CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

9 Février, 2015

Cours II
"Les systèmes de mémorisation liées à la chromatin (1)"

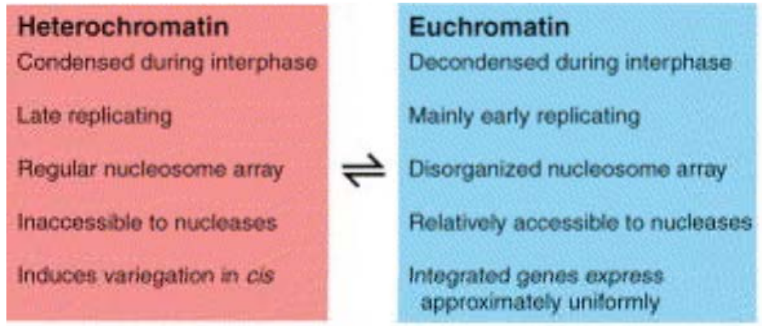
Seminaire: Prof. Robin ALLSHIRE (Université d'Edinburgh)
"Epigenetic inheritance of specialised chromatin states"

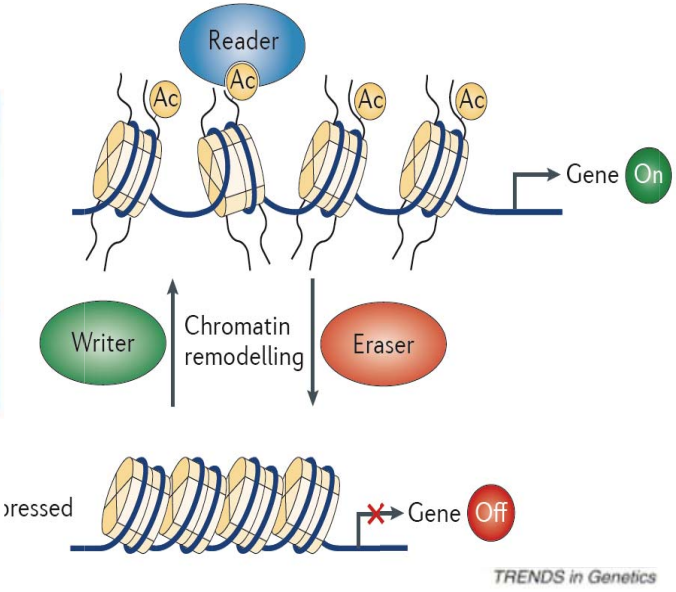
Chromatin: the physological template of the genome

SUMMARY of FIRST LECTURE

Discovery of the links between chromatin states and gene expression in model organisms (Drosophila, yeast, mouse…)

- **Cell staining methods:** correlations between transcription, cell cycle and chromatin
- **Genetics:** the exceptions to Mendel's rules where gene expression can vary within an individual tissue in a clonal way – eg PEV, X inactivation…)
- **Biochemistry**: links between histone modifications, gene expression and chromatin
- **Developmental biology:** chromatin can be a barrier or a facilitator of gene expression





Chromatin: the physological template of the genome

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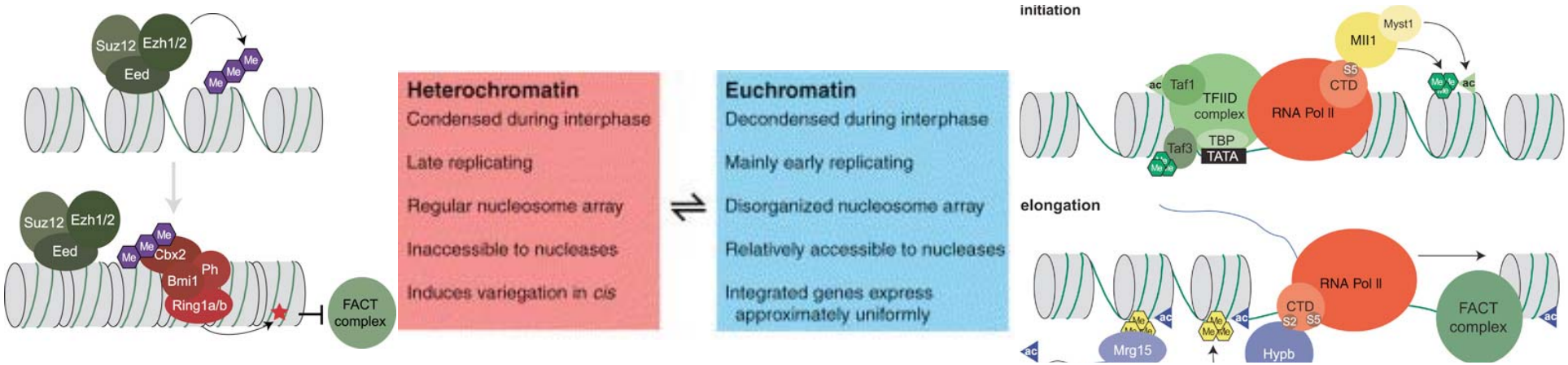


