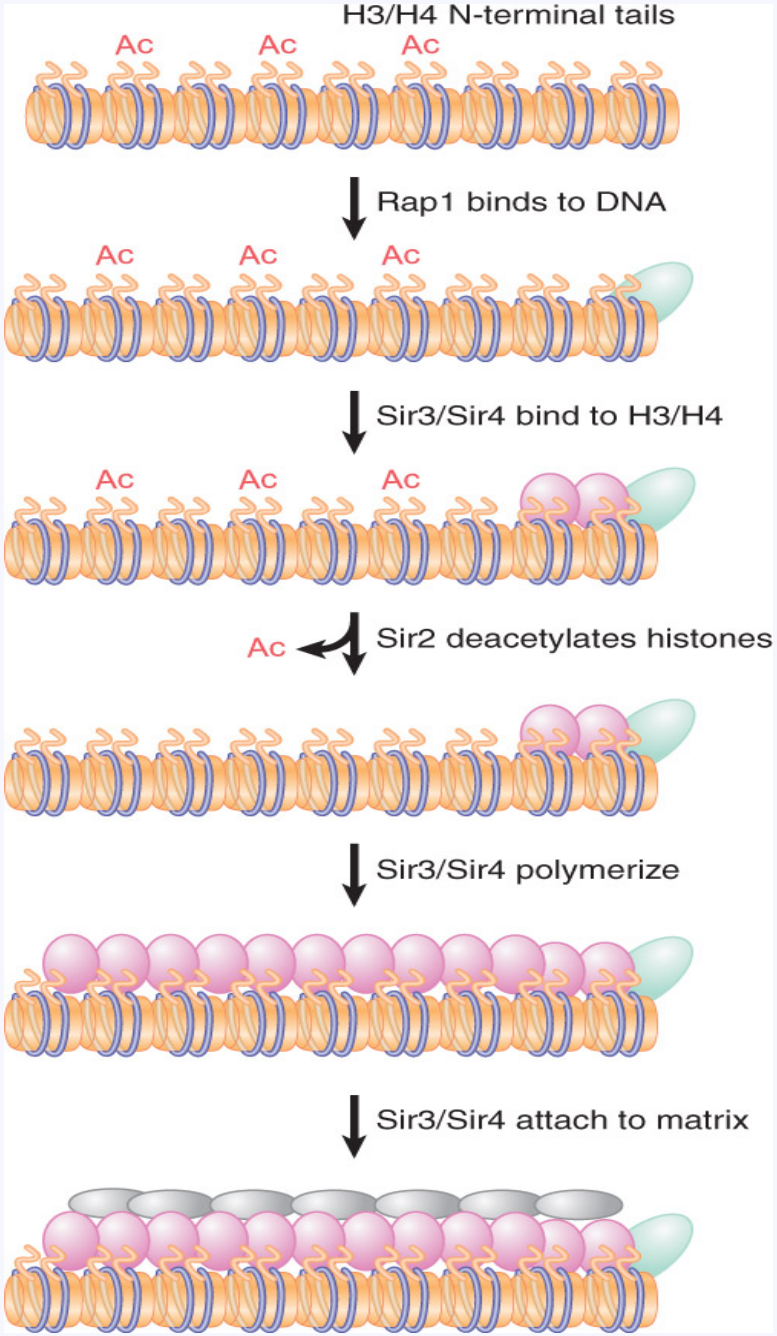
- **Rap1** recruits **Sir3** and **Sir4**, which interact with the N-terminal tails of **H3** and **H4**.
- **Sir2** deacetylates the N-terminal tails of **H3** and **H4** and promotes spreading of **Sir3** and **Sir4**.


activation in MATa cells:
80-90%
HMLα
MATa
HMRa
repression in MATα cells:
70-80%
HMLα
MATα
HMRa

Heterochromatin Depends on Interactions with Histones

- **Telomeric silencing** in yeast is analogous to **PEV**.
- **Rap1** initiates formation of heterochromatin in yeast by binding to specific target sequences in DNA.
- The targets of **Rap1** include telomeric repeats and silencers at *HML* and *HMR*.

- **Rap1** recruits **Sir3** and **Sir4**, which interact with the N-terminal tails of **H3** and **H4**.
- **Sir2** *deacetylates* the N-terminal tails of **H3** and **H4** and promotes the spreading of **Sir3** and **Sir4**.

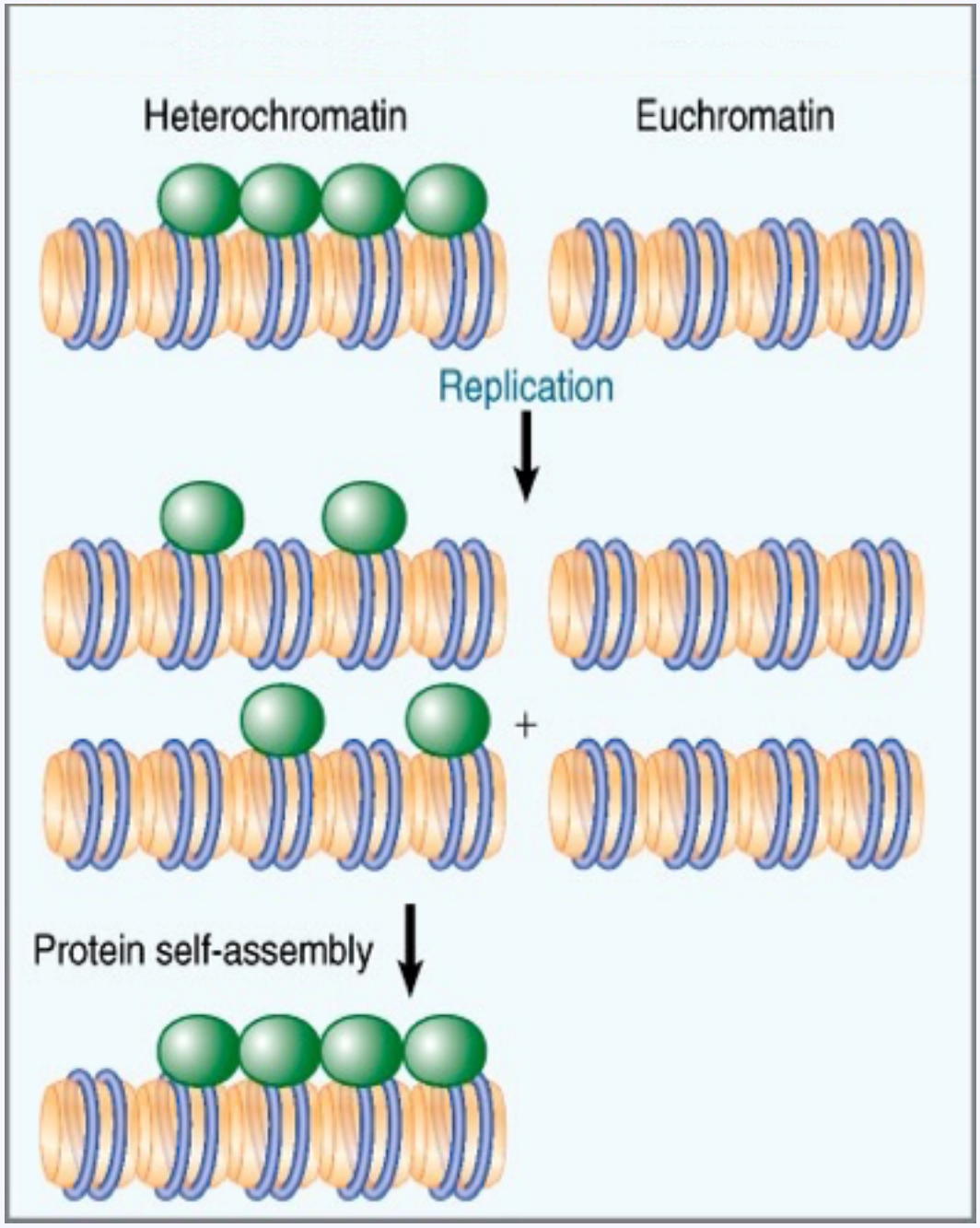


In yeast, **Rap1** is able to initiate the formation of heterochromatin by binding to specific target sequences in the telomeric regions of the DNA and to cis-acting silencer elements.

The proteins Sir3/Sir4 are recruited to the DNA sequences by **Rap1**, and act directly with the N-terminal tails of H3/H4.

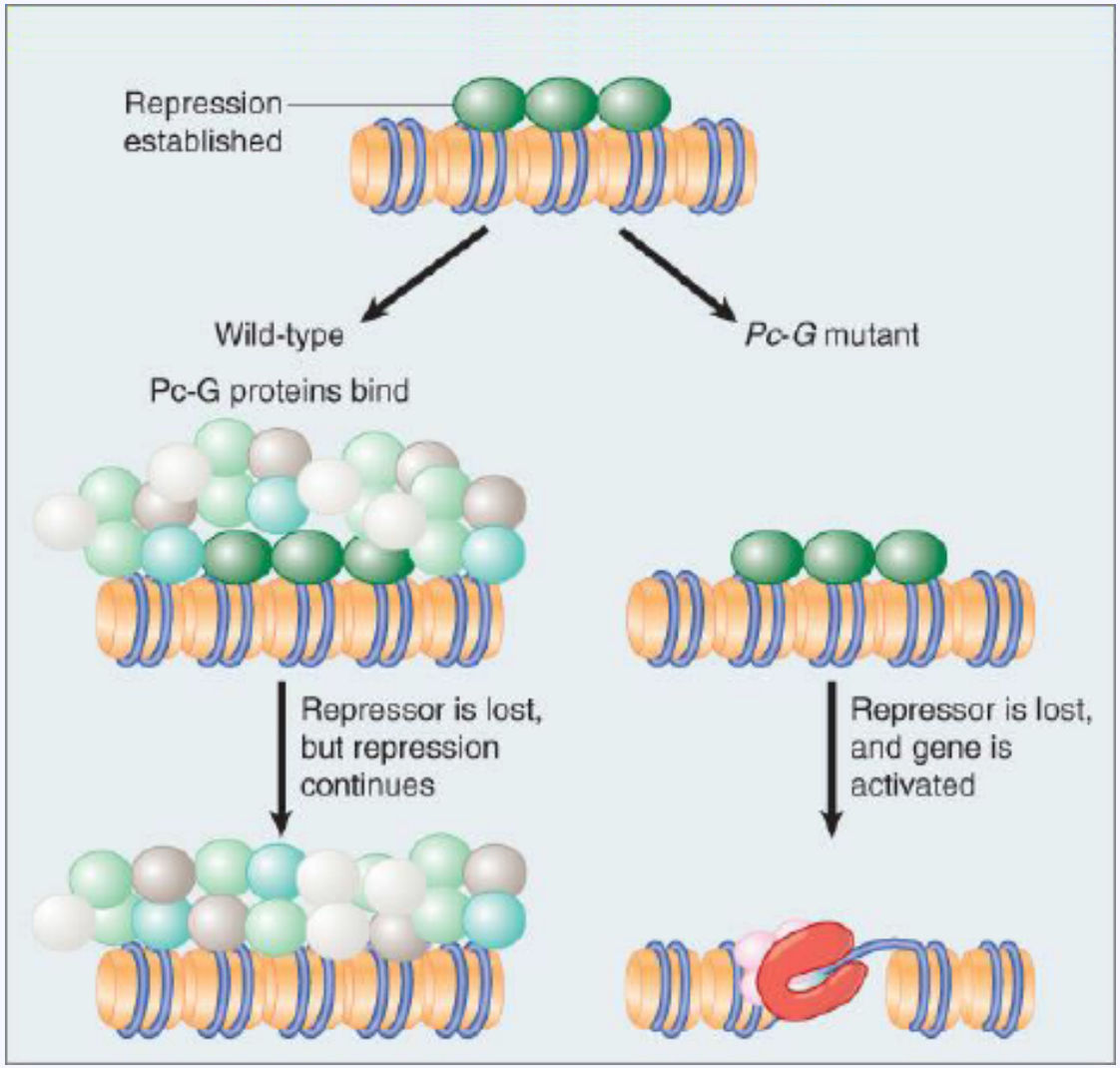
Sir2 then *deacetylates* the N-terminal tails of **H3** and **H4** and promotes spreading of **Sir3** and **Sir4**.

After which **Sir3/Sir4** continue to aggregate on the DNA and may act to tether the heterochromatin to the nuclear periphery.



Self-assembling complexes maintain heterochromatin

- Self-perpetuating structures that assemble on the DNA usually have a repressive effect by forming heterochromatic regions that prevent expression of genes within them.
- This depends on their ability to remain bound to the DNA following DNA replication.

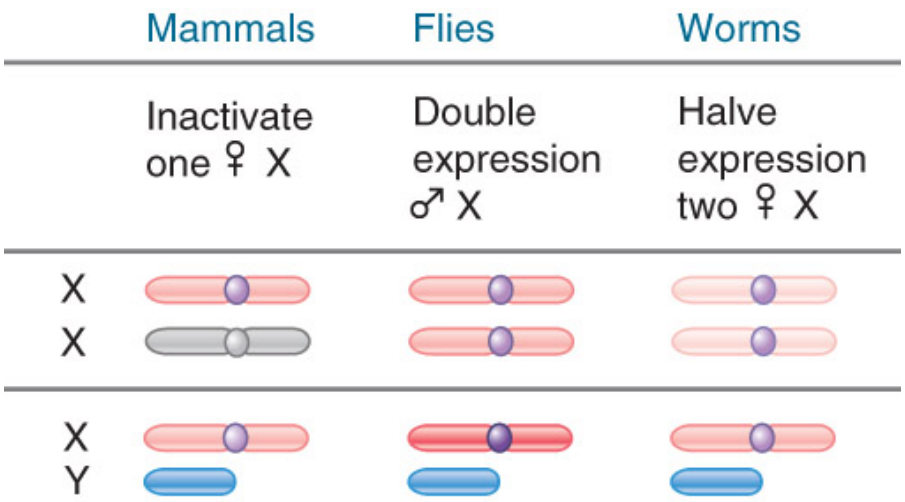


The Pc-G complex proteins maintain repression

Polycomb-group proteins are a family of protein complexes, first discovered in fruit flies, that can remodel chromatin such that "epigenetic silencing" of genes takes place. Thes Polycomb-group proteins are well known for silencing Hox genes through modulation of chromatin structure during embryonic development in fruit flies (*Drosophila melanogaster*).

X chromosome inactivation is an example of facultative heterochromatin.

- **dosage compensation –** Mechanisms employed to compensate for the discrepancy between the presence of two X chromosomes in one sex but only one X chromosome in the other sex.



Dosage compensation change X-expression

Mammals – one of the two female X chromosomes is inactivated

Drosophila –expression of single male X chromosome is doubled relative to the female X chromosome expression

C. elegans – expression of each female chromosome is halved relative to expression of single X in males.

X chromosome inactivation is an example of facultative heterochromatin.

- **dosage compensation –** Mechanisms employed to compensate for the discrepancy between the presence of two X chromosomes in one sex but only one X chromosome in the other sex.

X Chromosomes Undergo Global Changes

- **constitutive heterochromatin –** The inert state of permanently non-expressed sequences, such as satellite DNA.

- **facultative heterochromatin –** The inert state of sequences that also exist in active copies; for example, one mammalian X chromosome in females.

X Chromosomes Undergo Global Changes

- One of the two X chromosomes is inactivated at random in each cell during embryogenesis of eutherian mammals.

- **single X hypothesis –** The theory that describes the inactivation of one X chromosome in female mammals.

- In exceptional cases where there are >2 X chromosomes, all but one are inactivated (the **n–1 rule**).

XIST “Exists” to Silence

XIST, or **X-inactive specific transcript**, was discovered due to its specific expression from inactive female X chromosomes. This RNA has four unique properties (Borsani et al., 1991; Brockdorff et al., 1991, 1992; Brown et al., 1991, 1992; Clemson et al., 1996):

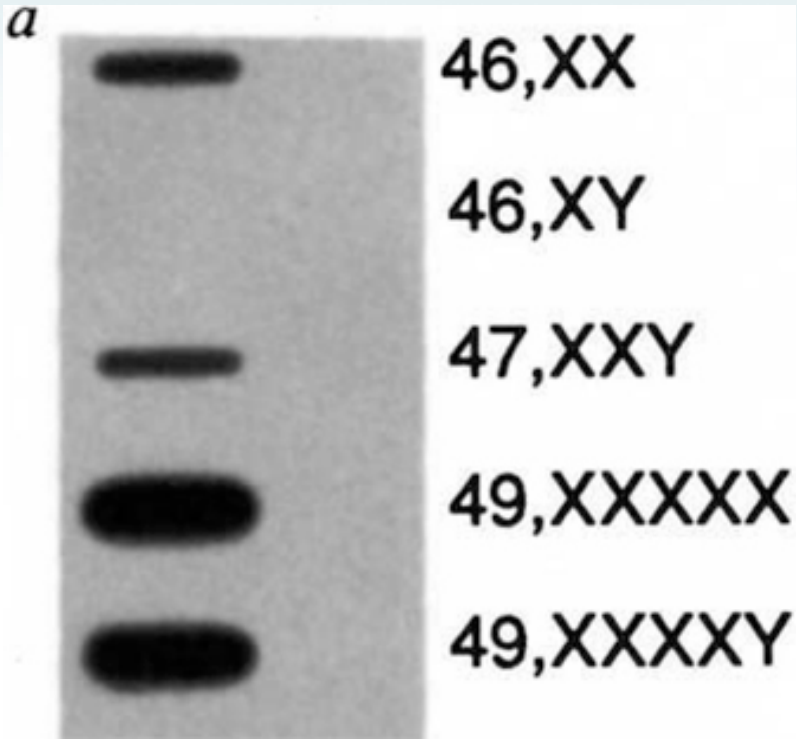
1.The **XIST** gene does not encode a protein but rather produces a **17 kilobase (kb) functional RNA molecule**. Hence, it is a noncoding RNA (Costa, 2008).

•XIST RNA is only expressed in cells containing at least two Xs and is not normally expressed in male cells **(Figure 2).** Higher XIST expression can be seen in cells with more X chromosomes, as a counting mechanism dictates that only one X per cell can remain active. In such cells, XIST is expressed from all supernumerary Xs.

•XIST RNA remains exclusively in the nucleus and is able to "coat" the chromosome from which it was produced

•Paradoxically, XIST RNA is expressed from an otherwise inactive X chromosome.

X Chromosomes Undergo Global Changes



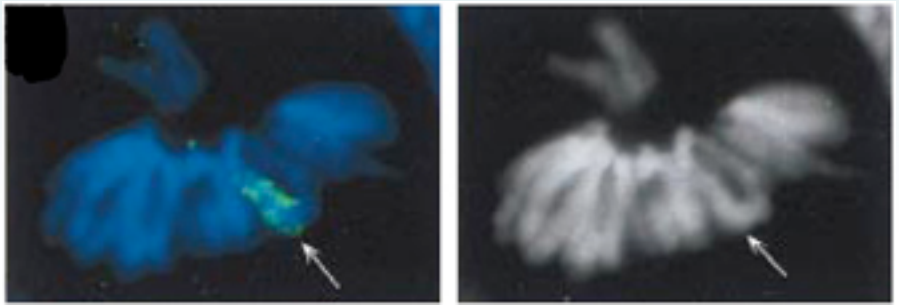


Figure 3: RNA fluorescent in situ hybridization on metaphase chromosomes showing that Xist (green) coats the X chromosome (arrow). DNA stained in blue.

Figure 2: Expression of the XIST gene in males, females and somatic cell hybrids.
Slot blot of total cellular RNA isolated from human lymphoblastoid cell lines or mouse-human somatic cell hybrids retaining either the active or inactive human X chromosome, hybridized with the 14A XIST cDNA proble. The probe hybridizes only to RNA samples from cell lines which contain an inactive X chromosome.

XIST “Exists” to Silence

XIST, or **X-inactive specific transcript**, was discovered due to its specific expression from inactive female X chromosomes. This RNA has four unique properties (Borsani et al., 1991; Brockdorff et al., 1991, 1992; Brown et al., 1991, 1992; Clemson et al., 1996):

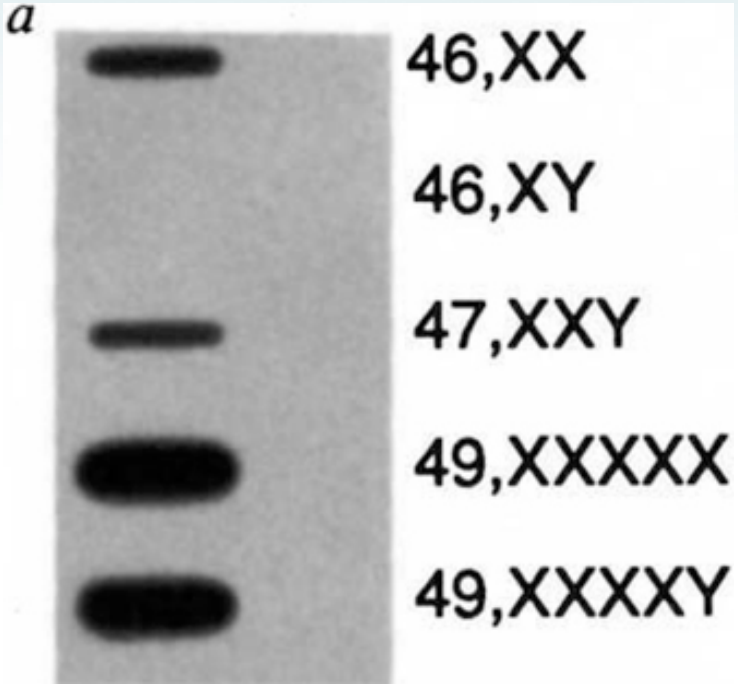
1.The XIST gene does not encode a protein but rather produces a 17 kilobase (kb) functional RNA molecule. Hence, it is a noncoding RNA (Costa, 2008).

•XIST RNA is only expressed in cells containing at least two Xs and is not normally expressed in male cells **(Figure 2).** Higher XIST expression can be seen in cells with more X chromosomes, as a counting mechanism dictates that only one X per cell can remain active. In such cells, XIST is expressed from all supernumerary Xs.

•XIST RNA remains exclusively in the nucleus and is able to "coat" the chromosome from which it was produced

•Paradoxically, **XIST RNA** is expressed from an otherwise effectively **inactive X** chromosome

X Chromosomes Undergo Global Changes



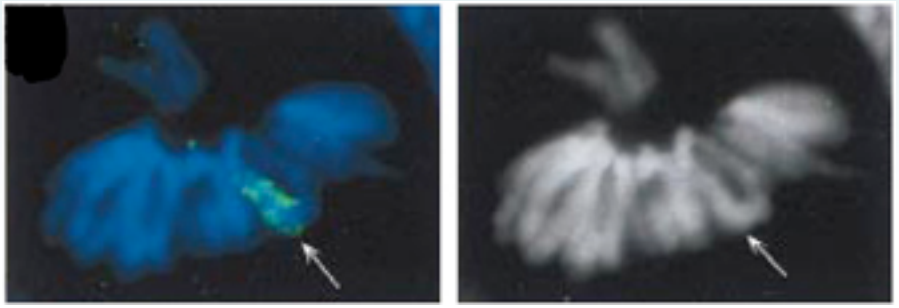


Figure 3: RNA fluorescent in situ hybridization on metaphase chromosomes showing that Xist (green) coats the X chromosome (arrow). DNA stained in blue.

Figure 2: Expression of the XIST gene in males, females and somatic cell hybrids.
Slot blot of total cellular RNA isolated from human lymphoblastoid cell lines or mouse-human somatic cell hybrids retaining either the active or inactive human X chromosome, hybridized with the 14A XIST cDNA proble. The probe hybridizes only to RNA samples from cell lines which contain an inactive X chromosome.

TSIX Antagonizes XIST

So, how is **XIST** expressed from one X chromosome while it remains silent on the other X? The answer to this question came just several years after the discovery of **XIST**, when **XIST**'s antisense partner— **TSIX** ("**XIST**" backwards)—was identified (Lee et al., 1999).

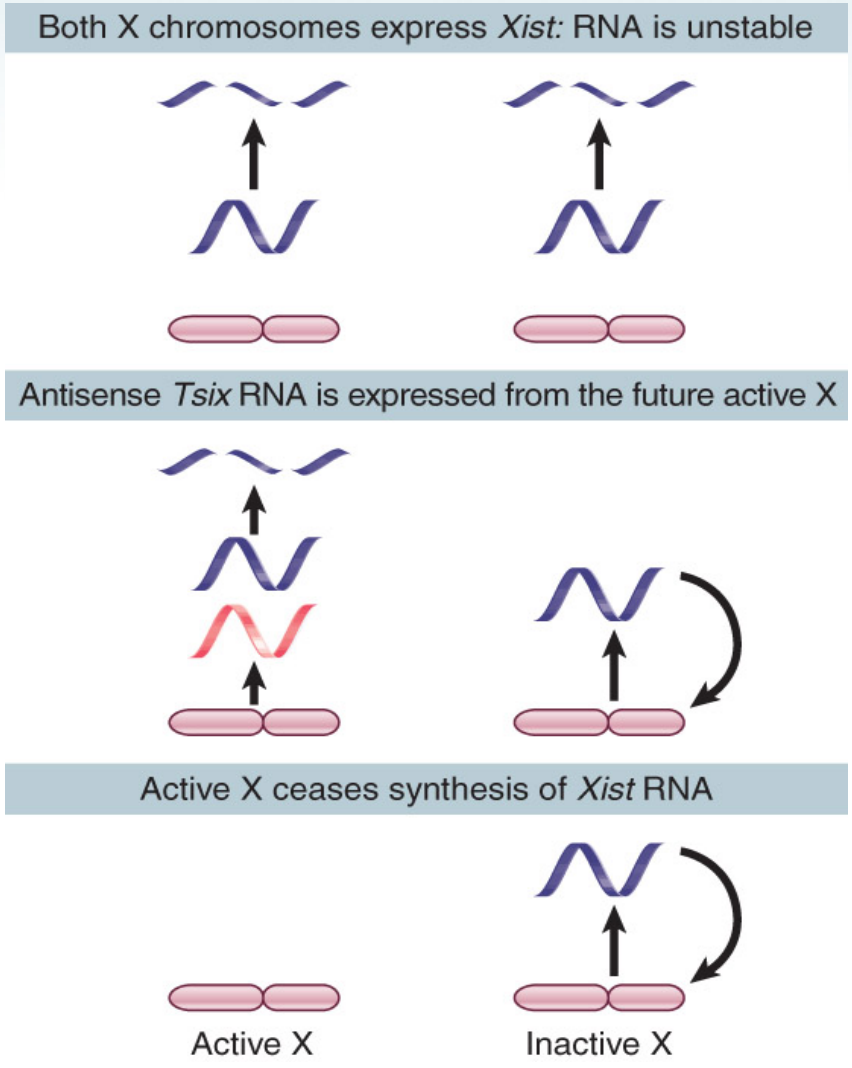
The term "antisense" refers to the fact that **TSIX** is complementary in sequence to **XIST**. TSIX is also a **long (40 kilobase), noncoding RNA (lncRNA)**, but it is transcribed in the opposite direction across the entire **XIST** gene. Like **XIST**, **TSIX** only acts on the chromosome that produces it.

Moreover, there is an inverse relationship between **TSIX** and **XIST** expression: When **TSIX** transcription is reduced on one X, **XIST** expression increases and leads to inactivation **of that same X chromosome** (Lee & Lu, 1999; Sado et al., 2001).

In contrast, overexpression of **TSIX** prevents any increases in **XIST** expression and blocks inactivation in cis or on that same X (Luikenhuis et al., 2001; Stavropoulos et al., 2001).

These observations suggest that expression of **TSIX** is required to antagonize **XIST** on the future active X.

X Chromosomes Undergo Global Changes



- The *Xic* (X inactivation center) is a *cis*-acting region on the X chromosome that is necessary and sufficient to ensure that only one X chromosome remains alive.
- *Xic* includes the *Xist* gene, which codes for an RNA that is found only on inactive X chromosomes.

Xist RNA inactivates one X chromosome

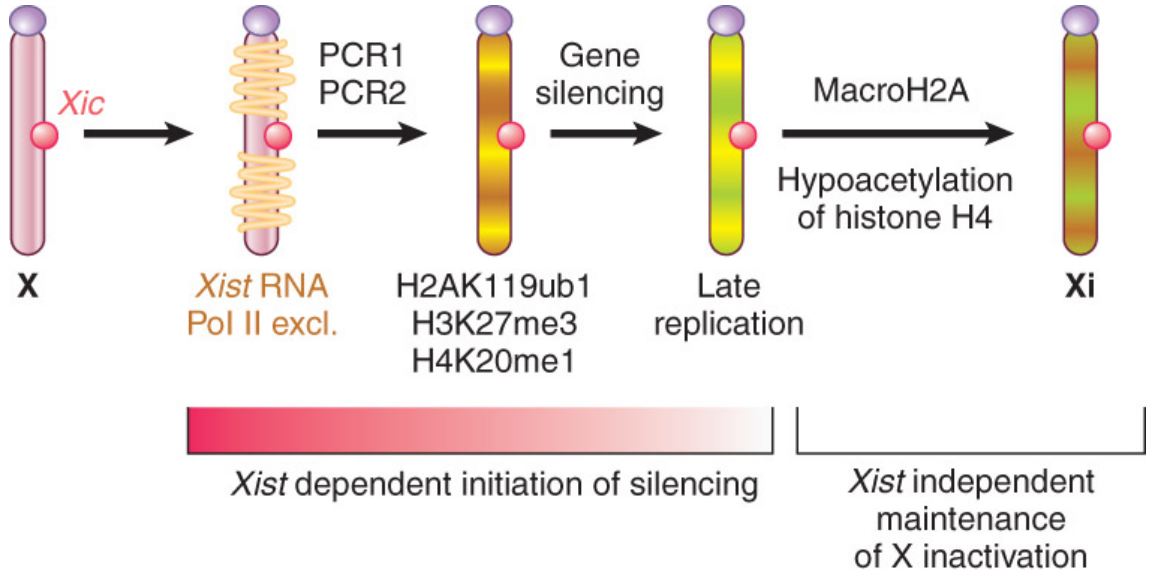
Furthermore. while it has been confirmed that **XIST** RNA is both necessary and sufficient for inactivation (Penny et al., 1996; Wutz & Jaenisch, 2000).....

It also recruits various silencing protein complexes to label the future inactive X chromosome.

Increased **XIST** expression represents a key initiation event in X inactivation, indicating the central role of this noncoding RNA.

In addition to silencing one of the two Xs, the cell must also make sure that the **other X remains active.** Thus, there must be a way for the two Xs to communicate with each other to designate mutually exclusive fates. Interestingly, recent evidence suggests that this communication is mediated by protein- and transcription-dependent pairing between the Xs during early development (Bacher et al., 2006; Xu et al., 2006, 2007). The random XCI story becomes even more complex with the discovery of various enhancers and modifiers that can alter or skew inactivation of one X chromosome over the other.

- *Xist* recruits Polycomb complexes, which modify histones on the inactive X.
- The mechanism that is responsible for preventing *Xist* RNA from accumulating on the active chromosome is unknown.



Silencing of one X chromosome by Xist.

Adapted from A. Wutz and J. Gribnau, Curr. Opin. Genet. Dev. 17 (2007): 387-393.

Furthermore. while it has been confirmed that XIST RNA is both necessary and sufficient for inactivation (Penny et al., 1996; Wutz & Jaenisch, 2000).....

It also recruits various silencing protein complexes to label the future inactive X chromosome.

Increased **XIST** expression represents a key initiation event in X inactivation, indicating the central role of this noncoding RNA.

In addition to silencing one of the two Xs, the cell must also make sure that the **other X remains active.** Thus, there must be a way for the two Xs to communicate with each other to designate mutually exclusive fates. Interestingly, recent evidence suggests that this communication is mediated by protein- and transcription-dependent pairing between the Xs during early development (Bacher et al., 2006; Xu et al., 2006, 2007).

The random XCI story becomes even more complex with the discovery of various enhancers and modifiers that can alter or skew inactivation of one X chromosome over the other.

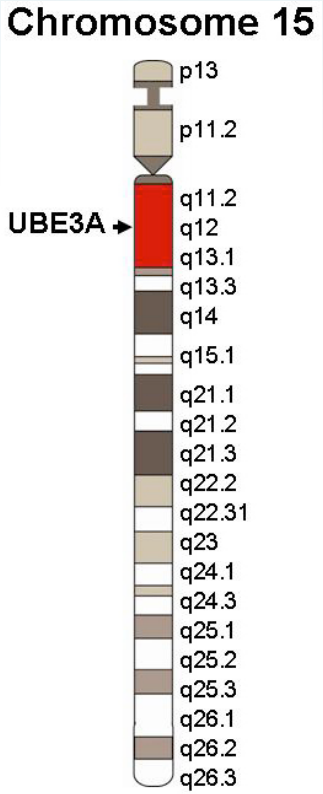
Epigenetics

- In the early 1980's Marcus Pembrey (then, a geneticist at University College London in the U.K.) and his colleagues (who studied the unusually detailed historical medical records of the isolated northern Swedish city of Overkalix) found that grandsons of men who experienced famine during mid-childhood went through puberty earlier and had longer lifespans, while the grandsons of men who were well fed in early childhood had an increased likelihood of diabetes.

- For females, the effect was similar but it was tied to the grandmother, rather than the grandfather.

- More contemporaneously, Pembrey has found that fathers who had started smoking before age 11 had sons who were significantly fatter than average. There was no similar effect on daughters

- **Angelman syndrome** results from a loss of gene activity in a specific part of chromosome 15 (the 15q11-q13 region).



  - This region on chromosome 15 contains a gene called **UBE3A** that, when mutated or absent, likely causes the characteristic features of this condition.
    Humans normally have two copies of the **UBE3A** gene, one from each parent.
  - Both copies of this gene are active in many of the body's tissues.
  - In the brain, however, only the the **maternal copy** is active. If the maternal copy is lost because of a chromosomal change or a gene mutation, a person will have no working copies of the **UBE3A** gene in the brain.

- **Prader-Willi syndrome** is caused by the loss of active genes in a specific part of chromosome 15, the 15q11-q13 region. People normally have two copies of this chromosome in each cell, one copy from each parent.
  - Prader-Willi syndrome occurs when the paternal copy is partly or entirely missing

Epigenetics

- Pembrey (a geneticist at University College London in the U.K.) and his colleagues (who studied the unusually detailed historical medical records of the isolated northern Swedish city of Overkalix) found that grandsons of men who experienced famine during mid-childhood went through puberty earlier and had longer lifespans, while the grandsons of men who were well fed in early childhood had an increased likelihood of diabetes.

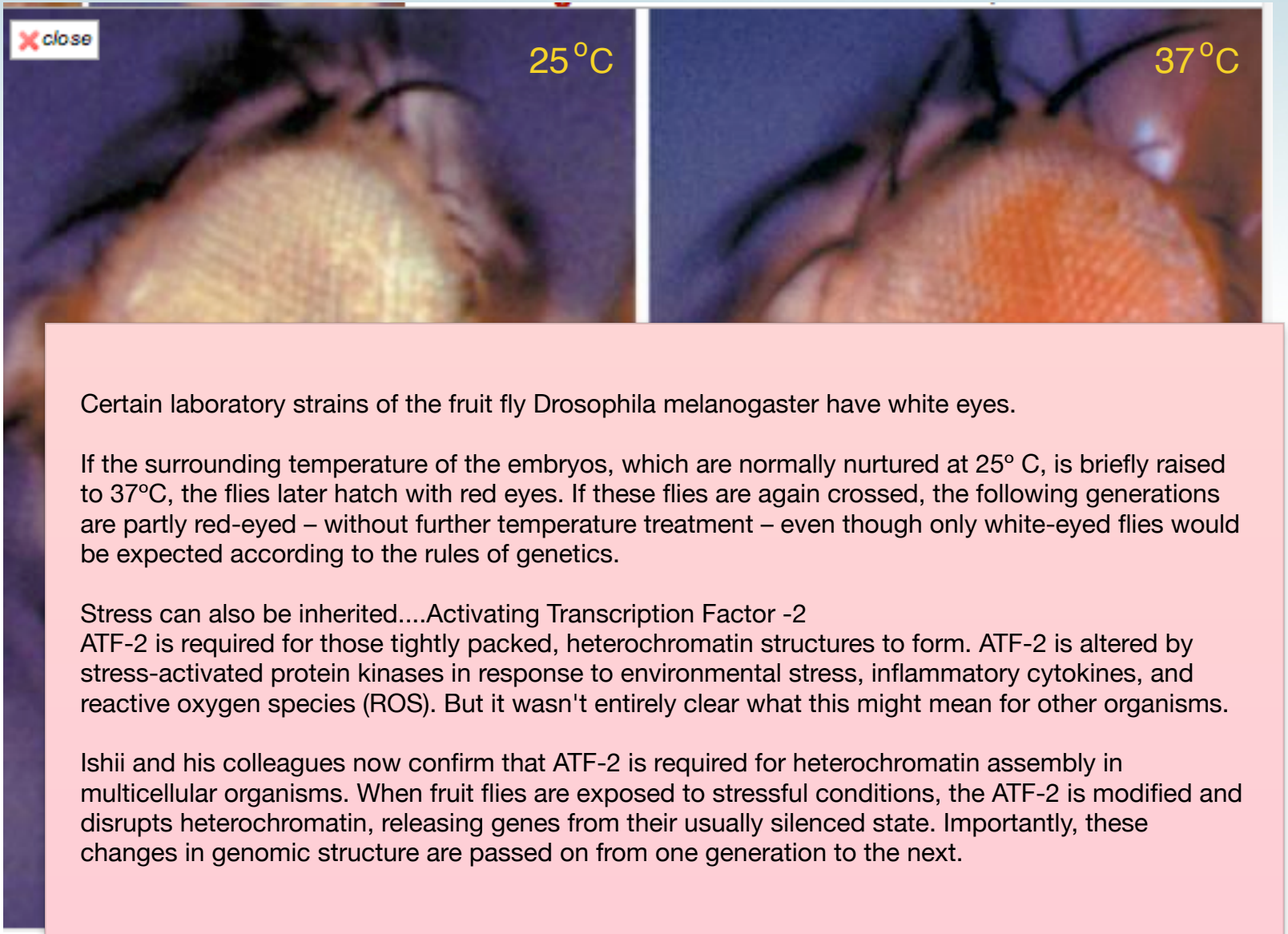
- For females, the effect was similar but it was tied to the grandmother, rather than the grandfather.

- More contemporaneously, Pembrey has found that fathers who had started smoking before age 11 (on average) had sons who were significantly more obese than average. There was no similar effect on daughters.

Epigenetic Effects Can Be Inherited

- Epigenetic effects can result from the modification of a nucleic acid **after** it has been synthesized **<u>or</u>** by the perpetuation of protein structures.

- Epigenetic effects may be inherited through generations (**transgenerational epigenetics**).



Certain laboratory strains of the fruit fly Drosophila melanogaster have white eyes.

If the surrounding temperature of the embryos, which are normally nurtured at 25° C, is briefly raised to 37°C, the flies later hatch with red eyes. If these flies are again crossed, the following generations are partly red-eyed – without further temperature treatment – even though only white-eyed flies would be expected according to the rules of genetics.

Stress can also be inherited....Activating Transcription Factor -2
ATF-2 is required for those tightly packed, heterochromatin structures to form. ATF-2 is altered by stress-activated protein kinases in response to environmental stress, inflammatory cytokines, and reactive oxygen species (ROS). But it wasn't entirely clear what this might mean for other organisms.

Ishii and his colleagues now confirm that ATF-2 is required for heterochromatin assembly in multicellular organisms. When fruit flies are exposed to stressful conditions, the ATF-2 is modified and disrupts heterochromatin, releasing genes from their usually silenced state. Importantly, these changes in genomic structure are passed on from one generation to the next.

- *Th*

determined by epigenetic factors. (Credit: Renato Paro/ETH Zürich)

Epigenetic effects of maternal diet...

ScienceDaily (Mar. 9, 2011) — Poor diet during pregnancy increases offspring's vulnerability to the effects of aging, new research has shown for the first time.

To test their theory, the researchers used a well-established rat model where, by altering the protein content of the mother's diet during pregnancy, the offspring develop type 2 diabetes in old age.

First, they studied the RNA from insulin secreting cells in the pancreas from offspring of normally fed as well as malnourished mothers in young adult life and in old age. When they compared the two, they found that there was a significant decrease in the expression of the **Hnf4a gene** in the offspring prone to type 2 diabetes. The expression of Hnf4a also decreased with age in both groups.

Second, they studied the DNA and found that the decrease of Hnf4a was caused by epigenetic changes..... .

Caring Mothers Reduce Response to Stress for Life

How a rat responds to stress depends on whether its mother cared for it properly as a pup, which marks its genes for life. Dr. Mae-Wan Ho *reports*

Maternal effects in the spotlight

Maternal effects on the development of offspring are well known. But they are thought to be due to nutritional and physiological factors affecting the foetus in the womb; and within the past few years, geneticists have discovered that diet and stress can profoundly change the pattern of gene expression in the offspring, affecting their health prospects as adults (see Diet trumping genes, *SiS* 20).

A team of researchers from the Douglas Hospital Research Centre and McGill University in Montreal Canada, and the Molecular Medicine Centre, in Edinburgh University Western General Hospital in the UK, now report a remarkable experiment in which the behaviour of the mother nursing her pups not only affects the pups' response to stress as adults, but are correlated with changes in gene expression states in brain cells that persist into adult life. Such changes are referred to as '**epigenetic**' as they do not involve alterations in the base sequence of DNA in the genome, only their off and on states; but they can persist in the brain cells and are passed on to all the daughter cells.

Caring mothers reduce stress response of pups

In the nest, the mother rat licks and grooms her pups and, while nursing, arches her back to groom and lick her pups. Some mothers (high performers) tend to do these acts more frequently than others (low performers). As adults, the offspring of high performers are less fearful and show more modest responses to stress in the hypothalamus-pituitary-adrenal (HPA) neuro-endocrine pathway.