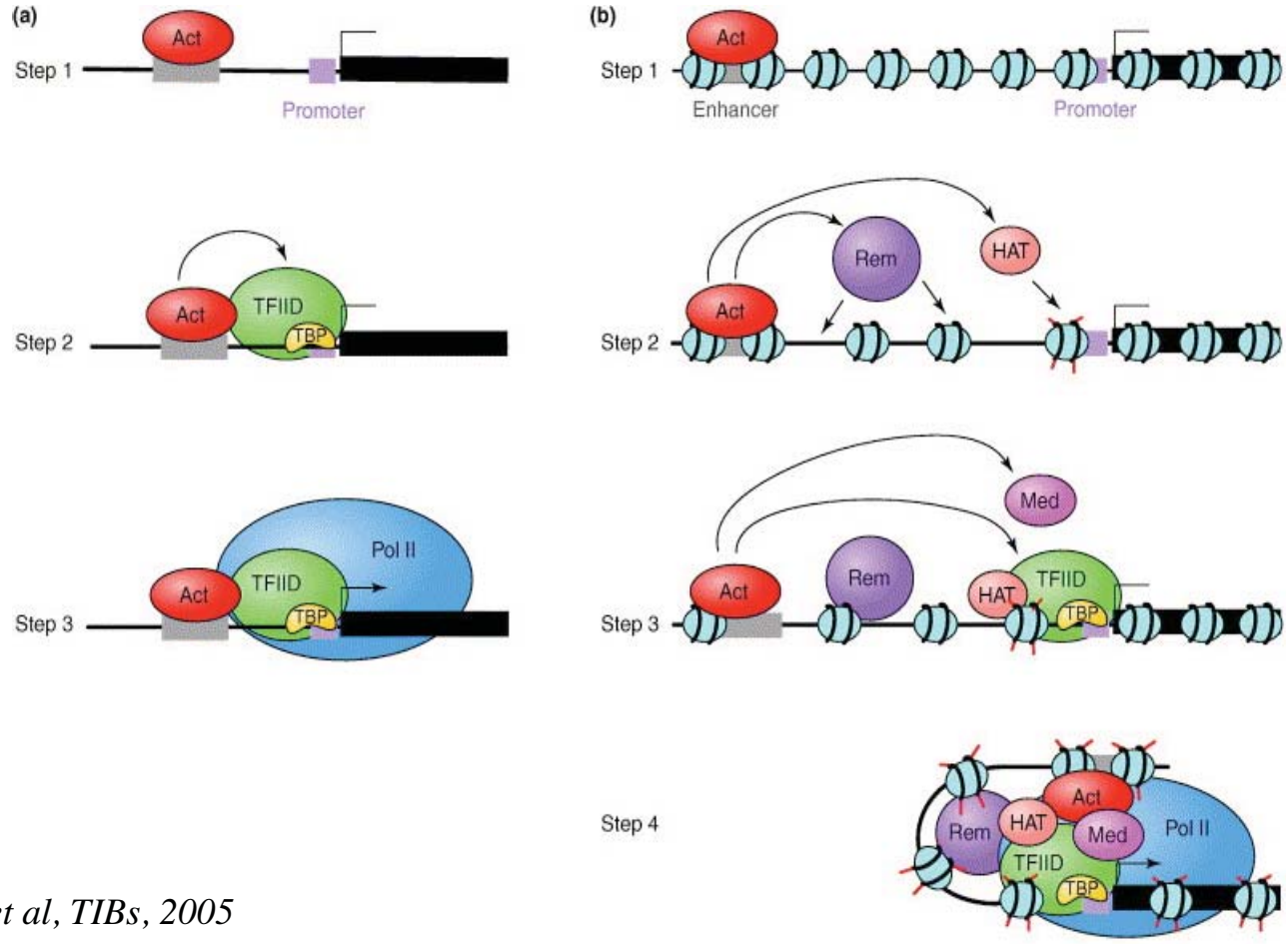
- **Different "flavors" of chromatin**: histone variants, histone modifications, histone modifiers (writers/erasers), histone binding proteins (readers), DNA methylation, non-coding RNAs…