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
- Chromatin Marking
- RNA-mediated EIS

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops -protein products (or modifications thereof) or mRNA
  - Bistability of the *lac* operon
  - *Candida albicans*, where an epigenetic switch underlies the transition between white and opaque cells—two states that are heritable for many generations
- 3 D templating -Structural Inheritance
- Chromatin Marking
- RNA-mediated EIS

Epigenetic properties of white–opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop

Rebecca E. Zordan, David J. Galgoczy, and Alexander D. Johnson

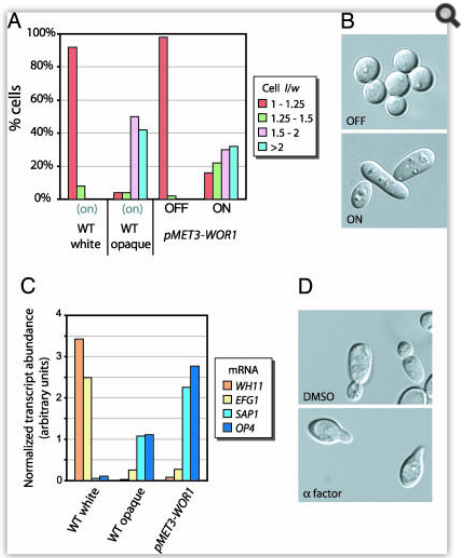
Additional article information

ABSTRACT

White–opaque switching in the human fungal pathogen *Candida albicans* is an alternation between two distinct types of cells, white and opaque. White and opaque cells differ in their appearance under the microscope, the genes they express, their mating behaviors, and the host tissues for which they are best suited. Each state is heritable for many generations, and switching between states occurs stochastically, at low frequency. In this article, we identify a master regulator of white–opaque switching (Wor1), and we show that this protein is a transcriptional regulator that is needed to both establish and maintain the opaque state. We show that in opaque cells, Wor1 forms a positive feedback loop: It binds its own DNA regulatory region and activates its own transcription leading to the accumulation of high levels of Wor1. We further show that this feedback loop is self-sustaining: Once activated, it persists for many generations. We propose that this Wor1 feedback loop accounts, at least in part, for the heritability of the opaque state. In contrast, white cells (and their descendents) lack appreciable levels of Wor1, and the feedback loop remains inactive. Thus, this simple model can account for both the heritability of the white and opaque states and the stochastic nature of the switching between them.

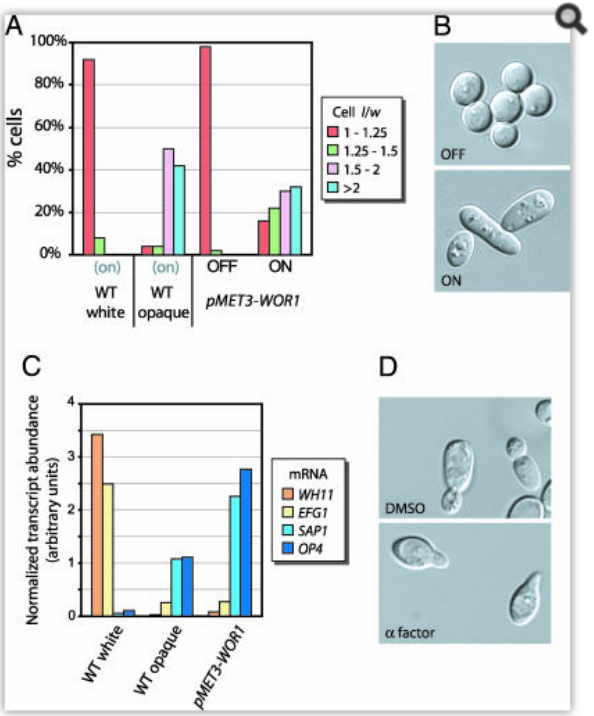
Keywords: phenotypic switching, transcriptional regulation, WOR1

Fig. 2.



Ectopic expression of *WOR1* in white cells drives the cells to the opaque phase. (*A* and *B*) Ectopic expression of WOR1 causes white cells to resemble opaque cells in appearance. Cell dimensions were measured in differential interference contrast images (*B*), and populations of cells were compared based on the distribution of length/width ratios for 50 cells per strain for each condition (*A*). (*C*) Ectopic expression of WOR1 in white cells causes them to express genes characteristic of opaque cells. Quantitative RT-PCR was used to monitor transcription of the white-specific genes *WH11* and *EFG1* and the opaque-specific genes SAP1 and OP4. All values were normalized to *PAT1*, a transcript that is not regulated by white–opaque switching. (*D*) Ectopic expression of WOR1 in white cells renders them sensitive to the mating pheromone α-factor. This specialized property of true opaque cells is visualized by the formation of mating projections on the ends of the cells (14). Cells were treated with α-factor (10 μg/ml in DMSO) or an equivalent amount of DMSO

Fig. 2.



Ectopic expression of *WOR1* in white cells drives the cells to the opaque phase. (*A* and *B*) Ectopic expression of WOR1 causes white cells to resemble opaque cells in appearance. Cell dimensions were measured in differential interference contrast images (*B*), and populations of cells were compared based on the distribution of length/width ratios for 50 cells per strain for each condition (*A*). (*C*) Ectopic expression of WOR1 in white cells causes them to express genes characteristic of opaque cells. Quantitative RT-PCR was used to monitor transcription of the white-specific genes *WH11* and *EFG1* and the opaque-specific genes SAP1 and OP4. All values were normalized to *PAT1*, a transcript that is not regulated by white–opaque switching. (*D*) Ectopic expression of WOR1 in white cells renders them sensitive to the mating pheromone α-factor. This specialized property of true opaque cells is visualized by the formation of mating projections on the ends of the cells (14). Cells were treated with α-factor (10 μg/ml in DMSO) or an equivalent amount of DMSO

Epigenetic properties of white–opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop

Rebecca E. Zordan, David J. Galgoczy, and Alexander D. Johnson

Additional article information

ABSTRACT

White–opaque switching in the human fungal pathogen *Candida albicans* is an alternation between two distinct types of cells, white and opaque. White and opaque cells differ in their appearance under the microscope, the genes they express, their mating behaviors, and the host tissues for which they are best suited. Each state is heritable for many generations, and switching between states occurs stochastically, at low frequency. In this article, we identify a master regulator of white–opaque switching (Wor1), and we show that this protein is a transcriptional regulator that is needed to both establish and maintain the opaque state. We show that in opaque cells, Wor1 forms a positive feedback loop: It binds its own DNA regulatory region and activates its own transcription leading to the accumulation of high levels of Wor1. We further show that this feedback loop is self-sustaining: Once activated, it persists for many generations. We propose that this Wor1 feedback loop accounts, at least in part, for the heritability of the opaque state. In contrast, white cells (and their descendents) lack appreciable levels of Wor1, and the feedback loop remains inactive. Thus, this simple model can account for both the heritability of the white and opaque states and the stochastic nature of the switching between them.

Keywords: phenotypic switching, transcriptional regulation, WOR1

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
  - Inheritance of alternative three-dimensional (3-D) structures through spatial templating: **Prions**
- Chromatin Marking
- RNA-mediated EIS

- **Prion –** A proteinaceous infectious agent that behaves as an inheritable trait, although it contains no nucleic acid.
  - Examples are:
  - **Bovine spongiform encephalopathy** (**BSE**), commonly known as **"mad cow disease"**, is a fatal, neurodegenerative disease in cattle that causes a spongy degeneration in parts of the brain and spinal cord.
  - **Sup35** in Yeast
  - **PrPSc**, the agent of *scrapie* in sheep and *bovine spongiform encephalopathy* in cattle, and **Psi**, which confers an inherited state in yeast.

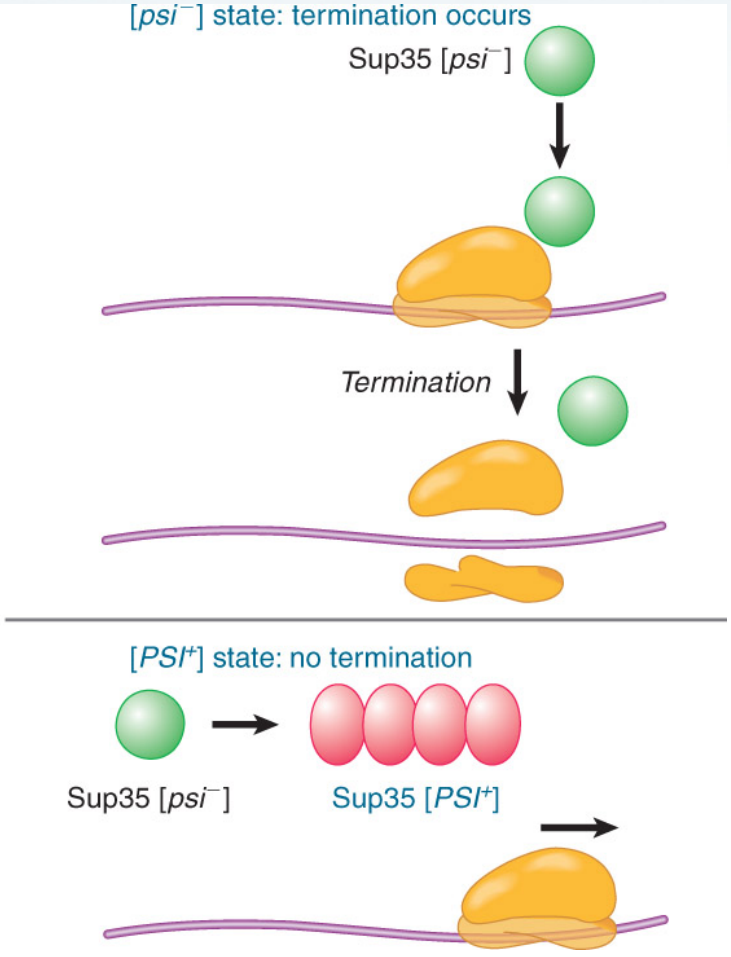
Yeast Prions Show Unusual Inheritance

- **amyloid fibers –** Insoluble fibrous protein polymers with a cross β-sheet structure, generated by prions or other dysfunctional protein aggregations (such as in Alzheimer's).

- Conversion between the two forms is influenced by chaperones.

- The wild-type form has the recessive genetic state *psi*$^{-}$ and the mutant form has the dominant genetic state *PSI*$^{+}$.

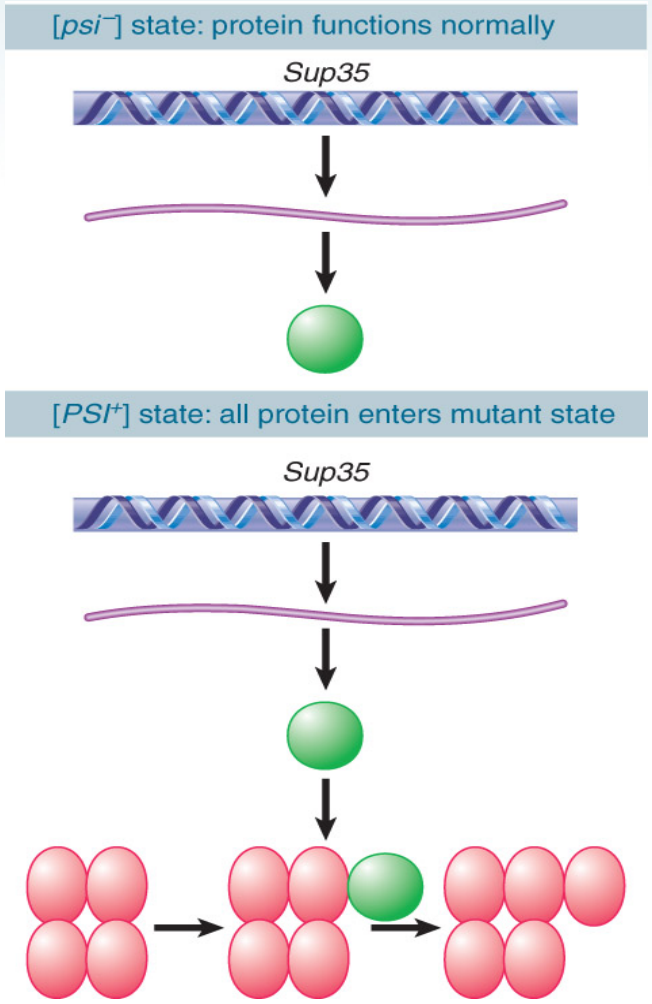
Yeast Prions Show Unusual Inheritance



- The **Sup35** protein in its wild-type soluble form is a termination factor for translation.
- Sup35 can also exist in an alternative form of oligomeric aggregates, in which it is not active in protein synthesis.

The [PSI+] state is due to protein aggregation as a function of the history of the strain

Yeast Prions Show Unusual Inheritance



- The presence of the oligomeric form causes newly synthesized protein to acquire the inactive structure.

The [PSI+] state is dominant

Prions Cause Diseases in Mammals

- Scrapie (in sheep) is a neurodegenerative disorder that is manifested by the inability of the sheep to remain upright.
- The protein responsible for scrapie exists in two forms and is normally expressed in the brain:
- the wild-type, non-infectious form **PrP^{C}** is susceptible to proteases
- the disease-causing form **PrP^{Sc}** is **resistant** to proteases
- Difference in the two forms appears to lie with a **change in conformation** rather than conventional alterations (i.e. methylation, acetylation, sequence mutation)

Prions Cause Diseases in Mammals

- The PrP^{Sc} protein can perpetuate itself by causing the newly synthesized PrP protein to take up the PrP^{Sc} form instead of the PrP^{C} form

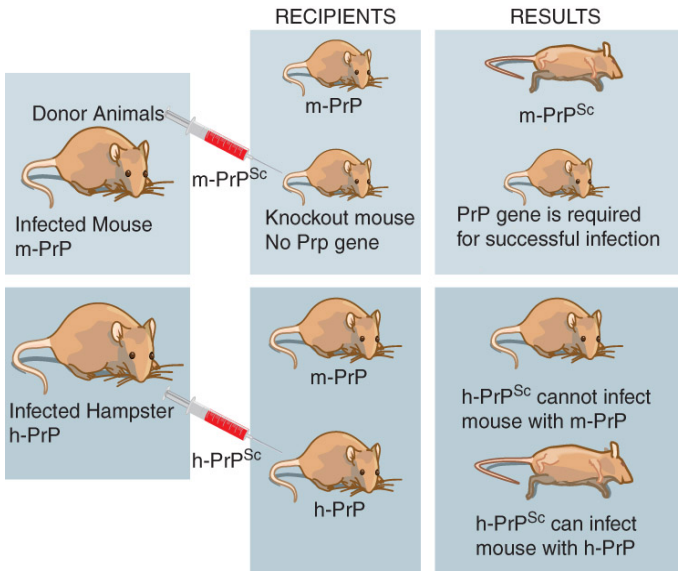
- Different prions may interact, leading to the formation of many different transmissible (cell- heritable and infectious) phenotypes

- Indeed, multiple strains of PrP^{Sc} may have different conformations of the protein -giving rise to variable degrees of severity of the phenotype.

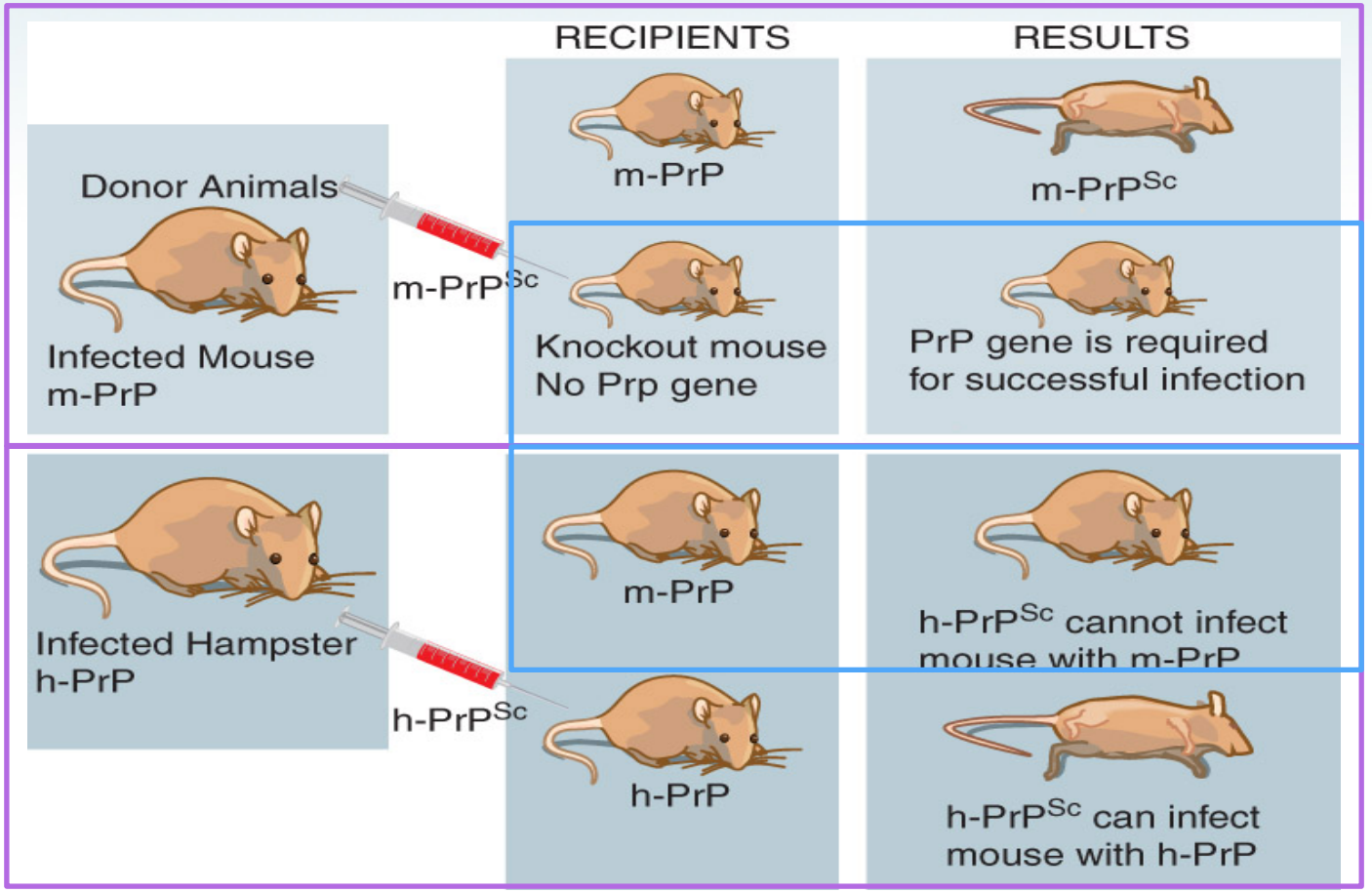
Prions Cause Diseases in Mammals

- The neurological disease, scapie can be transmitted to mice by injecting the purified PrP^{Sc} protein into mice.
- The recipient mouse must already have a copy of the ***PrP*** gene, coding for the mouse protein, present.

PrP interactivity is not always restricted to species



Prions Cause Diseases in Mammals



PrP interactivity is often restricted to species… but not always

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
- Chromatin Marking
  - Physical changes in DNA, histones and non-histonal proteins interacting with chromatin caused by chemical modification
  - Numerous examples.
- RNA-mediated EIS

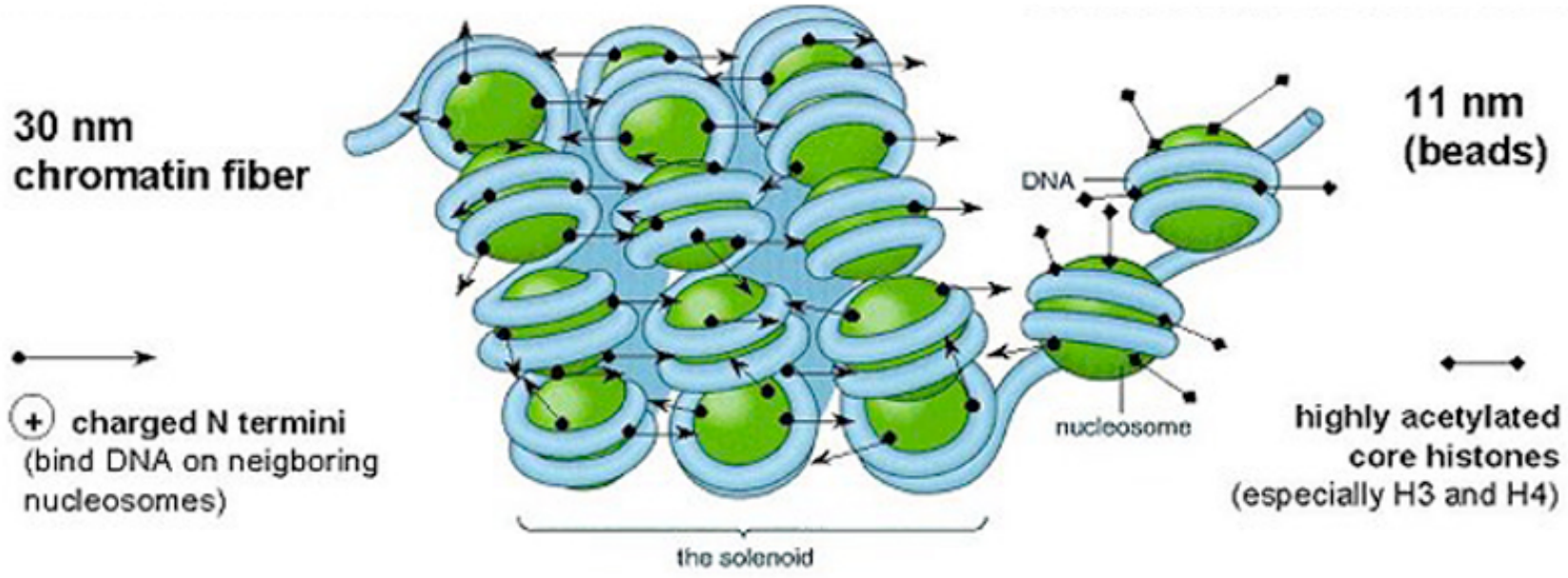
Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
- Chromatin Marking
- RNA-mediated EIS
  - RNA interference has been located in all eukaryote phyla.. with quite different mechanisms used by different cell types -lncRNA, siRNA, μRNA

Histone Acetylation Is Associated with Transcription Activation

- **Acetylation** is associated with activation of gene activity.
  - **Histone acetyltransferases (HATs)** vary in their target specificity.

- **Deacetylation** is associated with repression of gene activity.
  - **Histone deacetylase (HDACs) –** Enzyme that removes acetyl groups from histones; may be associated with repressors of transcription.
  - Deacetylases are very often present in complexes with repressor activity.


30 nm
chromatin fiber
11 nm
(beads)
DNA
nucleosome
+ charged N termini
(bind DNA on neigboring
nucleosomes)
highly acetylated
core histones
(especially H3 and H4)
the solenoid

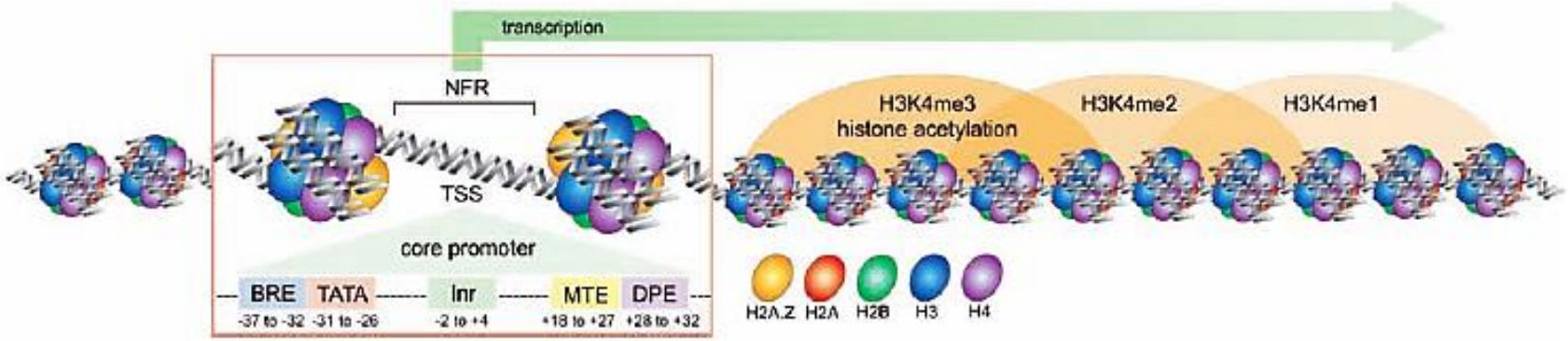
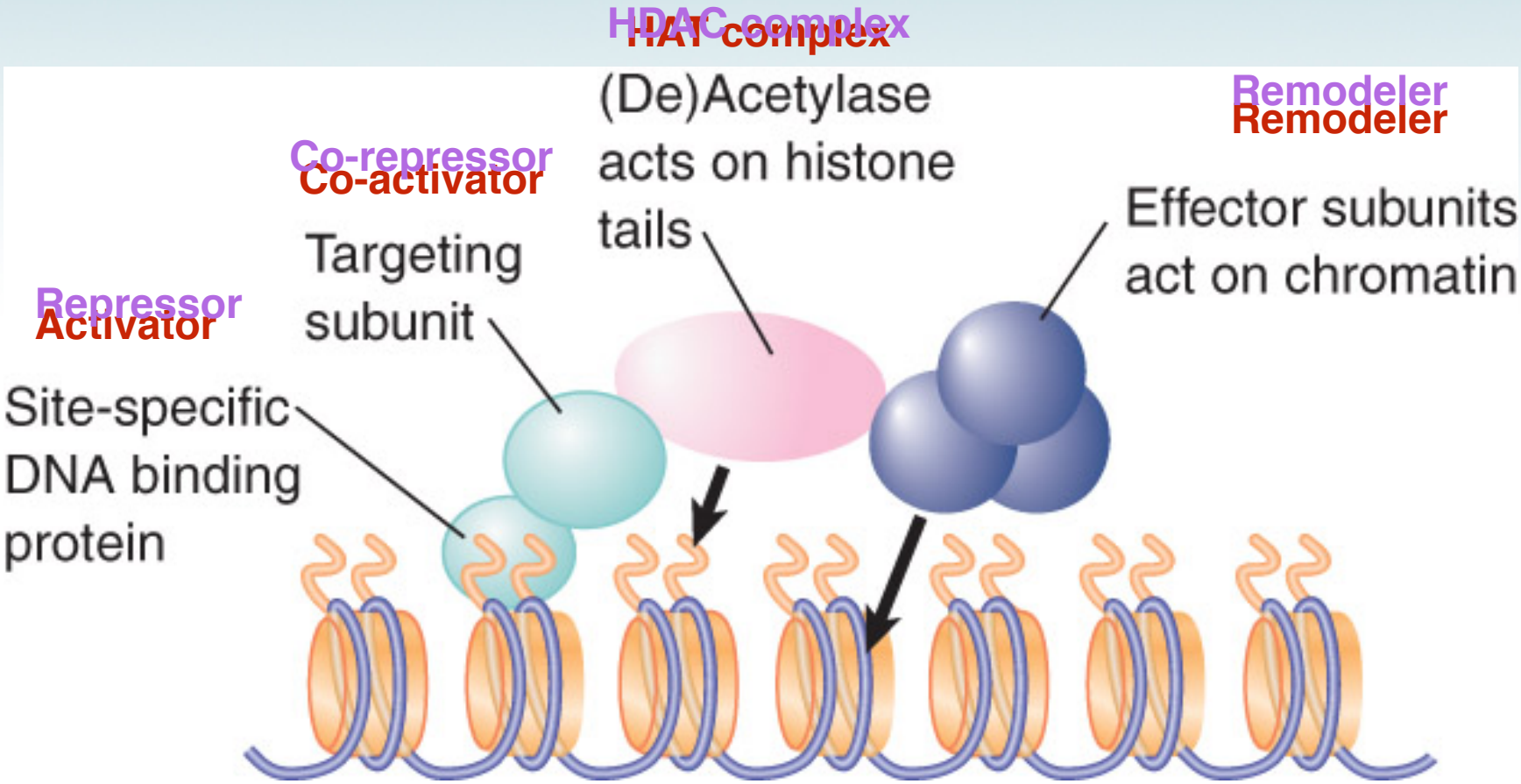


Figure 3. Signatures of active promoters. A nucleosome free region (NFR) surrounds the transcriptional start site (TSS) in the core promoter, which may contain core promoter elements, including BRE, TATA, Inr, MTE, DPE and others (positions are relative to the +1 TSS within the Inr; please see detailed explanation of these elements in the main text and in Table 1). The nucleosomes flanking the NFR contain the histone variant H2A.Z, while other nucleosomes contain normal H2A and other histone proteins that are subject to various modifications. Histone acetylation peaks just downstream of the promoter, while methylation of histone 3 lysine 4 is present in a gradient, from trimethylation (H3K4me3) at the promoter, to di- and then monomethylation (H3K4me2, H3K4me1) with increasing distance from the promoter into the transcribed region. This diagram is a composite of features determined in yeast, fly and mammalian systems; it is representative of some important characteristics of promoters identified in large-scale studies.

Nucleosomes are removed from the region around the start site but alternative histone components (H2AZ) are inserted into the “gate keeper” nucleosomes on each side of an open start site



Modifying complexes have several components

Curr Top Microbiol Immunol. 2003;274:237-68.

N-CoR-HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors.

Jones PL[1], Shi YB.

Author information

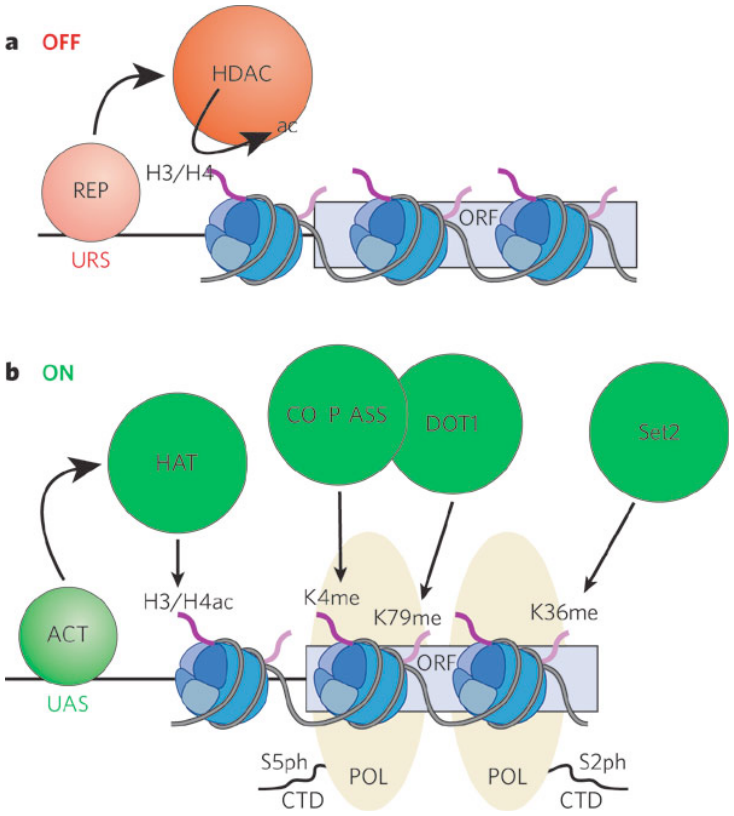
1 Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, B107 CLSL, 601 S. Goodwin Ave, Urbana, IL 61801, USA. pljones@life.uiuc.edu

Abstract

Many nuclear hormone receptors (NHRs) actively repress the expression of their primary response genes through the recruitment of transcriptional corepressor complexes to regulated promoters. N-CoR and the highly related SMRT were originally isolated and characterized by their ability to interact exclusivelywith the unliganded forms of NHRs and confer transcriptional repression. Recently, both the N-CoR and SMRT corepressors have been found to exist in vivo in multiple, distinct macromolecular complexes. While these corepressor complexes differ in overall composition, a general theme is that they contain histone deacetylase enzymatic activity. Several of these complexes contain additional transcriptional corepressor proteins with functional ties to chromatin structure. Together, these data suggest that modulation of chromatin structure plays a central role in N-CoR mediated transcriptional repression from unliganded NHRs.

PMID: 12596910 DOI: 10.1007/978-3-642-55747-7_9

[Indexed for MEDLINE]



- **a**, In the **OFF** state, the DNA-bound repressor (REP) at the upstream repressor site (URS) recruits negative modifiers, such as histone deacetylase (HDAC), which remove acetyl (ac) groups from histones.
- **b**, In the **ON** state, DNA-bound activator (ACT) at the upstream activator site (UAS) recruits positive modifiers, such as histone acetylases (HAT), at the promoter, while DNA-bound RNA polymerase (POL) recruits histone methylases at the ORF. Early during elongation, the C-terminal domain (CTD) polymerase repeat is phosphorylated at serine 5 (S5ph), leading to recruitment of the COMPASS complex (Set1, part of the COMPASS complex, methylates H3K4) and DOT1 (which methylates H3K79). Later in elongation the CTD repeat is phosphorylated at serine 2 (S2ph), leading to recruitment of Set2 (which methylates H3K36).

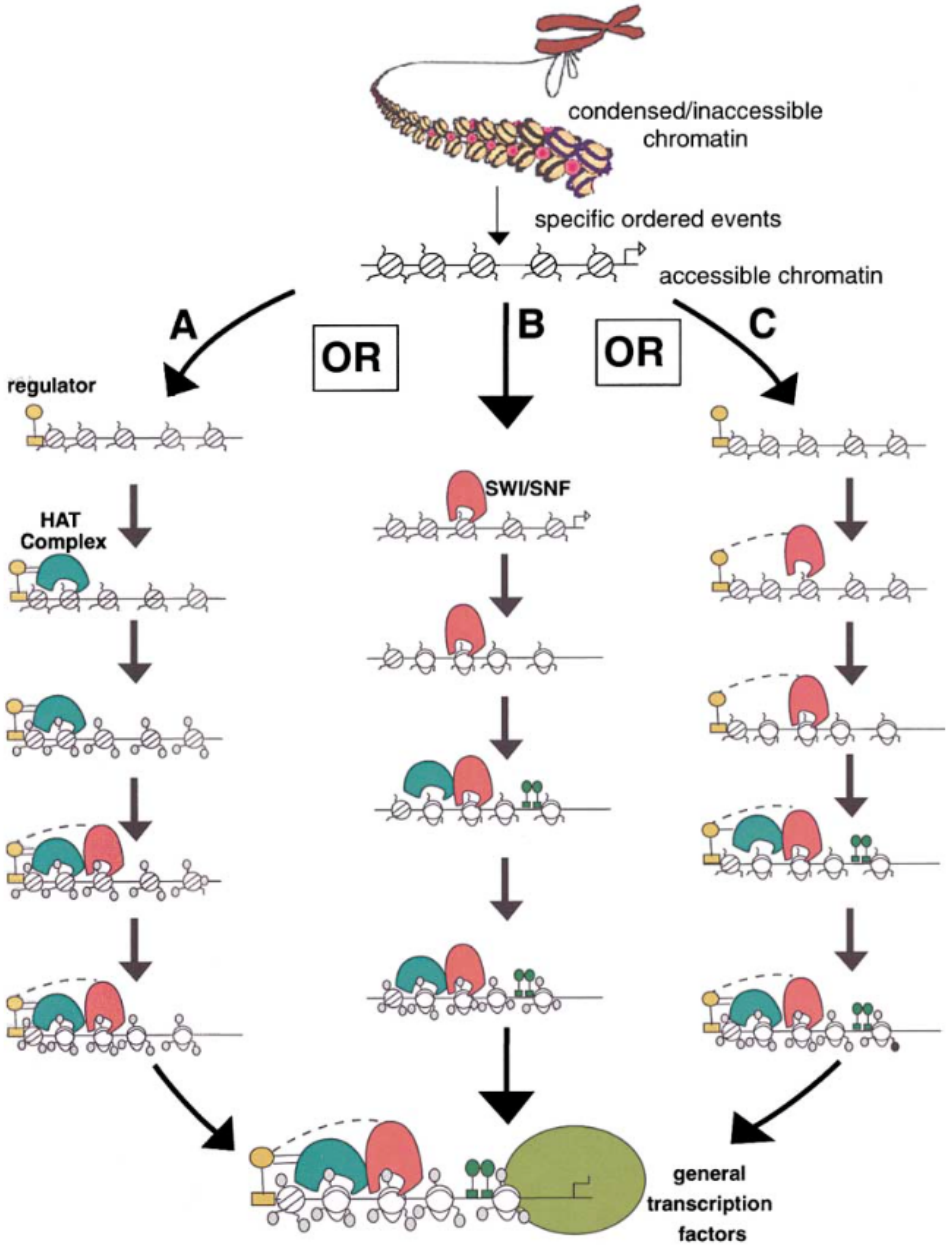


Figure 5. Models Depicting Different Orders of Action by Regulators and Chromatin-Remodeling Complexes

Regulators, HAT complexes, and ATP-dependent remodeling complexes can act in different orders (pathway A, B, or C) and still give the same end result: a template competent for transcription. Although not shown, it is also possible that binding by the general transcription factors precedes the action and recruitment of HAT complexes and ATP-dependent remodelers.

Narliker *et al.*, Cell (2002) **108:** 475-487

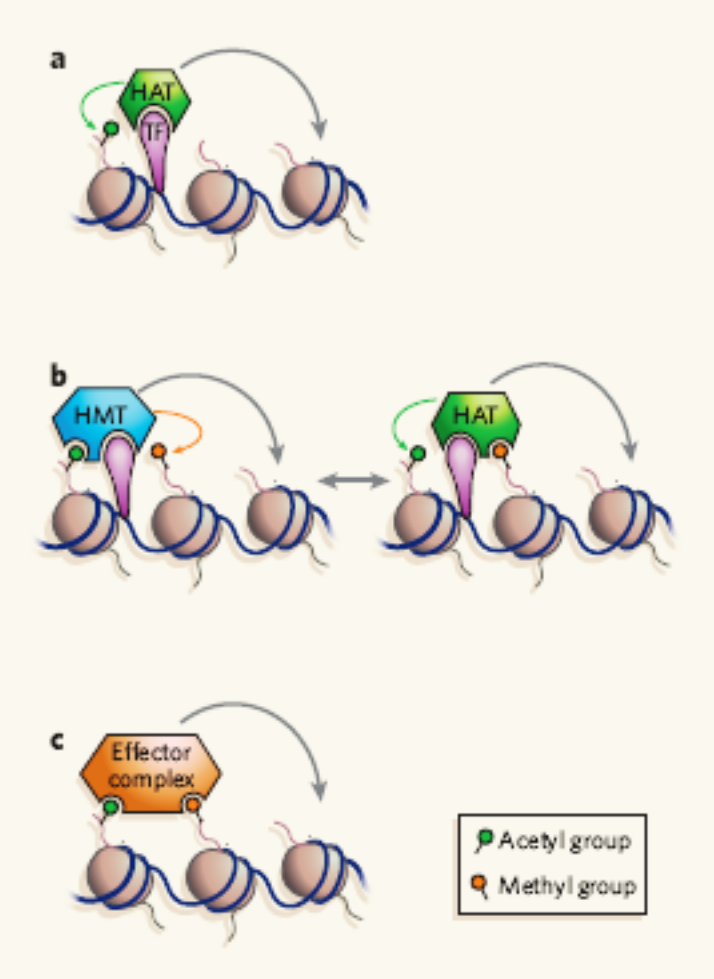


Figure 1 | Recruitment of chromatin regulators by nucleosomes. These hypothetical scenarios show nucleosomes as brown spheres around which the DNA (blue) is wound. **a**, A DNA-bound transcription factor protein (TF) may be the initial contact for chromatin regulators, in this case a histone acetyltransferase (HAT). The TF binds to specific DNA sequences so that the HAT complex is recruited to particular regions of the DNA. The HAT enzyme adds an acetyl group to the nearby histone tails and may have additional functions in chromatin regulation (black arrow). **b**, Regulators may also be targeted to particular DNA regions by a combinatorial interaction with a transcription factor and an acetylation mark. Here a histone methyltransferase complex (HMT) adds a methyl group to nearby histone tails, among other possible effects (black arrow). The alternating recruitment of HAT and HMT reinforces the complex histone modification pattern. **c**, A combination of histone modification marks, in the absence of a transcription factor, may suffice to stably recruit a regulatory protein complex to a regulatory site.

Becker, Nature, News & Views (2006) 443: 31

Binge Drinking Can Lead to Harmful Epigenetic Changes

THE EPIGENETICS OF ALCOHOLISM

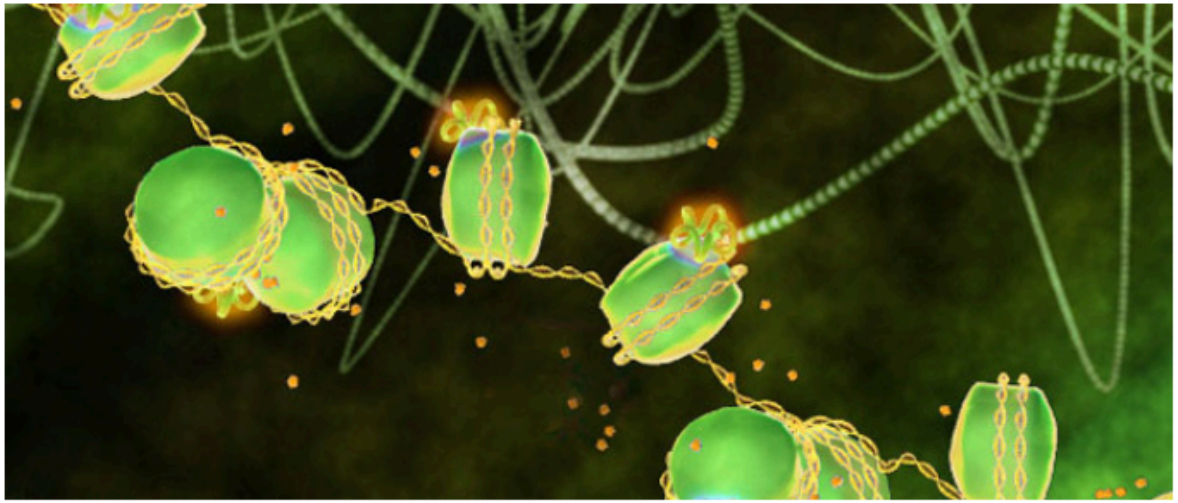
December 30, 2014 Bailey Kirkpatrick News & Reviews

The researchers fed rats either ethanol in a liquid diet or an ethanol-free isocaloric liquid diet. Then, they gave the rats either ethanol or water in three binge administrations 12 hours apart. Liver samples were analyzed for histone modifications and liver damage was assessed.

In their paper published in ***Hepatology International***, the team concluded that the profile of site-specific histone modifications were altered as a result of ethanol binge administration after chronic ethanol. Specifically, the researchers indicated that "chronic ethanol administration alone caused an increase in histone H3 ser10 and ser28 (H3S10 or S28) phosphorylation, and binge ethanol reduced their levels." They also found that "histone H3 lysine-9 acetylation (H3AcK9) was not increased after chronic ethanol", but histone H3 lysine-9 acetylation "increased significantly after acute binge and chronic ethanol binge." After chronic ethanol binge, they discovered that the mice had increased protein levels, specifically an increase of histone methyltransferase, GCN5, and a modest increase of histone deacetylase, HDAC3, in the nucleus.

Histone variant H2A.Z underlies inactivation of activity-dependent gene expression in learned behavior

August 23, 2016 Caitlin Aamodt For The Scientist



Work from David Sweatt's group was the first to implicate the histone variant H2A.Z in cognition. They found that in the rodent hippocampus dynamic transcriptional activation after contextual fear conditioning is mediated by H2A.Z eviction immediately downstream of the transcription start site (TSS)[2]. When H2A.Z levels were depleted via siRNA knockdown they observed an improvement in fear memory, suggesting that H2A.Z is a negative regulator of memory formation. New research from Azad Bonni's team recently published in the journal Science sheds further light on how this occurs [6]. Their findings suggest that H2A.Z is critical for switching off genes after they have been activated by cellular activity.