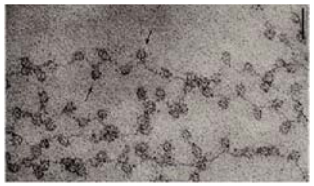
Chromatin: the physological template of the genome

Eukaryotic gene expression occurs in a chromatin context

Transcription from Naked DNA

Transcription in Chromatin





From Szutorisz et al, TIBs, 2005

Key:

Act Sequence-specific activator

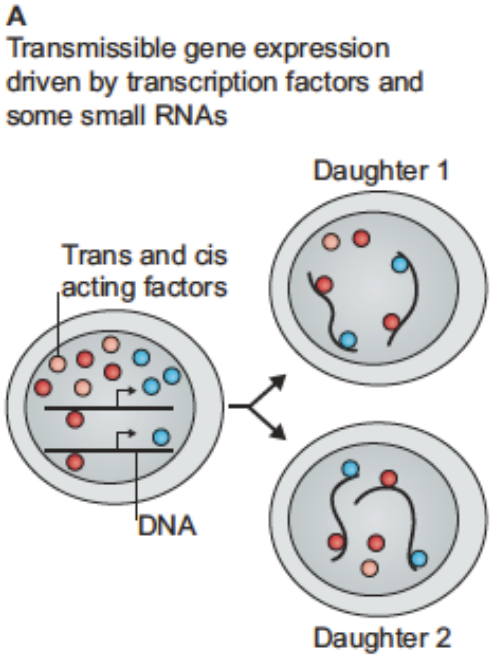
Rem ATP-dependent chromatin remodelling complex

Med Mediator

HAT Histone acetyltransferase

Chromatin: a carrier of cellular memory?

Truly epigenetic factors have to:
- be maintained through cell division;
- template their own duplication;
- be heritable in the absence of ongoing inducing signals



A. Fisher andN. Brockdorff, 2013

Mark Ptashne: On the use of the word 'epigenetic'.
Curr Biol. 2007, 17:R233-6.

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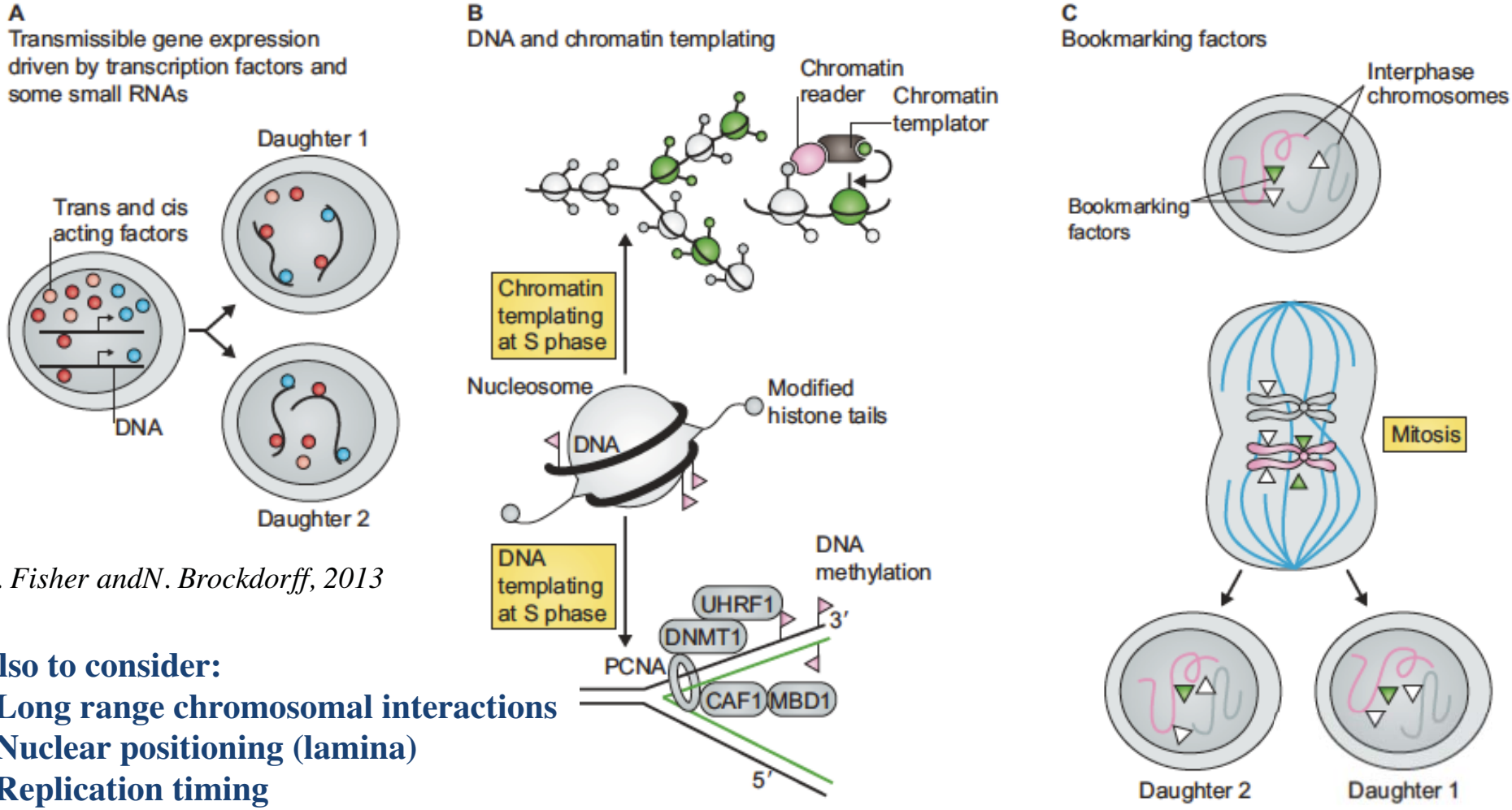
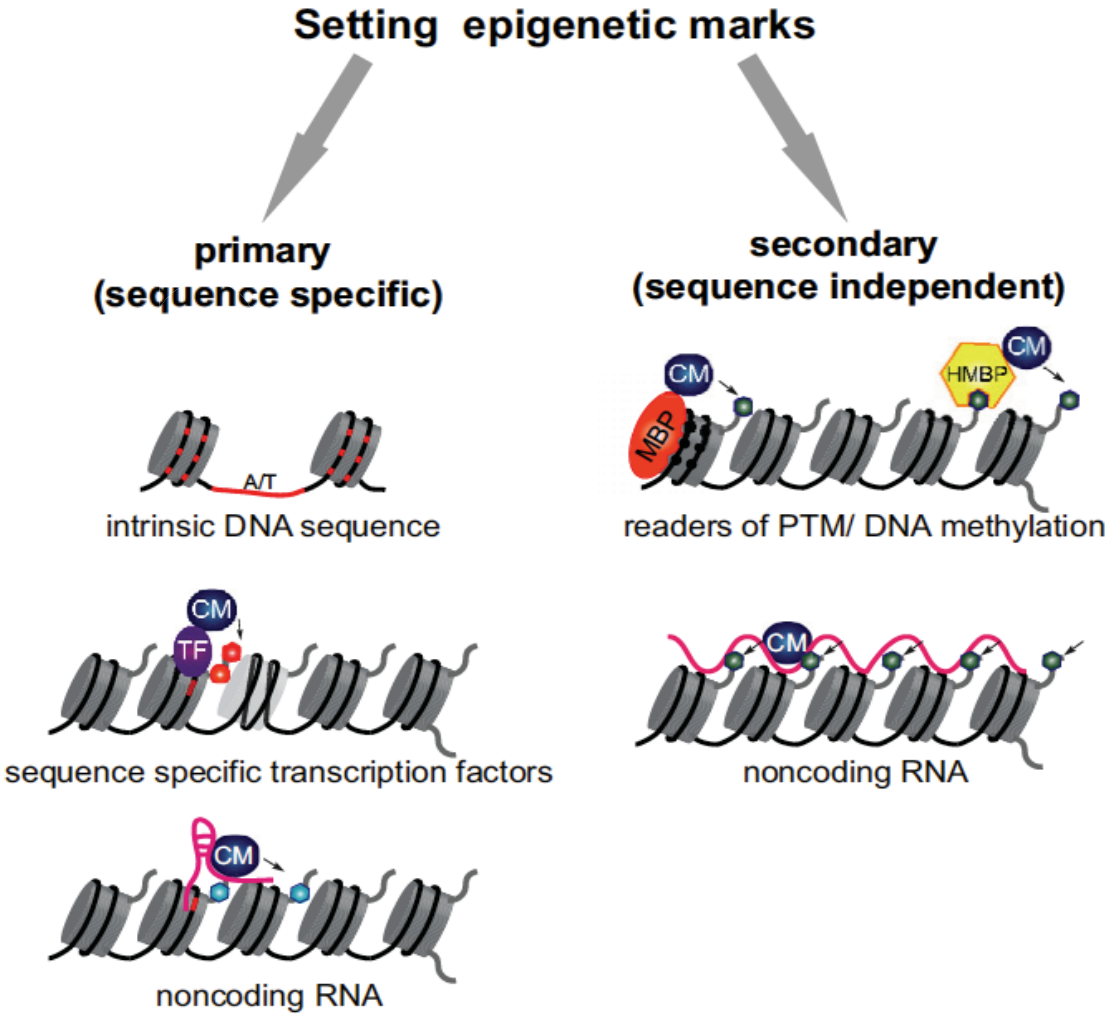