The Epigenetics Behind the Flu

November 17, 2015 Natalie Crowley Educationally Entertaining

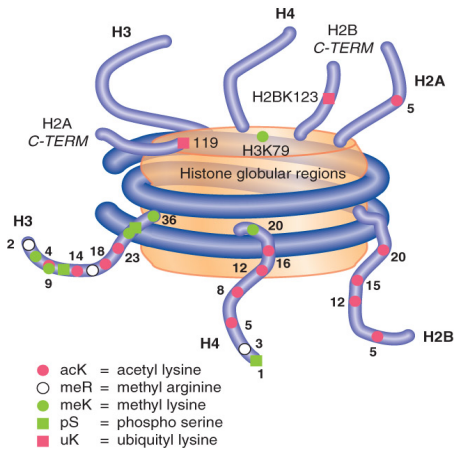
In a healthy person, the immune system is equipped to handle millions of bacteria, microbes, viruses, toxins, and parasites that invade the body — and it does this daily. But, the flu virus is craftier. Rather than overpowering the immune system, it succeeds by "tricking it". An epigenetic way it can do this is by producing a protein that mimics the tail of a histone. Researchers at The Rockefeller University found that the immunosuppressive NS1 protein of the influenza A virus (H3N2) contained the same sequence of amino acids as the tail domain of a DNA packaging protein in humans called histone H3. Histones play an important role in gene activation and the tails provide a scaffold for the assembly of protein complexes that control gene activity. NS1's ability to copy the histone H3 tail allows it to access the core of the gene regulatory machinery, target the PAF1 transcription elongation complex (hPAF1C), and block the antiviral gene function. [4] The NS1 protein is found in a majority of influenza A viruses (those generally responsible for the large flu epidemics), but their main sequence varies from strain to strain. Some flu strains such as the H1N1 virus do not appear to have an NS1 tail. It may be the diversity of the NS1 tail that explains how viruses can go undetected for some time within the human body. While more research is needed, the findings from this study could be applied to the development of new epigenetic drugs specific to influenza anti-inflammatory responses.

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
- Chromatin Marking
- RNA-mediated EIS

Four Recognized Mechanisms of Cellular Epigenetic Inheritance

- Self sustaining feedback loops
- 3 D templating -Structural Inheritance
- **Chromatin Marking**
  - Physical changes in DNA, histones and non-histonal proteins interacting with chromatin caused by chemical modification
  - Numerous examples.
- RNA-mediated EIS



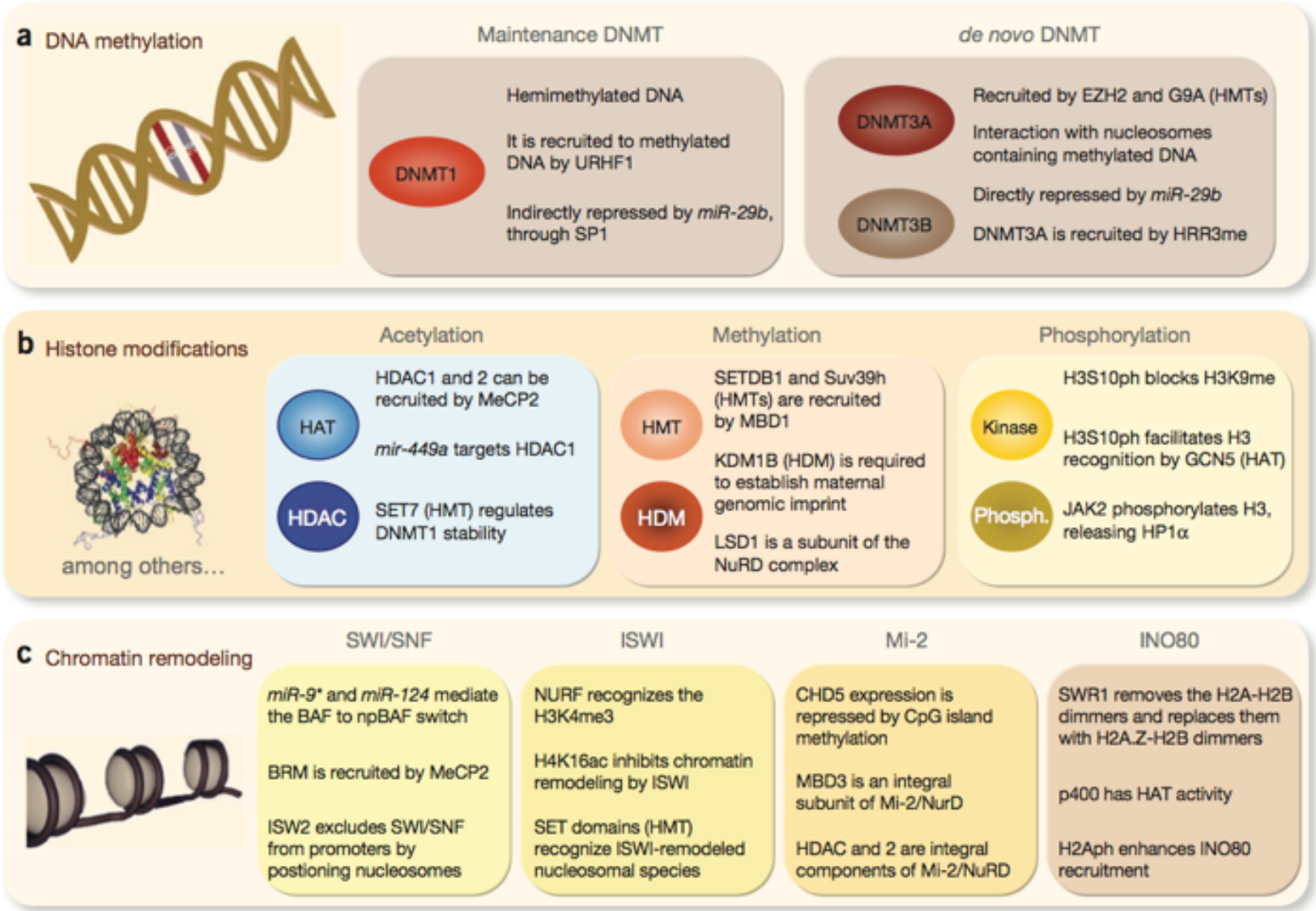
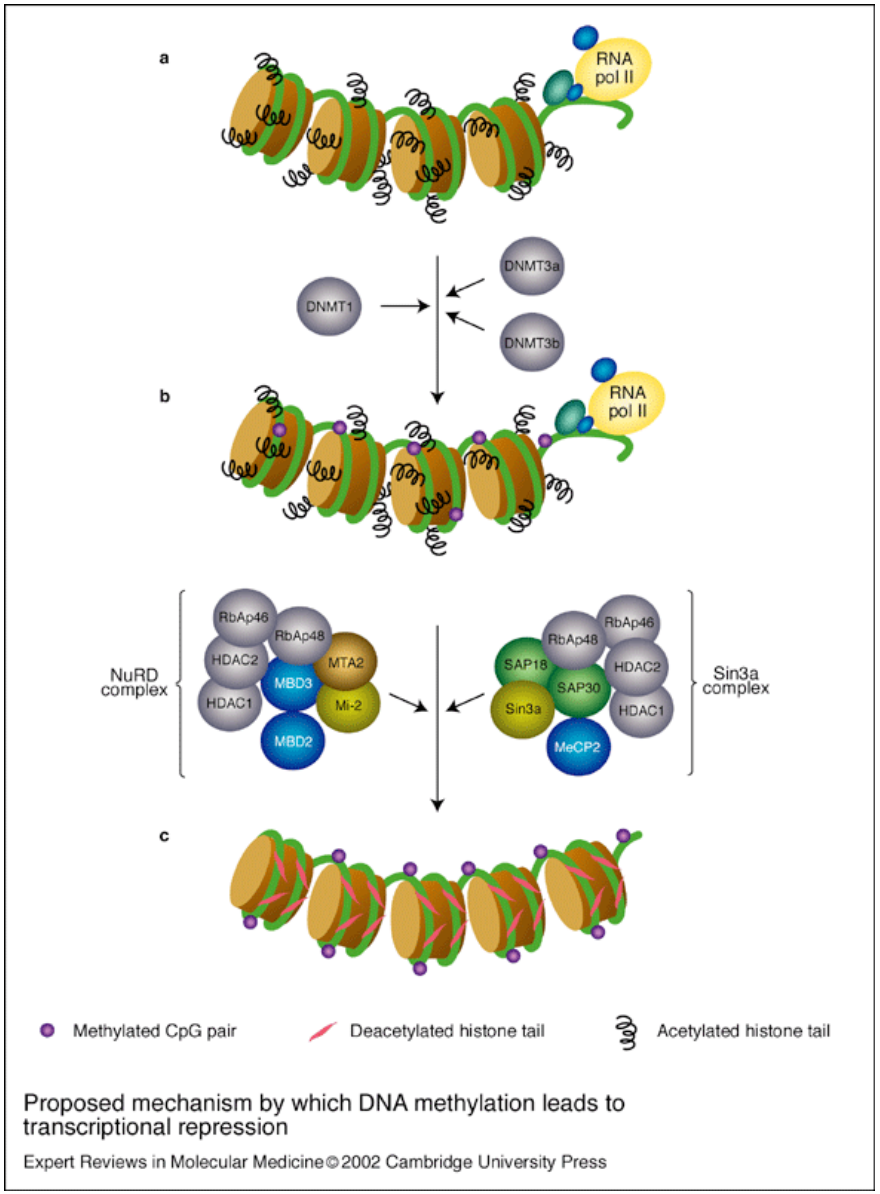


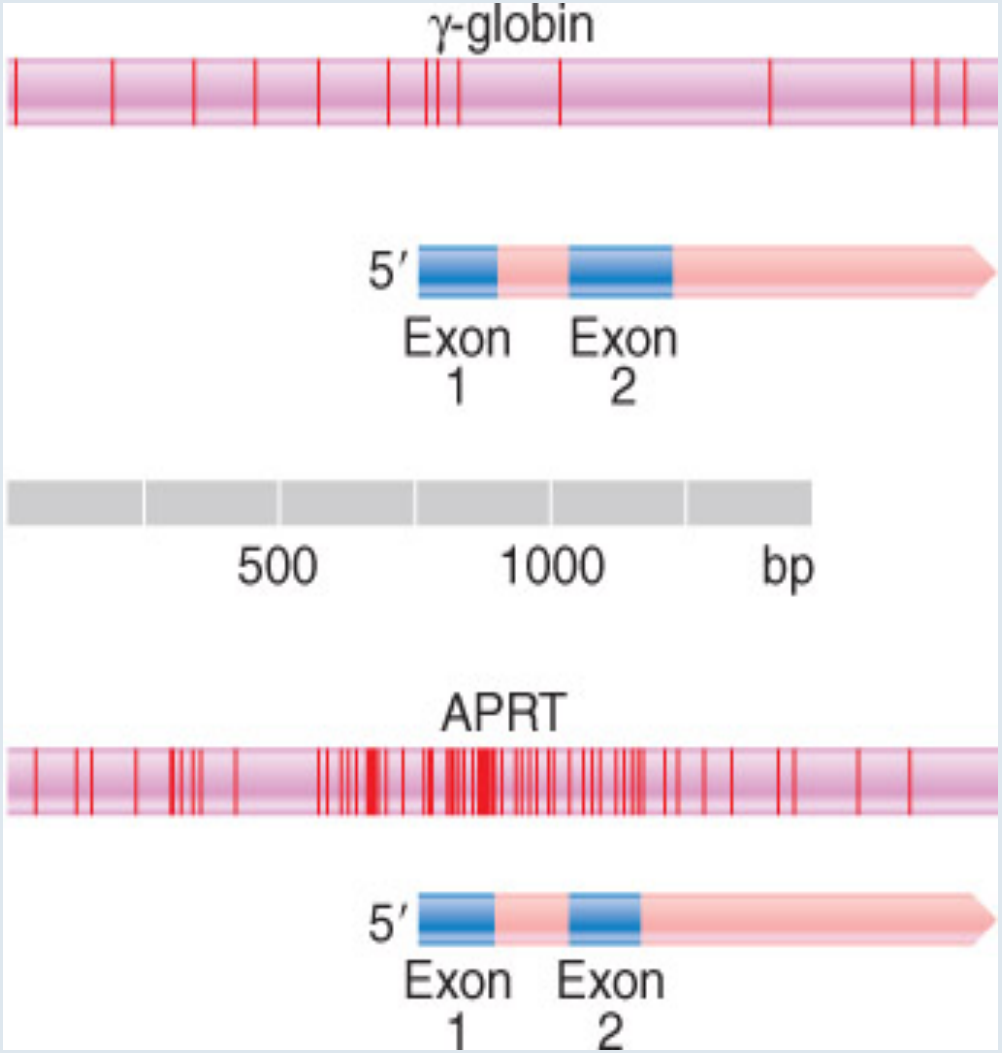
Figure 2 Epigenetic machinery and interplay among epigenetic factors. Epigenetic marks are catalyzed by different epigenetic complexes, whose principal families are illustrated here. (**a–c**) Epigenetic regulation depends on the interplay among the different players: DNA methylation (**a**), histone marks (**b**) and nucleosome positioning (**c**). The interaction among the different factors brings about the final outcome. This figure illustrates selected examples of the possible interrelations among the various epigenetic players.



- **Figure 2. Proposed mechanism by which DNA methylation leads to transcriptional repression.**
- **(a)** Transcriptionally active chromatin is predominantly **unmethylated** and has high levels of acetylated histone tails (short black squiggles).
- **(b)** Methylation at CpG dinucleotides can be carried out by one of the three known human DNA methyltransferases (**DNMT1**, 3a and 3b), resulting in DNA with high levels of CpG methylation (purple circles), but still containing predominantly acetylated histone tails. DNA in this form would still be expected to be transcriptionally competent.
- **(c)** Methylated DNA is targeted by **methyl-binding domain** (**MBD**) proteins such as **MBD2** and **MeCP2**, which are found associated with large protein complexes such as the **NuRD** complex (**MBD2**) and the **Sin3a** complex (**MeCP2**).
- Histone deacetylase (HDAC1 and 2) and **chromatin-remodelling activities** (Mi-2 and Sin3a) within these complexes result in alterations in chromatin structure, producing chromatin that is refractory to transcriptional activation (pink streaks represent deacetylated histone tails).

Proposed mechanism by which DNA methylation leads to transcriptional repression

G. Strathdee and R. Brown



DNA methylation is probably the best-understood system of Epigenetic inheritance as it is a genetic modification found in Eubacteria, Archaebacteria, and Eukaryota. It is involved in many important functions

In eukaryotes, methylation usually occurs on the cytosines in **CpG doublets** as well as **CpNpG** triplets in plants.

Since **CpG** and **CpNpG** are palindromes… they provide perfect template for “mirroring” regulatory markers one at of the DNA strands.

In vertebrate genomes, CpG dinucleotides are relatively depleted, except in specific DNA regions with a high den- sity of this dinucleotide.

The typical density of CpG doublets in mammalian DNA is ~1/100 bp, as seen for alpha - globin genes.

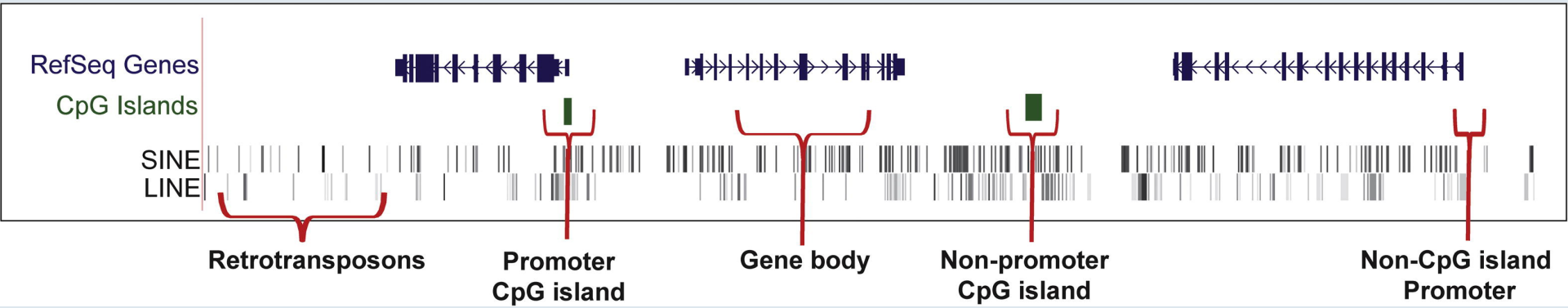


Fig. 1. Graphic representation of the different compartments where DNA methylation occurs in relation to CpG islands and genes. For clarity, only SINE and LINE retrotransposons are shown in the figure to represent the repetitive DNA compartment.

Table 2. DNA methylation in different genomic compartments in normal and cancer and its consequence.

Compartment	Methylation in normal	Methylation in cancer	Consequence
Promoter CpG island	1–2% methylated, corresponding to X-inactivation, imprinting and germ cell specific expression	Increase methylation of 1–10% of loci	Gene silencing
Non-promoter CpG island	Variably methylated, often tissue specific	Increased methylation	Unknown
non-CpG island promoter	Methylated or unmethylated	Methylated or unmethylated	Associated with expression but may be secondary to transcription programs
Gene body	Methylated in active genes	Poorly studied	May be secondary to transcription programs
Retrotransposons	Mostly methylated	Frequently hypomethylated	Chromosome instability

M.R.H. Estécio, Jean-Pierre J. Issa / FEBS Letters 585 (2011) 2078–2086

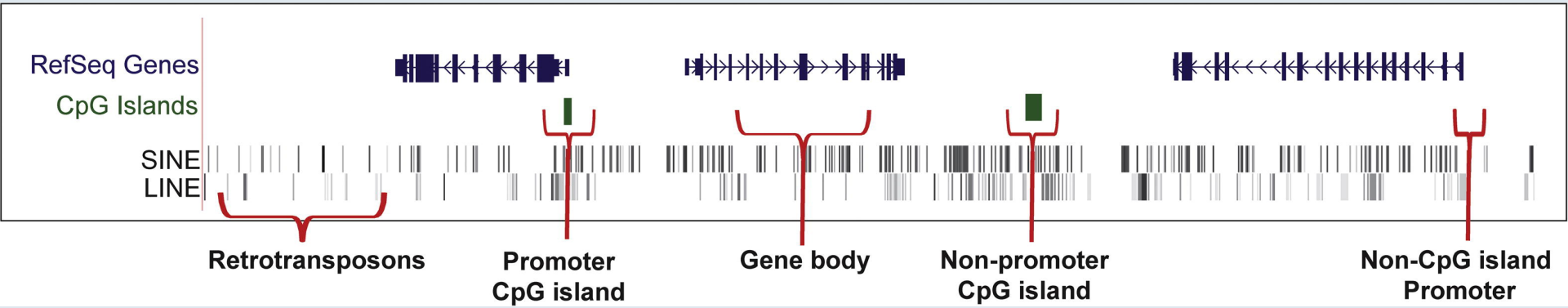


Fig. 1. Graphic representation of the different compartments where DNA methylation occurs in relation to CpG islands and genes. For clarity, only SINE and LINE retrotransposons are shown in the figure to represent the repetitive DNA compartment.

Table 1
Massively parallel sequencing-based methods to study DNA methylation in high coverage or whole-genome resolution.

Technique	Detection	Description	Ref.
HELP-Seq	Restriction enzyme	*Hpa*II restriction enzyme is used to eliminate the methylated fraction of the genome, and the enrichment for unmethylated fragments is compared to DNA digested with MspI	[133]
MeDIP-Seq	Antibody	Methylated DNA is captured using anti-5-methylcytosine antibodies, followed by massively parallel sequencing. Regions enriched for captured tags are classified as methylated, rendering qualitative maps with little or no quantitative characteristics	[134]
MethylC-Seq	Bisulfite treatment	The genome is fragmented by sonication, and modified adaptors are ligated to the DNA prior to bisulfite-conversion. It is the only truly genome-wide method applied to the human genome at the moment, but the still high cost of the method limits its application to large group of samples. This technique may become the standard for methylome analysis with the introduction of cheaper, faster sequencers	[16]
Padlock, BSPPs	Bisulfite treatment	Genomic DNA is treated with bisulfite, and selected targets are collected using molecular inversion probes. The fact that only selected areas are evaluated in high depth for DNA methylation can be either a strength or a weakness of the method, depending on the researcher's objective	[58,135]
RRBS	Restriction enzyme + bisulfite treatment	Genomic DNA is fragmented using *Msp*I, and fragments of a certain size range are purified from gel electrophoresis. The purified DNA is ligated to modified adaptors, bisulfite-treated, and then sequenced after library preparation	[36]

van de Lagemaat *et al.*
Epigenetics & Chromatin (2018) 11:59
https://doi.org/10.1186/s13072-018-0230-0

Epigenetics & Chromatin

RESEARCH Open Access

CpG binding protein (CFP1) occupies open chromatin regions of active genes, including enhancers and non-CpG islands

Louie N. van de Lagemaat[1], Maria Flenley[2], Magnus D. Lynch[2,3,7], David Garrick[4], Simon R. Tomlinson[5], Kamil R. Kranc[5,6] and Douglas Vernimmen[1*]

Abstract

Background: The mechanism by which protein complexes interact to regulate the deposition of post-translational modifications of histones remains poorly understood. This is particularly important at regulatory regions, such as CpG islands (CGIs), which are known to recruit Trithorax (TrxG) and Polycomb group proteins. The CxxC zinc finger protein 1 (CFP1, also known as CGBP) is a subunit of the TrxG SET1 protein complex, a major catalyst of trimethylation of H3K4 (H3K4me3).