A. Fisher andN. Brockdorff, 2013

Also to consider:
- **Long range chromosomal interactions**
- **Nuclear positioning (lamina)**
- **Replication timing**

Setting Chromatin Marks

Chromatin modifying activities, chromatin associated proteins, DNA methyl binding proteins can be targeted either in a primary (DNA or RNA seq) or secondary manner during development, in response to signalling (eg hormonal), upon DNA damage….

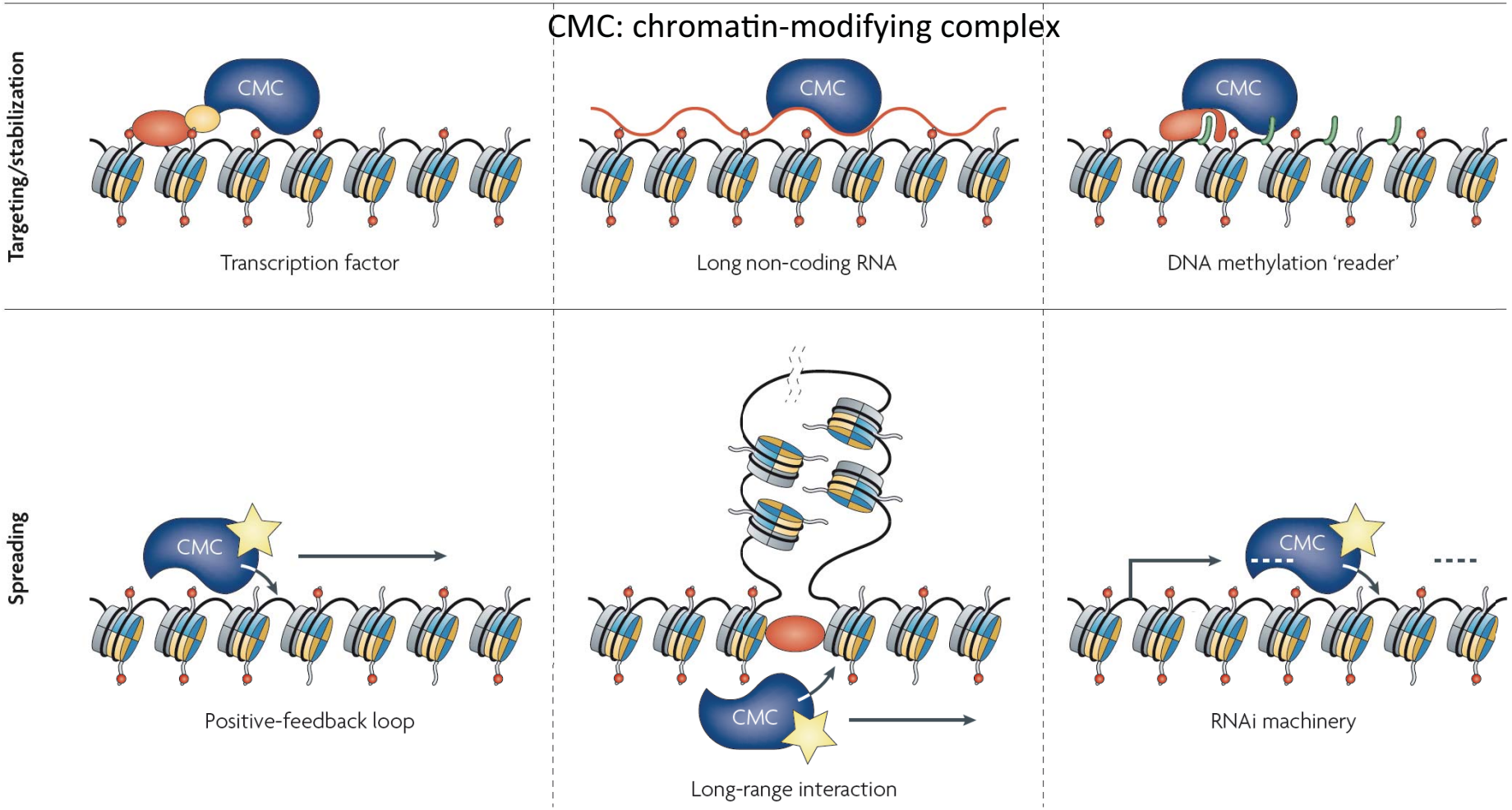


From: Hassler and Egger (2012), *Biochimie* 94, 2219-2230

Perpetuating Chromatin Marks

To what extent chromatin marks are truly 'epigenetic' in the heritable sense remains very much an open question.