Results: Here, we used ChIP followed by high-throughput sequencing (ChIP-seq) to analyse genomic occupancy of CFP1 in two human haematopoietic cell types. We demonstrate that CFP1 occupies CGIs associated with active transcription start sites (TSSs), and is mutually exclusive with H3K27 trimethylation (H3K27me3), a marker of polycomb repressive complex 2. Strikingly, rather than being restricted to active CGI TSSs, CFP1 also occupies a substantial fraction of active non-CGI TSSs and enhancers of transcribed genes. However, relative to other TrxG subunits, CFP1 was specialised to TSSs. Finally, we found enrichment of CpG-containing DNA motifs in CFP1 peaks at CGI promoters.

Conclusions: We found that CFP1 is not solely recruited to CpG islands as it was originally defined, but also other regions including non-CpG island promoters and enhancers.

Keywords: CFP1, CpG islands, Trithorax group proteins, Epigenetics, Enhancers

Proceedings of the National Academy of Sciences of the United States of America

PNAS

Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga *, Esteban Ballestar *, Maria F. Paz *, Santiago Ropero *, Fernando Setien *, Maria L. Ballestar †, Damia Heine-Suñer ‡, Juan C. Cigudosa §, Miguel Urioste ¶, Javier Benitez ¶, Manuel Boix-Chornet †, Abel Sanchez-Aguilera †, Charlotte Ling ‖, Emma Carlsson ‖, Pernille Poulsen **, Allan Vaag **, Zarko Stephan ††, Tim D. Spector ††, Yue-Zhong Wu ‡‡, Christoph Plass ‡‡, and Manel Esteller * , §§

Author Affiliations

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

Abstract

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.

DNA methylation | epigenetics | histones

This Issue
July 26, 2005
vol. 102 no. 30
Masthead (PDF)
Table of Contents

PREV ARTICLE NEXT ARTICLE

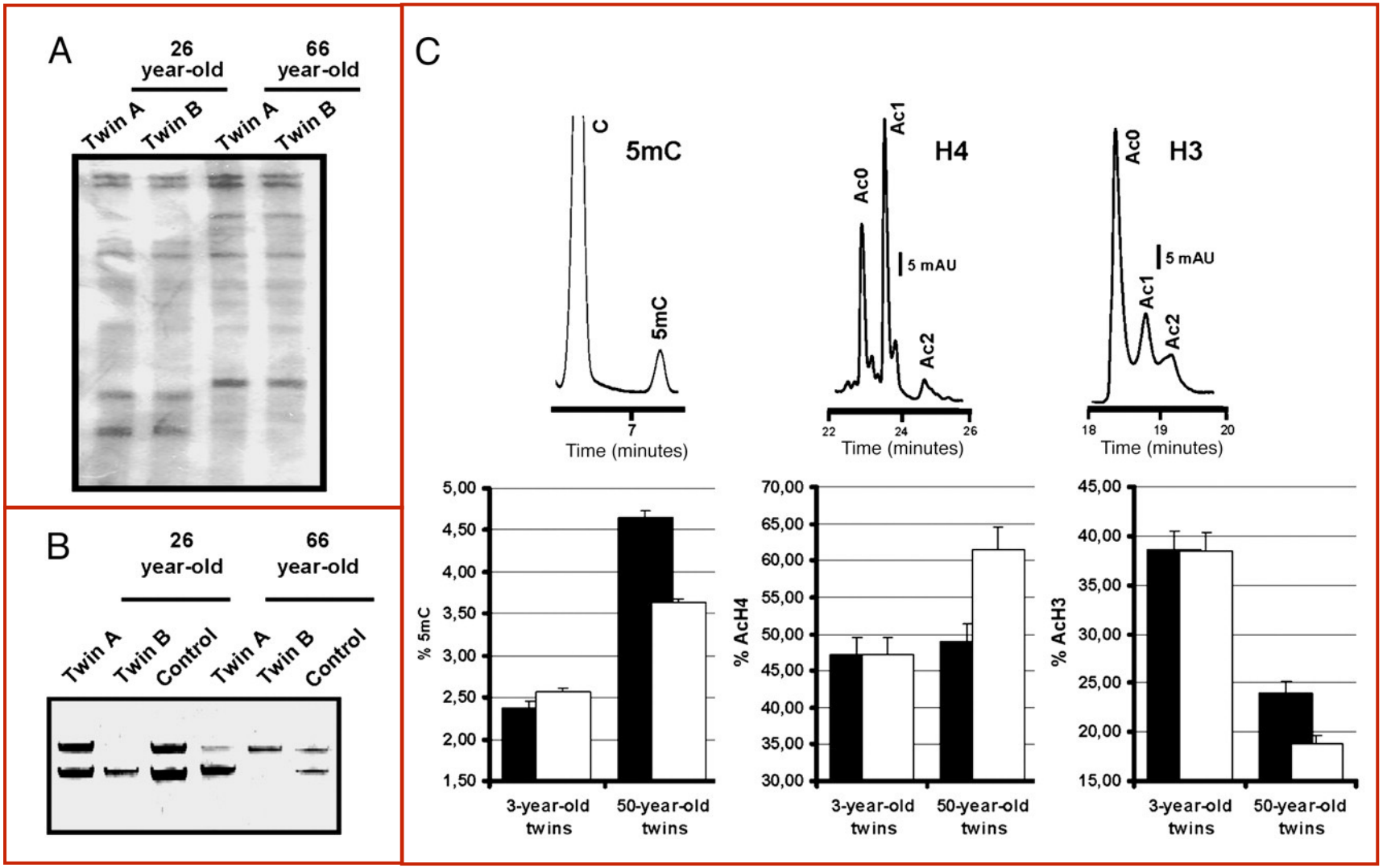
Published online before print July 11, 2005, doi: 10.1073/pnas.0500398102
PNAS July 26, 2005 vol. 102 no. 30 10604-10609

Classifications
Biological Sciences
Medical Sciences

Access

- Abstract *Free*
- Full Text (HTML)
- Full Text (PDF)
- Full Text + SI (Combined PDF)
- Figures Only
- Supporting Information
- Related Articles

Epigenetic differences arise in MZ twins.



Fraga M F et al. PNAS 2005;102:10604-10609

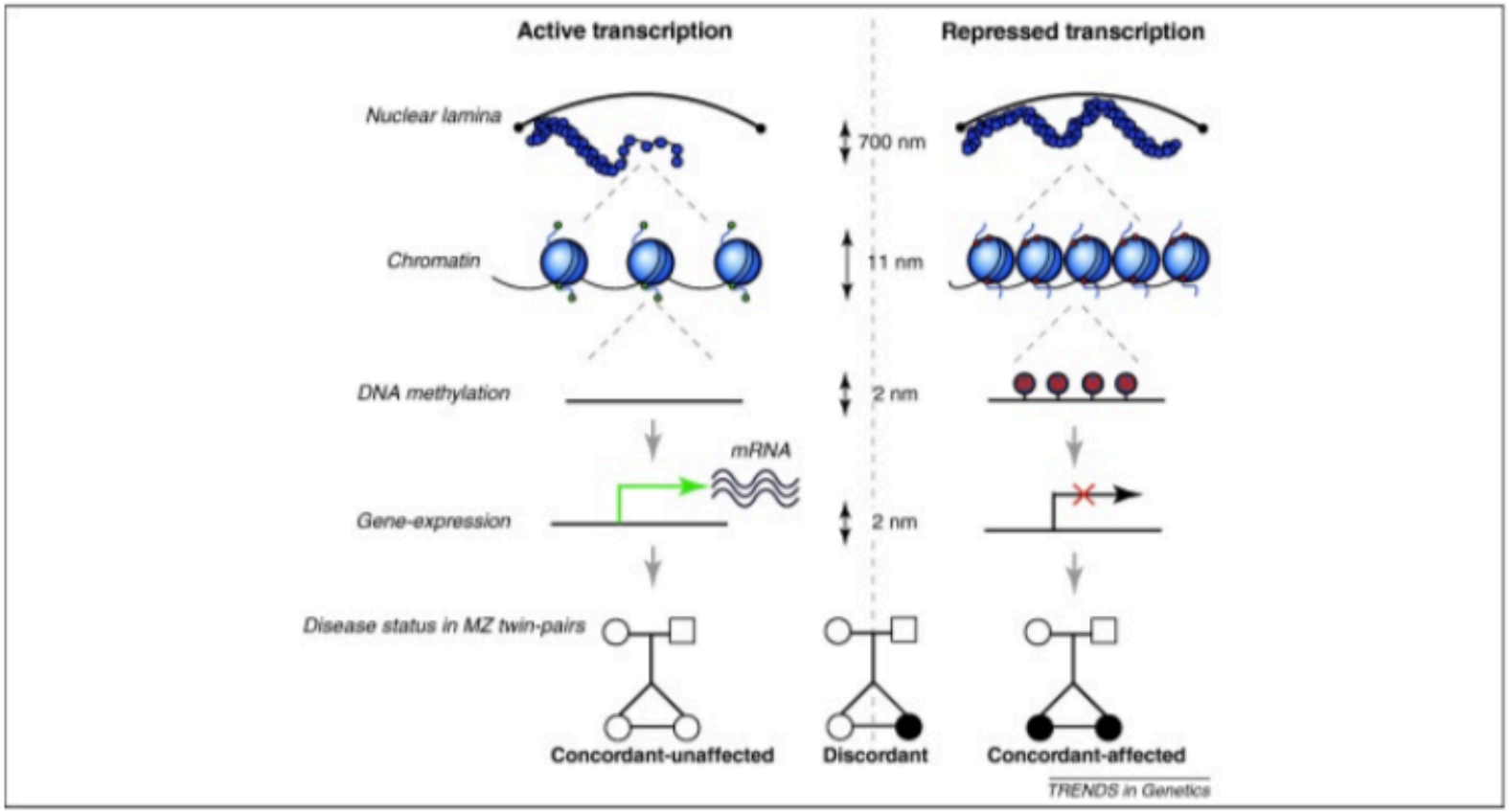
Epigenetic differences arise in MZ twins.

(A) Two representative examples of the determination of monozygosity using microsatellite markers.

(B) Quantification of X chromosome inactivation by PCR amplification of the androgen receptor locus after digestion with the DNA methylation-sensitive and -insensitive restriction enzymes HpaII and MSp I, respectively. Two examples of a different pattern of X inactivation between MZ twin pairs are shown.

(C) (C Upper) Quantification of global 5mC DNA content (Left), histone H4 acetylation (Center), and histone H3 acetylation (Right) by HPLC and high-performance capillary electrophoresis.

(D) (C Lower) Comparison of epigenetic values between the siblings of each 3- and 50-year-old twin pair. Results are expressed as mean ± SD.



Epigenetic changes and their effects on transcription and disease. Epigenetic variants across multiple levels of chromatin structure, shown here at different levels of cell resolution in nanometers (nm), associate with gene expression and disease status in a sample of MZ twins. Top, higher-order chromatin loop configurations and attachment to the nuclear lamina can represent active and repressed chromatin domains that associate with differential gene expression. The next level represents the chromatin 'beads on a string' configuration, which reflects structural organization into loosely structured (active) and densely packed (repressed) chromatin states. Histone modifications associated with active transcription (green) and transcription silencing (red) are indicated by colored dots. The next levels of cell resolution depict DNA methylation (red M) in the promoter regions of the silenced genes and the corresponding differences in gene expression. Bottom, possible effects of these changes on disease status in a sample of MZ twins, highlighting unaffected-concordant, discordant, and disease-concordant MZ twins.

Trends Genet. 2011 Mar; 27(3): 116–125.

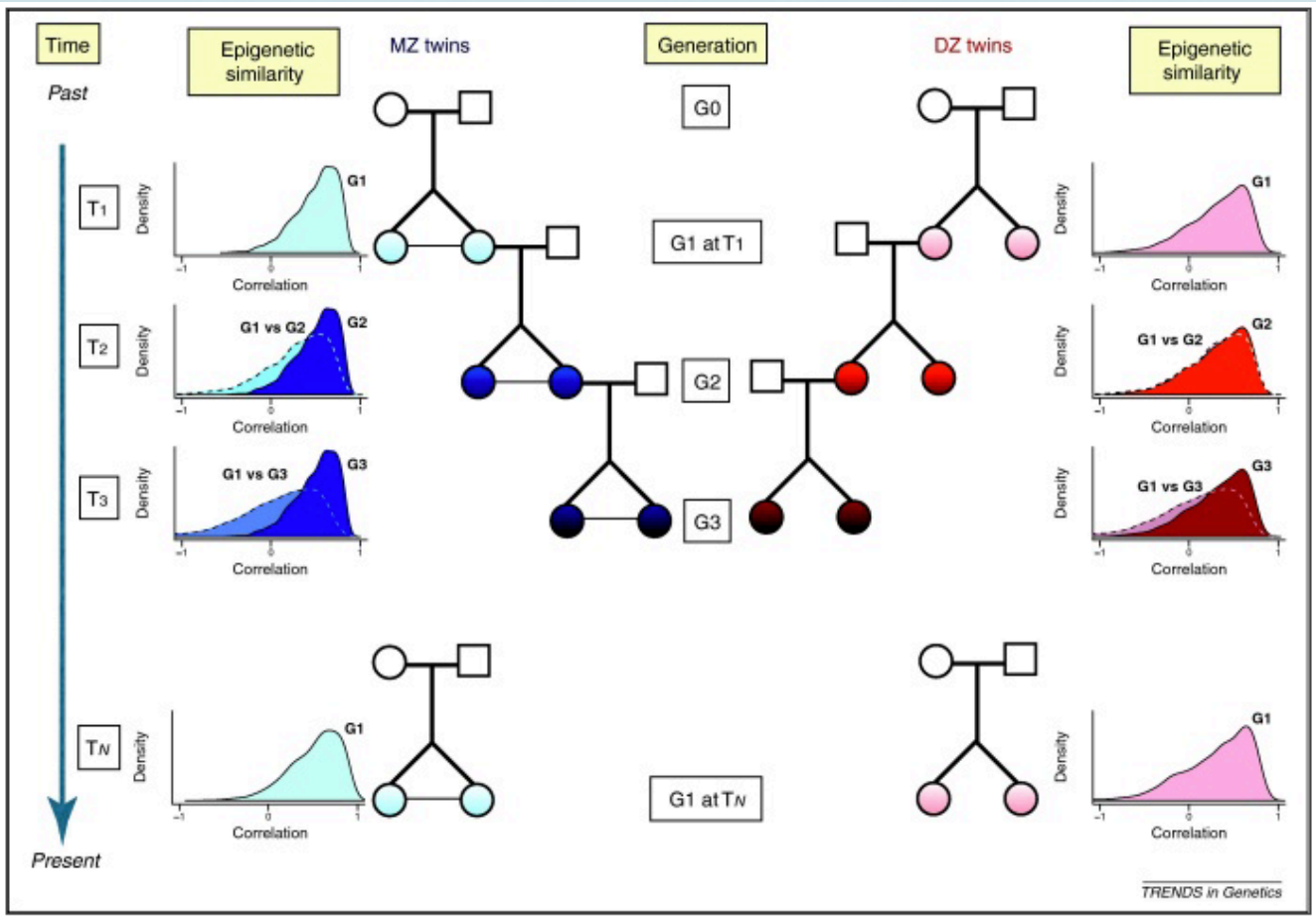


Figure 2
Transgenerational changes in epigenetic variation in twins. The figure provides illustrative examples of epigenetic heritability estimates in hypothetical families that include pairs of MZ or DZ twins across three generations. This highlights the idea ...

There is evidence that the rate of transmission of **epigenetic marks lessens with each generation** [57], suggesting that epigenetic profiles are likely to be more similar in families within generations as opposed to between generations

This would be in contrast to transgenerational inheritance of **DNA methylation patterns in plants,** which can be relatively stable for up to **eight generations** [48,50],

Trends Genet. 2011 Mar; 27(3): 116–125.


eight

Epigenetic inheritance at the agouti locus in the mouse

Hugh D. Morgan[1], Heidi G.E. Sutherland[2], David I.K. Martin[3] & Emma Whitelaw[1]

Epigenetic modifications have effects on phenotype, but they are generally considered to be cleared on passage through the germ line in mammals, so that only genetic traits are inherited. Here we describe the inheritance of an epigenetic modification at the agouti locus in mice. In viable yellow (A^{vy}/a) mice, transcription originating in an intra-cisternal A particle (IAP) retrotransposon inserted upstream of the agouti gene (*A*) causes ectopic expression of agouti protein, resulting in yellow fur, obesity, diabetes and increased susceptibility to tumours[1]. The pleiotropic effects of ectopic agouti expression are presumably due to effects of the paracrine signal on other tissues. A^{vy} mice display variable expressivity because they are epigenetic mosaics for activity of the retrotransposon: isogenic A^{vy} mice have coats that vary in a continuous spectrum from full yellow, through variegated yellow/agouti, to full agouti (pseudoagouti). The distribution of phenotypes among offspring is related to the phenotype of the dam; when an A^{vy} dam has the agouti phenotype, her offspring are more likely to be agouti[2,3]. We demonstrate here that this maternal epigenetic effect is not the result of a maternally contributed environment. Rather, our data show that it results from incomplete erasure of an epigenetic modification when a silenced A^{vy} allele is passed through the female germ line, with consequent inheritance of the epigenetic modification. Because retrotransposons are abundant in mammalian genomes, this type of inheritance may be common.

A is responsible for the wild-type coat colour of mice, as it encodes a paracrine signalling protein that causes hair follicle melanocytes to switch from the synthesis of eumelanin (black) to phaeomelanin (yellow). In mice that carry the *A* allele, transcription during the mid-portion of the hair growth cycle produces a sub-apical yellow band on a black hair. A ventral-specific promoter is responsible for a yellow pelage on the belly of the mouse[4]. The A^{vy} allele is one of four (the others being A^{iapy}, A^{iy} and A^{hvy}) alleles carrying IAP (Fig. 1*a*; refs 1,5,6). Mosaic activity of the IAP is responsible for the range of phenotypes in these mice. In A^{iapy} and A^{hvy} mice, the phenotype correlates with methylation of the IAP (refs 6,7). In A^{vy} mice, a yellow maternal phenotype was reported to shift the proportion of phenotypes in offspring, producing fewer pseudoagouti pups[2,3]. This was attributed to an effect of maternal metabolism on the phenotype of offspring. Alternatively, the maternal effect might result from the persistence of epigenetic modifications through meiosis, and we designed experiments to distinguish the two possibilities.

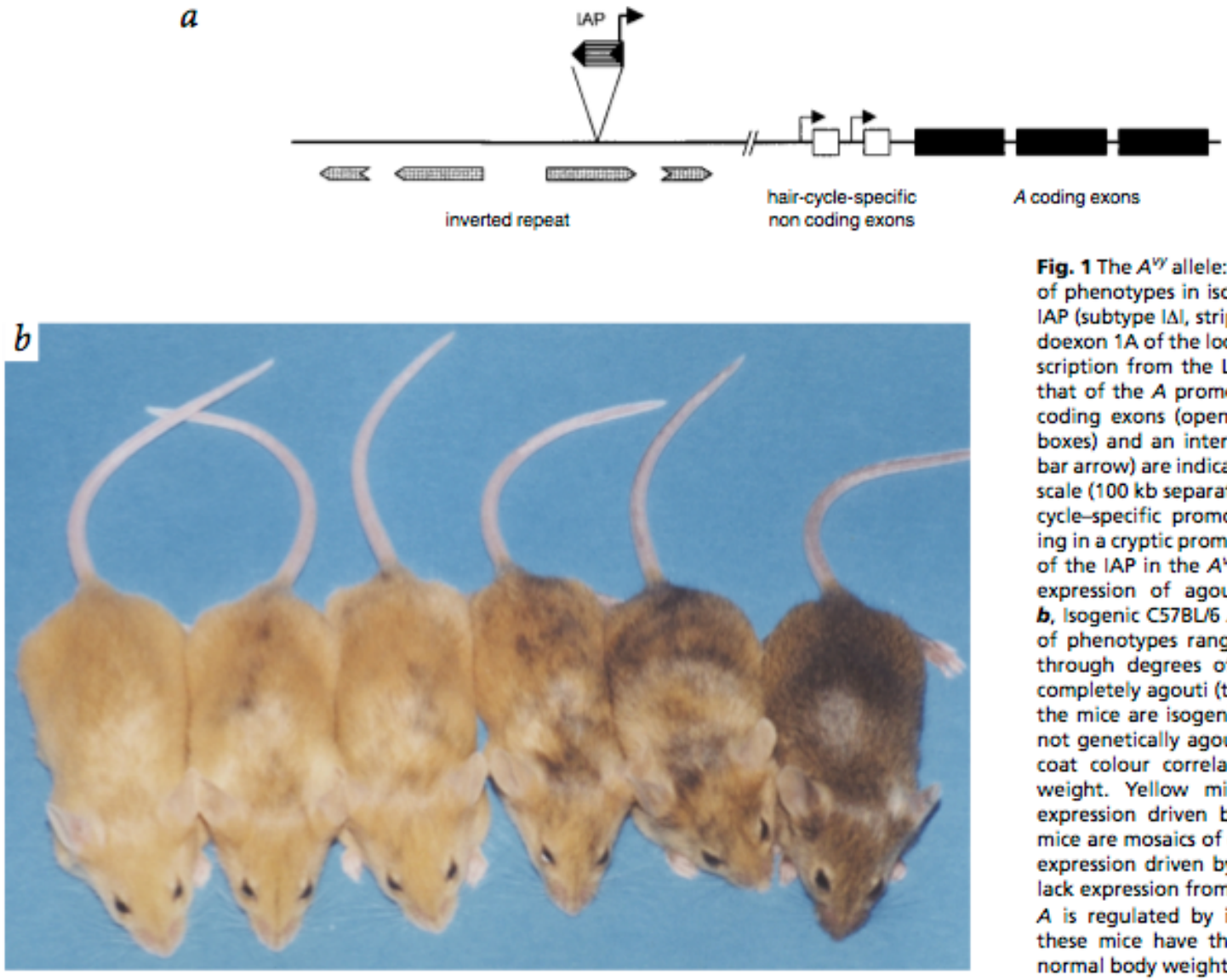


Fig. 1 The A^{vy} allele: map of the *A* locus and range of phenotypes in isogenic A^{vy} mice. ***a***, A^{vy} has an IAP (subtype IΔI, striped box) inserted in the pseudoexon 1A of the locus, with the direction of transcription from the LTRs (arrowhead) opposite to that of the *A* promoters. Hair-cycle–specific noncoding exons (open boxes), coding exons (filled boxes) and an interrupted inverted repeat (grey bar arrow) are indicated. The locus is not shown to scale (100 kb separates the insertion site and hair-cycle–specific promoters). Transcription originating in a cryptic promoter (arrowhead) in the 3′ LTR of the IAP in the A^{vy} allele results in constitutive expression of agouti in multiple tissues[1,2,4,25]. ***b***, Isogenic C57BL/6 A^{vy}/a mice show a continuum of phenotypes ranging from completely yellow, through degrees of yellow/agouti mottling, to completely agouti (termed pseudoagouti because the mice are isogenic with fully yellow mice and not genetically agouti). The extent of the yellow coat colour correlates closely with adult body weight. Yellow mice have pancellular agouti expression driven by the inserted IAP. Mottled mice are mosaics of cells that have or lack ectopic expression driven by the IAP. Pseudoagouti mice lack expression from the cryptic promoter, so that *A* is regulated by its hair-cycle promoters, and these mice have the wild-type coat colour and normal body weight[1–3].