One of more of these processes can participate in maintaining a chromatin mark over time….



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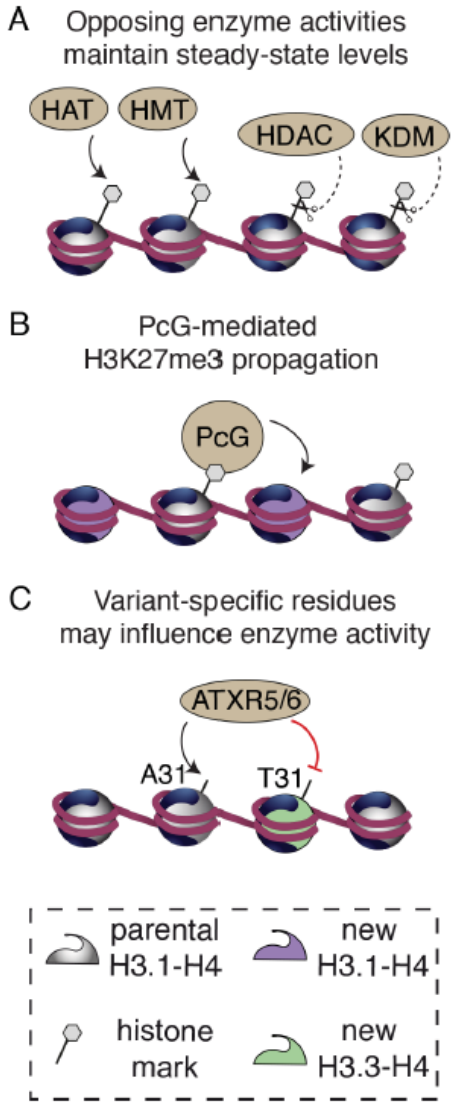
Opposing enzyme activities, (methylation/demethylation; acetylation/deacetylation), chromosome remodeling versus tethering of factors

Together can maintain **steady state levels**

In addition, chromatin states can be **maintained** or **erased** when cells duplicate their genome (DNA replication) and divide (mitosis)

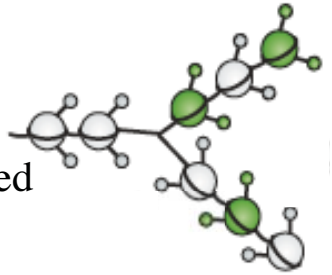


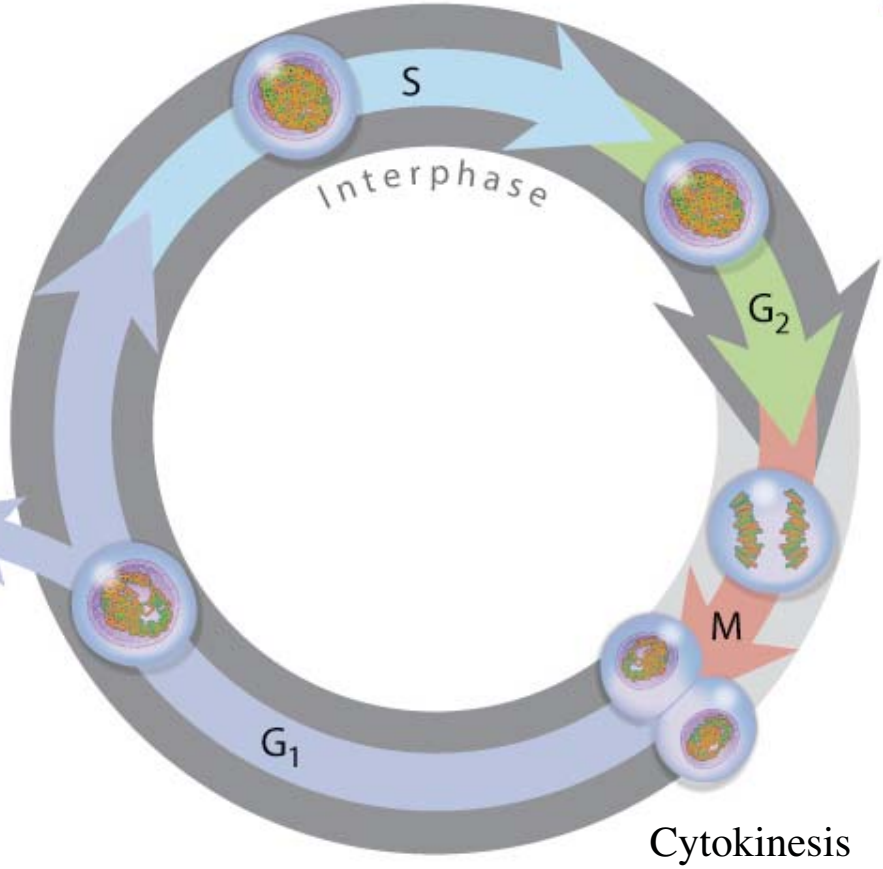
From: Gurard-Levin and Almouzni, 2014

Cellular memory through the cell cycle

S-phase:

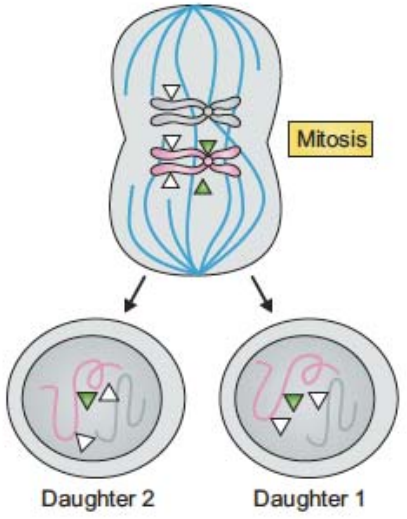
DNA synthesis
Each of the chromosomes is duplicated





G_2-phase:

preparation for cell division, protein synthesis



G_0

Cell arrest
Some G_0 cells are quiescent
ie can never re-enter cell cycle

Cytokinesis

G_1 cell growth, protein synthesis

E. Heard, February 9th, 2015

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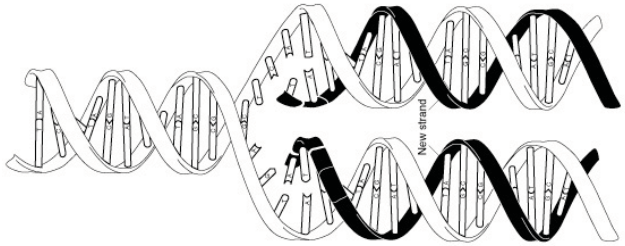
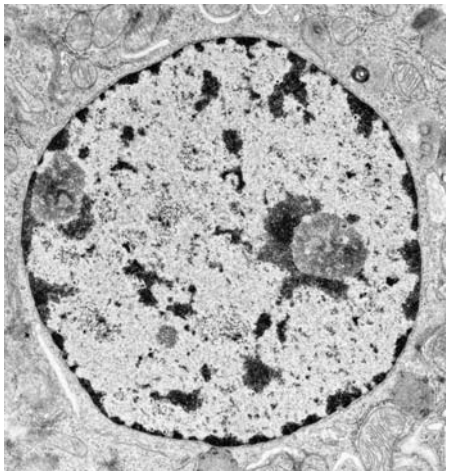
DNA replication

During S phase the cell must completely and accurately replicate a heterogeneously chromatin-packaged genome