The *Agouti* Mouse Models

The best-characterized example of non-genomic inheritance of epigenetic marks via the gametes is exemplified by the **agouti viable yellow** (*Avy*) mouse.64 The ***agouti* gene** (***Agrp***) encodes a paracrine factor that stimulates the production of a yellow pigment (phaeomelanin) rather than the black pigment (eumalenin) in the follicles of the hair.

Transcription of the gene from a wild-type allele (*A*) occurs during specific stages of development, is restricted to hair follicles, and results in the characteristic brown color of wild-type mice. In contrast, the *Avy* allele results from the insertion of an intracisternal A particle (IAP) transposon upstream of the *agouti locus*. The IAP acts as a cryptic promoter that stimulates constitutive and ectopic expression of the ***agouti* gene**. This aberrant expression leads to yellow fur, obesity, and high prevalence of tumor formation.

Typically, transposons are **heavily methylated to block their activity.** Nevertheless, the IAP of the *Avy* allele shows various degrees of methylation, from unmethylated (yellow mice) to heavily methylated (brown mice) -through various methylation densities (mottled combination of brown and yellow patches). Since the degree of methylation of the IAP correlates with the expression of the *agouti* gene (and fur color), it is relatively simple to assess whether parental methylation of the IAP is transmitted to the offspring. The authors first showed that the agouti traits were transmitted through the maternal line to the offspring. To demonstrate that inheritance of the phenotype was mediated by epigenetic mechanisms, they transplanted fertilized eggs from yellow dams into pseudoagouti mice. Amazingly, the offspring from these pregnancies retained the agouti phenotype. Or, in other words, the offspring inherited and maintained the **maternal epigenetic marks** via the gametes (**oocytes**). This was the first reported example of transgenerational epigenetic inheritance via the gametes (oocytes) in mammals. Nevertheless, in a follow-up study it was questioned whether the inherited epigenetic mark was **DNA methylation.** The reason is that the maternal pattern of DNA methylation, present in oocytes and the zygote, was completely erased in the blastocysts. Therefore, either histone marks and/or other oocyte factors must mediate this type of epigenetic transmission.

Chapter 21 - Transgenerational Epigenetic Inheritance of Type 2 Diabetes

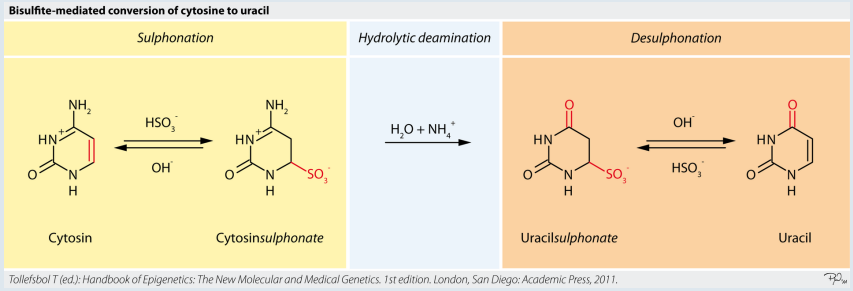
Author links open overlay panel
Josep C.Jiménez-Chillarón∗RubénDíaz∗†MartaRamón-Krauel∗SílviaRibó∗
Show more
https://doi.org/10.1016/B978-0-12-405944-3.00021-0

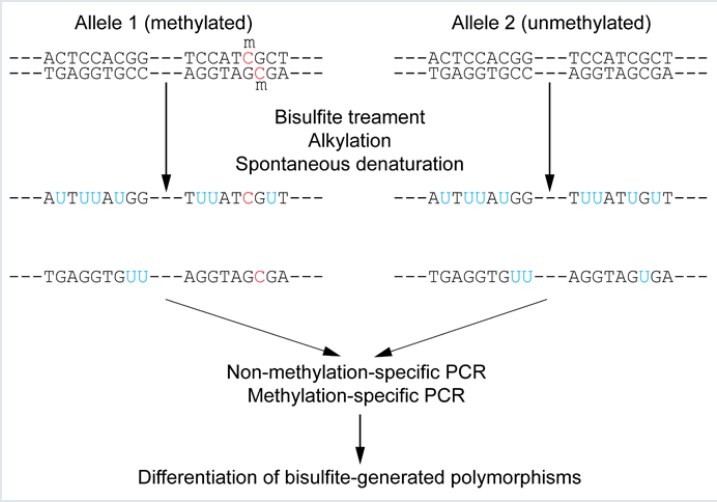
Abstract
Transgenerational inheritance of phenotypes refers to the transfer of information from one generation to the next by non-genomic mechanisms, such as epigenetics. Epigenetics can be defined as the study of heritable traits that do not involve changes in the DNA sequence. Epigenetic marks include DNA methylation, histone modifications and, recently, a variety of non-coding RNAs. Transgenerational epigenetic inheritance of diabetes therefore refers to the transfer of diabetes risk from parents to offspring through epigenetic modifications. This process implies that environmental factors can induce epigenetic modifications in cells from the germline which: i) escape the epigenetic reprogramming events occurring during gametogenesis and the first post-zygotic divisions, ii) are inherited into the next generation offspring, and iii) are stably maintained in tissues, where they influence gene expression and, ultimately, increase diabetes risk later in life. This chapter reviews the available evidence to support transgenerational epigenetic inheritance of type 2 diabetes and associated co-morbidities in humans and model organisms.

- *ScienceDaily (Sep. 20, 2011)* — The first comprehensive inventory of epigenetic changes over several generations shows that these often do not last and therefore probably have limited effects on long-term evolution, according to scientists in Germany.
- Scientists at the Max Planck Institute of Developmental Biology in Germany have now produced the first comprehensive inventory of spontaneous epigenetic changes.
- Using ***Arabidopsis*** (the workhorse of modern plant genetics) the researchers determined how often and where in the genome epigenetic modifications occur -- and how often they disappear again. They found that epigenetic changes are many orders of magnitude more frequent than conventional DNA mutations, but also often short lived. They are therefore probably much less important for long-term evolution than previously thought.
- To determine the rate and distribution of methylation changes in the genome, the German biologists looked at ten Arabidopsis lines...ensuring that all offspring came from the same stock, but had been propagated independently for **30 generations** by self-fertilization.
- "For each line, we were able to look at about **14 million cytosines**," said Claude Becker, a member of the Tübingen team. On average, every plant had almost **3 million methylated cytosines.** The vast majority of these were the same in all lines, but about **6 percent** had changed since the lines had become separated. At these positions, at least one of the individuals was different, with either methylation gained or lost relative to the ancestor. Each of the lines had about **30,000 such epimutations**, which was **1,000 times more than DNA mutations.**

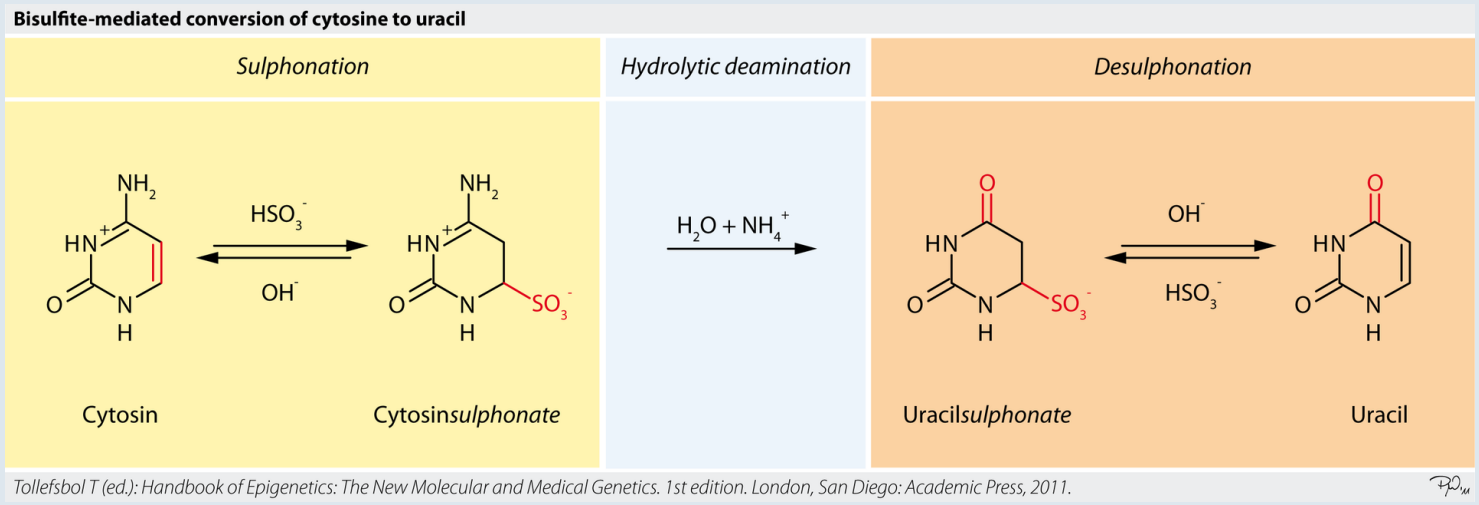
- *ScienceDaily (Sep. 20, 2011)* — The first comprehensive inventory of epigenetic changes over several generations shows that these often do not last and therefore probably have limited effects on long-term evolution, according to scientists in Germany.
- Scientists at the Max Planck Institute of Developmental Biology in Germany have now produced the first comprehensive inventory of spontaneous epigenetic changes.
- Using *Arabidopsis* (the workhorse of modern plant genetics) the researchers determined how often and where in the genome epigenetic modifications occur -- and how often they disappear again. They found that epigenetic changes are many orders of magnitude more frequent than conventional DNA mutations, but also often short lived. They are therefore probably much less important for long-term evolution than previously thought.
- To determine the rate and distribution of methylation changes in the genome, the German biologists looked at ten Arabidopsis lines...ensuring that all offspring came from the same stock, but had been propagated independently for **30 generations** by self-fertilization.
- "For each line, we were able to look at about **14 million cytosines**," said Claude Becker, a member of the Tübingen team. On average, every plant had almost **3 million methylated cytosines.** The vast majority of these were the same in all lines, but about **6 percent** had changed since the lines had become separated. At these positions, at least one of the individuals was different, with either methylation gained or lost relative to the ancestor. Each of the lines had about **30,000 such epimutations**, which was **1,000 times more than DNA mutations.**

Use of Bisulphite reactions to discriminate between methylated and non-methylated sequences.

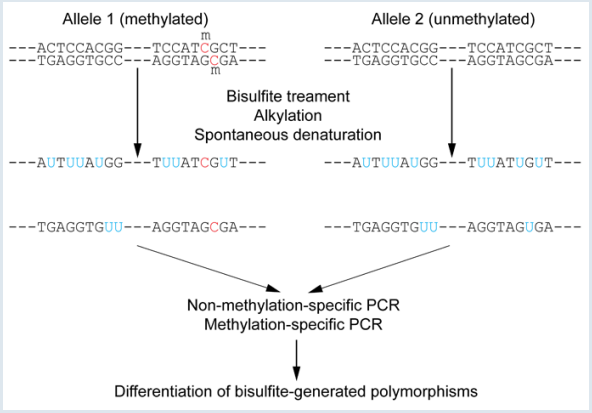




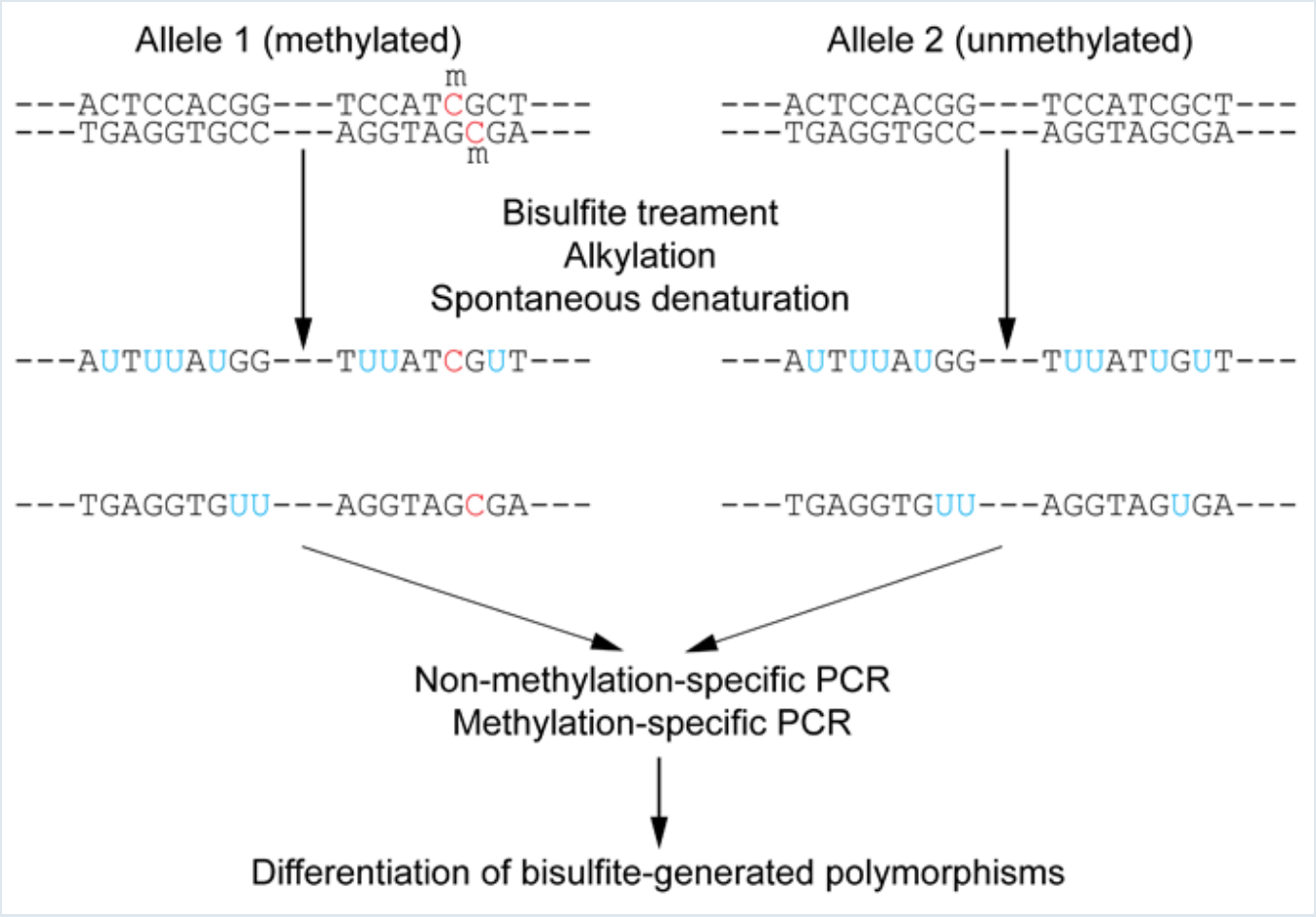
Use of Bisulphite reactions to discriminate between methylated and non-methylated sequences.

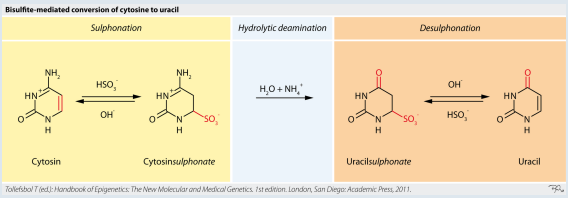


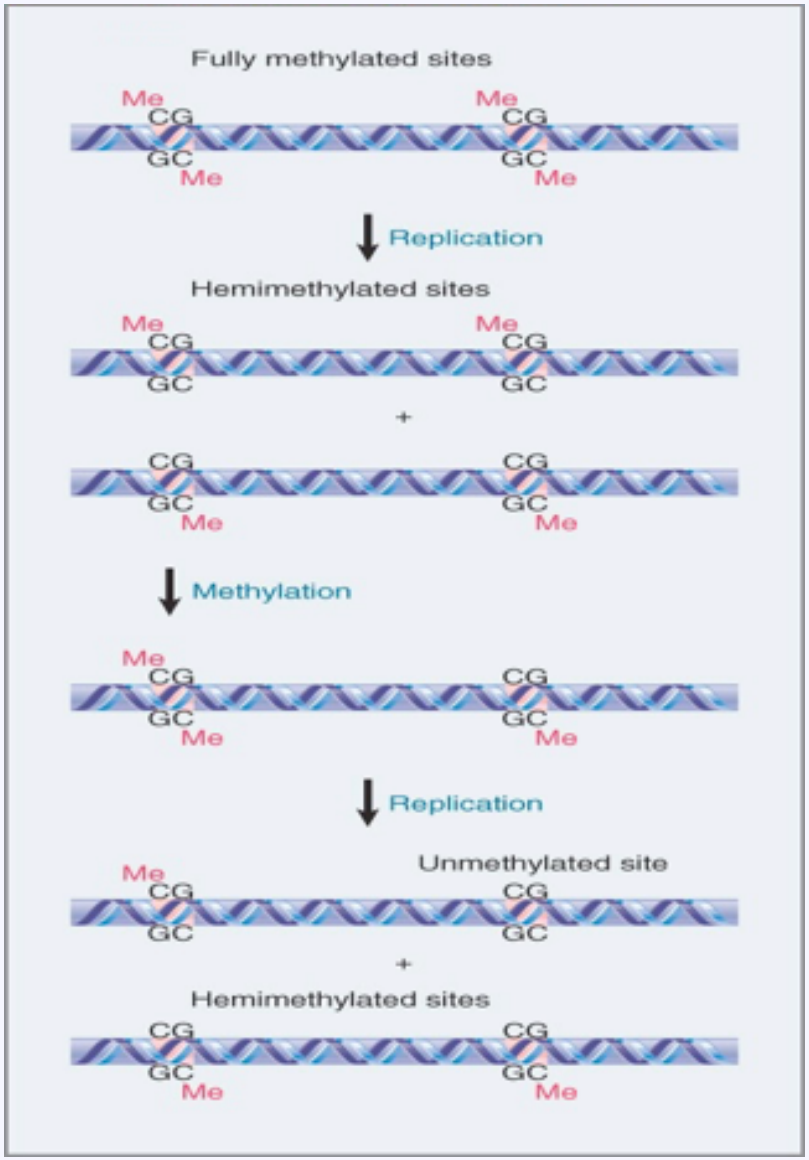
Tollefsbol T (ed.): Handbook of Epigenetics: The New Molecular and Medical Genetics. 1st edition. London, San Diego: Academic Press, 2011.



Use of Bisulphite reactions to discriminate between methylated and non-methylated sequences.







Most epigenetically important methyl groups in DNA are found on **cytosines** on both strands of the **CpG doublets**.

Replication converts a fully methylated site to a hemi-methylated site.

DNA Methylation and Demethylation in Mammals*

Zhao-xia Chen and Arthur D. Riggs[1]

+ Author Affiliations

↵[1] To whom correspondence should be addressed. E-mail: ariggs@coh.org.

Abstract

Cell type-specific DNA methylation patterns are established during mammalian development and maintained in adult somatic cells. Understanding how these patterns of 5-methylcytosine are established and maintained requires the elucidation of mechanisms for both DNA methylation and demethylation. The enzymes involved in the *de novo* methylation of DNA and the maintenance of the resulting methylation patterns have been fairly well characterized. However, important remaining challenges are to understand how DNA methylation systems function *in vivo* and in the context of chromatin. In addition, the enzymes and mechanisms for demethylation remain to be elucidated. There is still no consensus as to how active enzymatic demethylation is achieved in mammalian cells, but recent studies implicate base excision repair for genome-wide DNA demethylation in germ cells and early embryos.

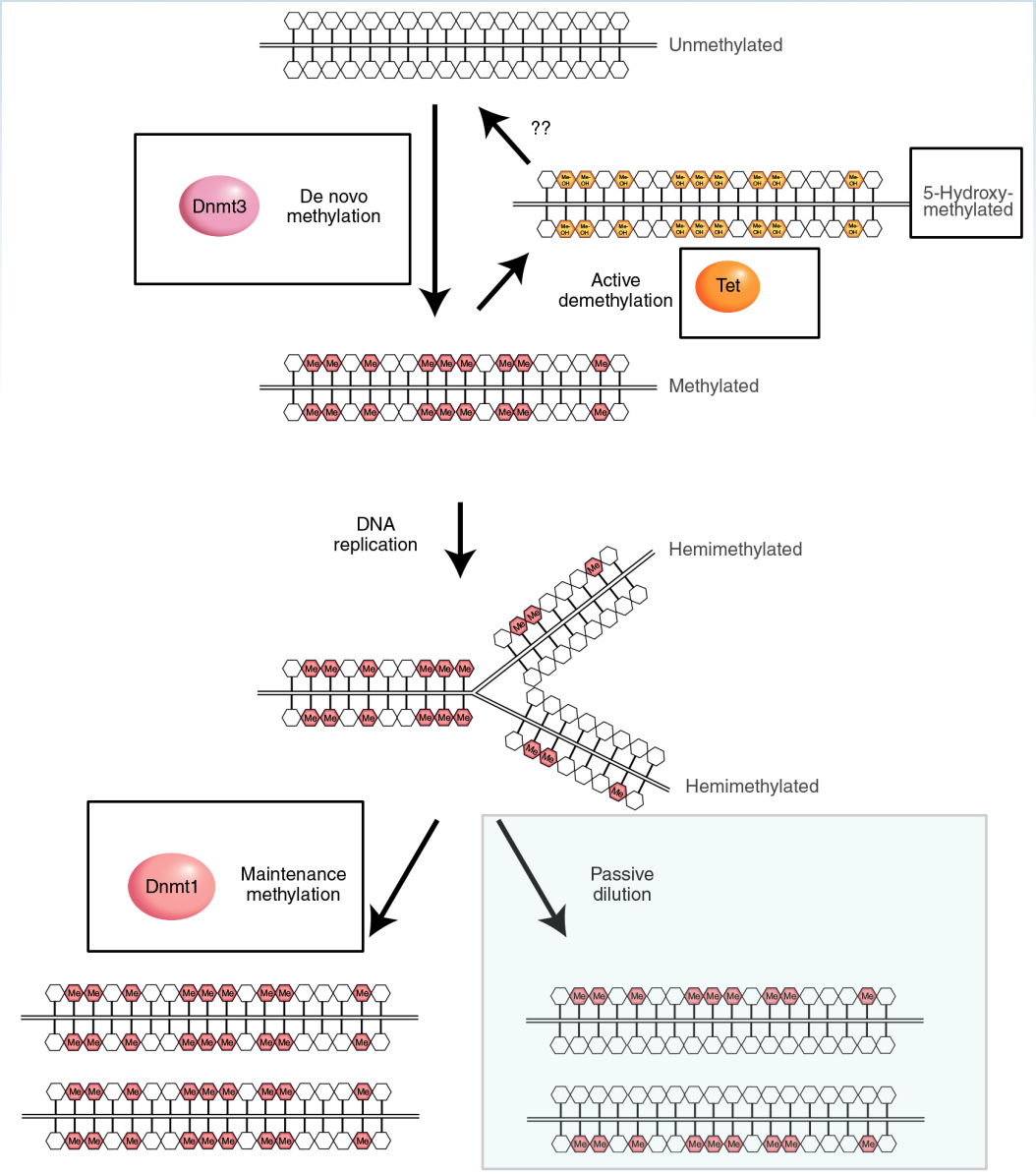


Figure 2. De novo methylation and maintenance methylation of DNA. A stretch of genomic DNA is shown as a line with self-complementary CpG pairs marked as vertical strokes. Unmethylated DNA (*top*) becomes methylated "de novo" by Dnmt3a and Dnmt3b to give symmetrical methylation at certain CpG pairs. On semiconservative DNA replication, a progeny DNA strand is base-paired with one of the methylated parental strands (the other replication product is not shown). Symmetry is restored by the maintenance DNA methyltransferase, Dnmt1, which completes half-methylated sites, but does not methylate unmodified CpGs.

Methylation requires not only ***de novo* activity**, but also **maintenance activity** -following replication

The fidelity of transmission in cell lineages ranges from about 1% variation per cell generation up to the very high fidelity of 10^{-6} variations per cell generation

Nature. 2008 Mar 6;452(7183):45-50.

Cyclical DNA methylation of a transcriptionally active promoter.

Métivier R, Gallais R, Tiffoche C, Le Péron C, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G.

Université de Rennes I, CNRS, UMR 6026 Equipe SPARTE, IFR 140 GFAS, Campus de Beaulieu, 35042 Rennes cedex, France.
Raphael.Metivier@univ-rennes1.fr

Processes that regulate gene transcription are directly under the influence of the genome organization. The epigenome contains additional information that is not brought by DNA sequence, and generates spatial and functional constraints that complement genetic instructions. DNA methylation on CpGs constitutes an epigenetic mark generally correlated with transcriptionally silent condensed chromatin. Replication of methylation patterns by DNA methyltransferases maintains genome stability through cell division. Here we present evidence of an unanticipated dynamic role for DNA methylation in gene regulation in human cells. Periodic, strand-specific methylation/demethylation occurs during transcriptional cycling of the pS2/TFF1 gene promoter on activation by oestrogens. DNA methyltransferases exhibit dual actions during these cycles, being involved in CpG methylation and active demethylation of 5mCpGs through deamination. Inhibition of this process precludes demethylation of the pS2 gene promoter and its subsequent transcriptional activation. Cyclical changes in the methylation status of promoter CpGs may thus represent a critical event in transcriptional achievement.

Molecules and Cells
Molecules and Cells
http://www.molcells.org

Mol Cells. 2011 Apr 30; 31(4): 343–349.
doi: 10.1007/s10059-011-0044-4
PMCID: PMC3933963
PMID: 21359677

Deacetylation and Methylation at Histone H3 Lysine 9 (H3K9) Coordinate Chromosome Condensation during Cell Cycle Progression

Jin-Ah Park,[1,4] Ae-Jin Kim,[1,4] Yoonsung Kang,[2] Yu-Jin Jung,[1] Hyong Kyu Kim,[3] and Keun-Cheol Kim[1,*]

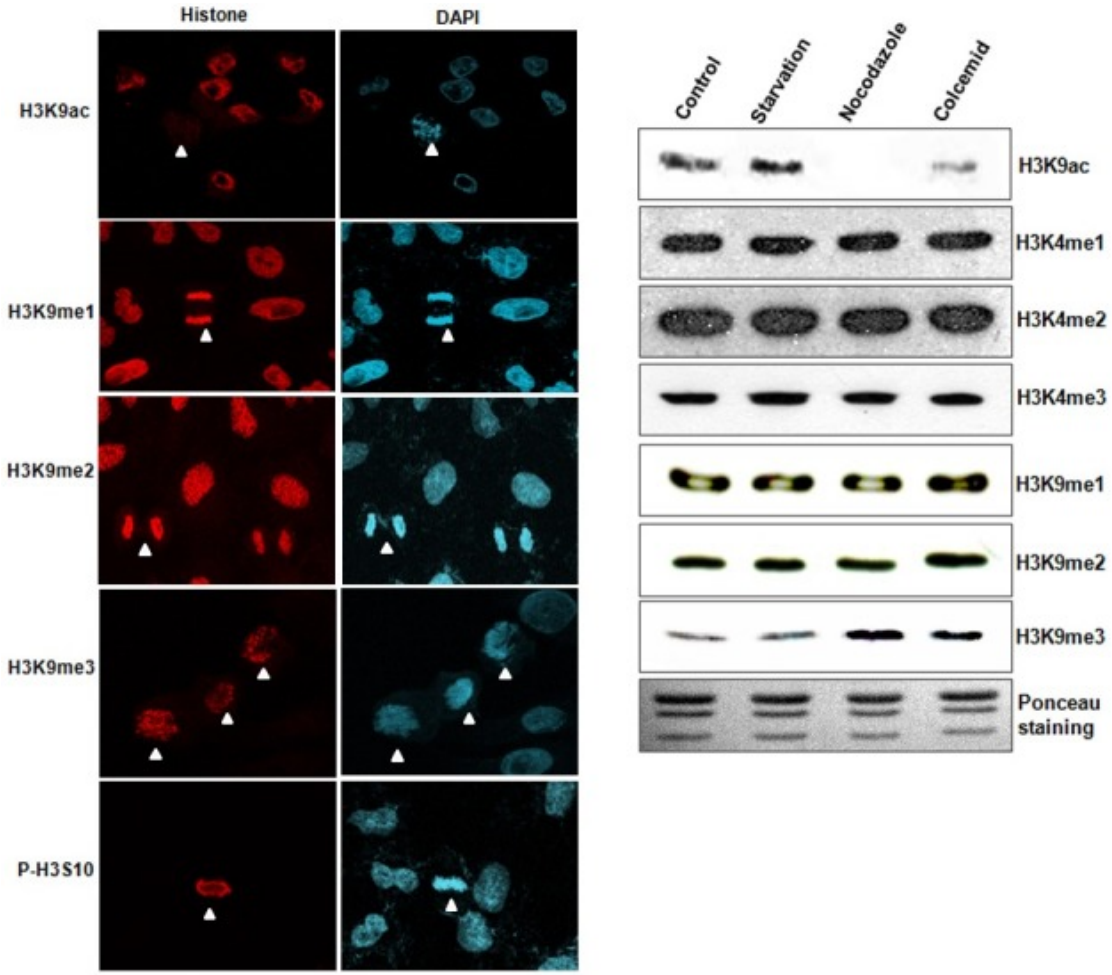
Abstract

Go to:

Interphasic chromatin condenses into the chromosomes in order to facilitate the correct segregation of genetic information. It has been previously reported that the phosphorylation and methylation of the N-terminal tail of histone H3 are responsible for chromosome condensation. In this study, we demonstrate that the deacetylation and methylation of histone H3 lysine 9 (H3K9) are required for proper chromosome condensation. We confirmed that H3K9ac levels were reduced, whereas H3K9me3 levels were increased in mitotic cells, via immunofluorescence and Western blot analysis. Nocodazole treatment induced G2/M arrest but co-treatment with TSA, an HDAC inhibitor, delayed cell cycle progression. However, the HMTase inhibitor, AdoX, had no effect on nocodazole-induced G2/M arrest, thereby indicating that sequential modifications of H3K9 are required for proper chromosome condensation. The expression of SUV39H1 and SETDB1, H3K9me3-responsible HMTases, are specifically increased along with H3K9me3 in nocodazole-arrested buoyant cells, which suggests that the increased expression of those proteins is an important step in chromosome condensa-tion. H3K9me3 was highly concentrated in the vertical chromosomal axis during prophase and prometaphase. Collectively, the results of this study indicate that sequential modifications at H3K9 are associated with correct chromosome condensation, and that H3K9me3 may be relevant to the condensation of chromosome length.

Keywords: chromosome condensation, deacetylation, H3K9, methylation, SETDB1, SUV39H1

Fig. 1.



Changes of histone modifications during cell cycle progression. (A) Histone modifications (red) were determined by immunostaining in culturing A549 cells. DNA was DAPI-stained for assumptions of mitotic stage. Acetylation of H3K9 (H3K9ac) was reduced in the mitotic cells, but trimethyation of H3K9 (H3K9me3) was increased in the mitotic cells. However, monomethylation of H3K9 (H3K9me1) and dimethylation of H3K9 (H3K9me2) were not changed under identical conditions. P-H3S10 is shown as a mitotic control. White arrow head; mitotic cells. (B) Western blot analyses were conducted using synchronized HeLa cells. H3K9me3 and H3K9ac expressions were altered in the nocodazole and colcemid treatment groups, but not in the serum starvation group.

Biochim Biophys Acta. 2014 Dec; 1839(12): 1362–1372.
doi: 10.1016/j.bbagrm.2014.02.007
PMCID: PMC4316174
PMID: 24560929

Understanding the relationship between DNA methylation and histone lysine methylation ☆

Nathan R. Rose[1] and Robert J. Klose*

‣ Author information ‣ Article notes ‣ Copyright and License information Disclaimer

Abstract

Go to:

DNA methylation acts as an epigenetic modification in vertebrate DNA. Recently it has become clear that the DNA and histone lysine methylation systems are highly interrelated and rely mechanistically on each other for normal chromatin function *in vivo*. Here we examine some of the functional links between these systems, with a particular focus on several recent discoveries suggesting how lysine methylation may help to target DNA methylation during development, and *vice versa*. In addition, the emerging role of non-methylated DNA found in CpG islands in defining histone lysine methylation profiles at gene regulatory elements will be discussed in the context of gene regulation. This article is part of a Special Issue entitled: Methylation: A Multifaceted Modification — looking at transcription and beyond.

Keywords: DNA methylation, Histone lysine methylation, Epigenetics, CpG island, Embryonic development

There is an emerging realisation that **DNA methylation** and **histone lysine methylation** in mammals are highly interrelated.

Targeting of DNA methylation is mechanistically linked to **H3K9 methylation**.

Uhrf1 acts as a link between **H3K9 methylation** and **maintenance methylation** during DNA replication.

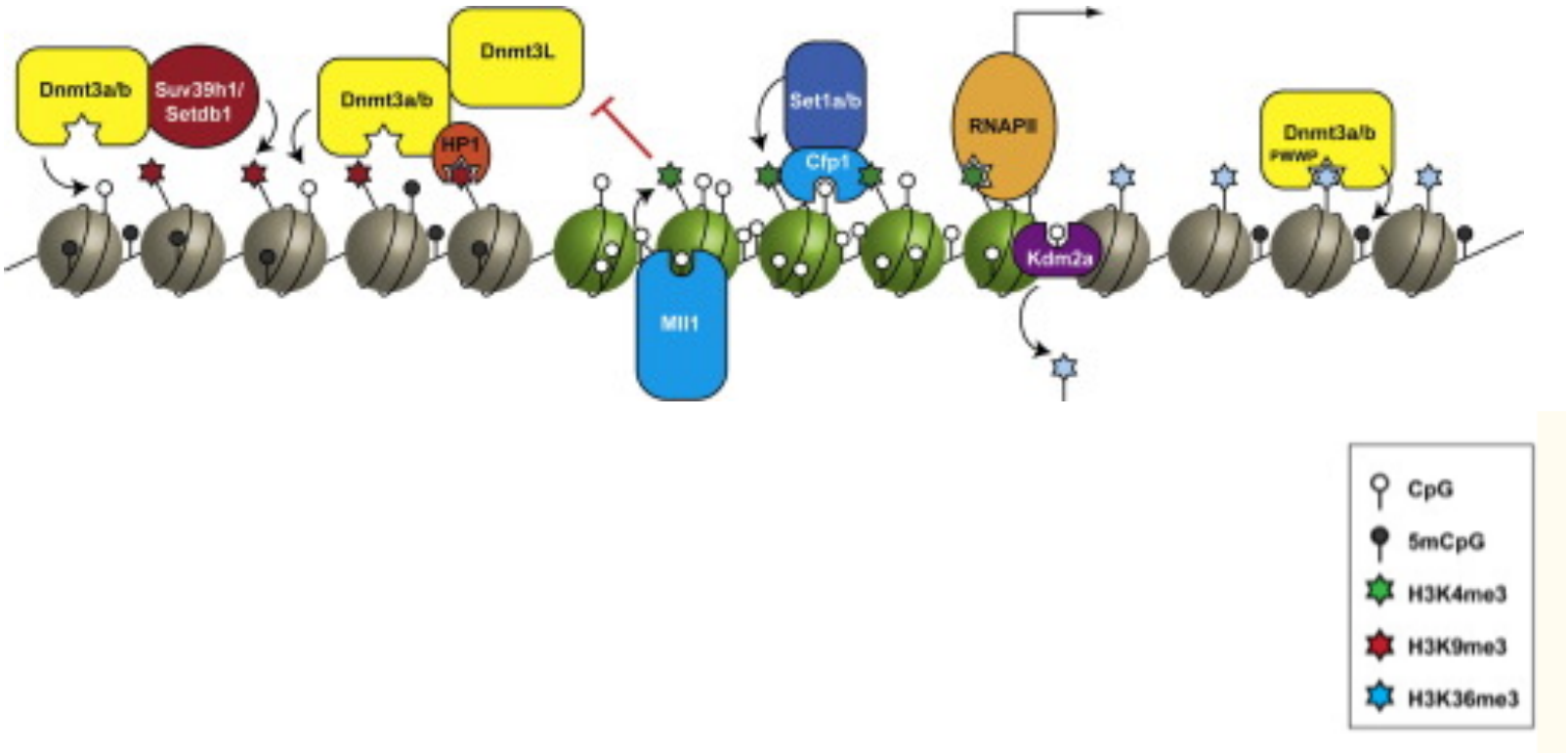
See previous slides…

Targeting of **Dnmt3a/b** is influenced by **H3K4** and **H3K36 methylation**.

Non-methylated DNA at **CpG islands** influences histone methylation through **ZF-CxxC proteins.**

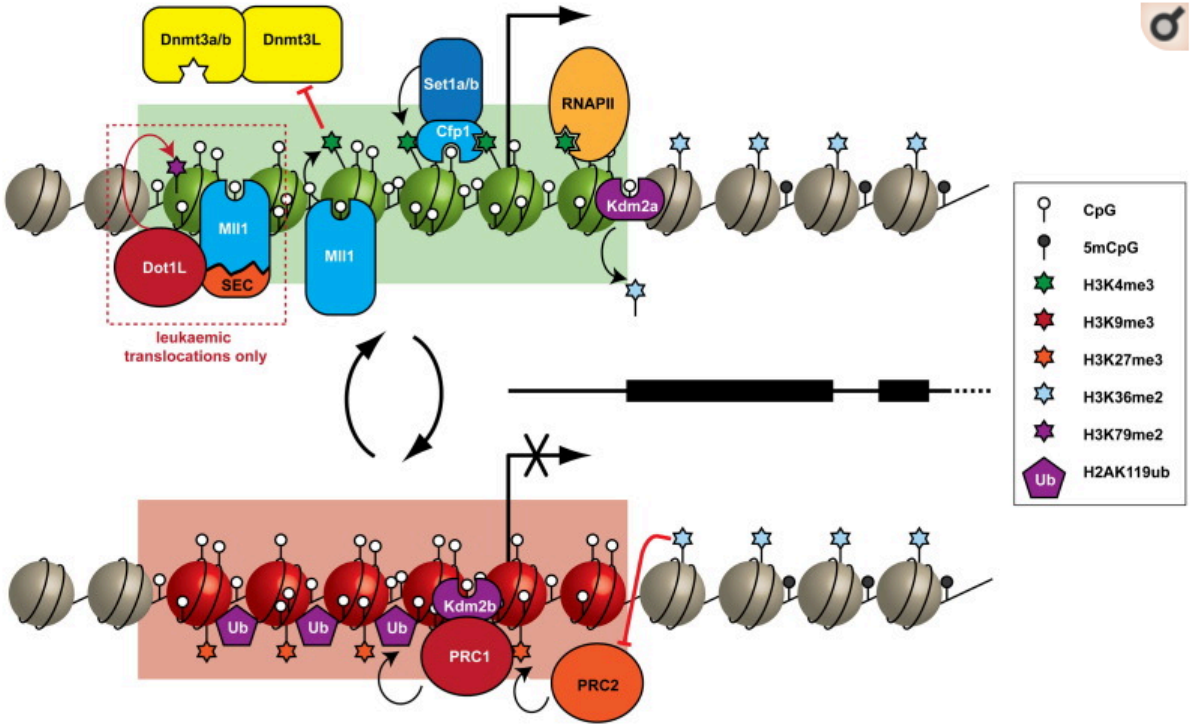
Understanding the relationship between DNA methylation and histone lysine methylation☆

Author links open overlay panel
Nathan R.Rose1Robert J.Klose



Understanding the relationship between DNA methylation and histone lysine methylation☆

Author links open overlay panel
Nathan R.Rose1Robert J.Klose



CpG islands are associated with distinctive chromatin environments.

(a) **CpG islands** associated with actively transcribed genes **recruit H3K4 methyltransferases** (Mll1/2, Set1a/b) through interactions between ZF-CxxC domains and non-methylated CpG dinucleotides. RNA PolII associates with H3K4me3, while Kdm2a/b remove H3K36me2 from CpG islands.
CpG islands also block DNA methylation by preventing the binding of **Dnmt3L/Dnmt3a/b** to histone tails that are methylated at H3K4.

(b) **CpG island** associated genes may be repressed by the **polycomb complexes PRC1 and PRC2.** PRC1 can be recruited to CpG islands *via* interaction with Kdm2b to ubiquitylate H2AK119. PRC2 is targeted *via* unknown mechanisms to methylate H3K27 at CpG islands, though it may be excluded from non-CpG island regions by its reduced ability to methylate substrates that have H3K36 methylation.

Understanding the relationship between DNA methylation and histone lysine methylation☆

Author links open overlay panel
Nathan R.Rose1Robert J.Klose

Epigenetic Effects Can Be Inherited

- Epigenetic effects can result from modification of a nucleic acid **after** it has been synthesized **or** by the perpetuation of protein structures.

- Epigenetic effects may be inherited through generations (**transgenerational epigenetics**).