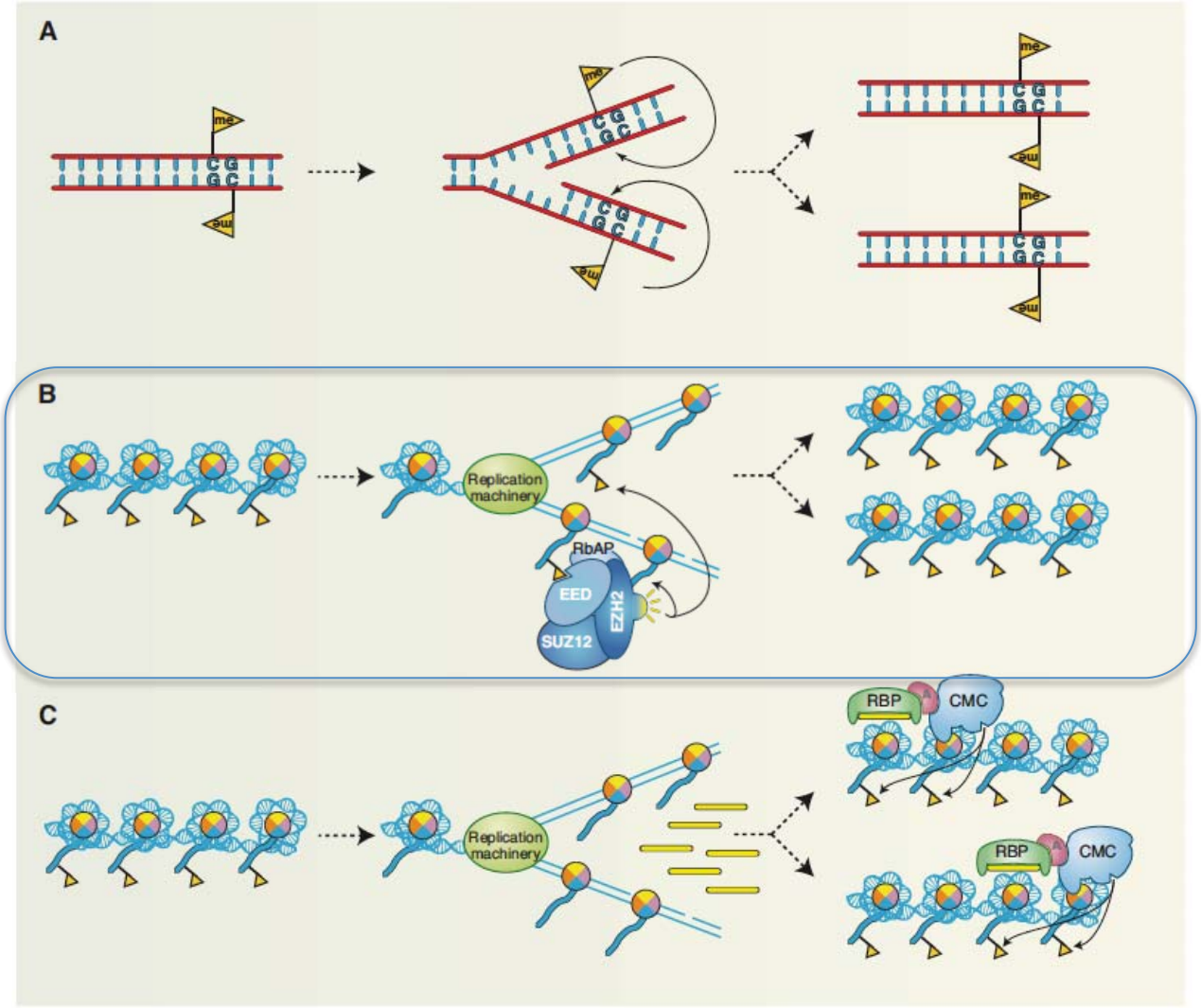


Semi-conservative DNA replication
Each strand of DNA remains intact and serves as a template for synthesis of a complementary strand, as predicted by Watson and Crick (1953) and proven by Meselson and Stahl1 (1958)



- DNA replication poses a particular challenge for chromatin state maintenance.
- Chromatin undergoes destabilization and re-assembly on the 2 daughter strands.
- Accurate DNA and chromatin replication is critical for maintenance of genomic and epigenomic integrity.
- S-phase is a window of opportunity to change the chromatin landscape

Epigenetic inheritance and DNA replication



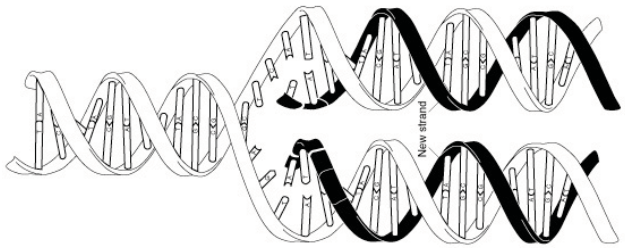
✓

?

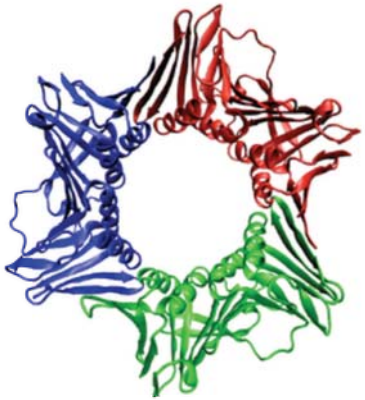
✓

Chromatin replication

The DNA "replisome" also provides the scaffold for replicating chromatin



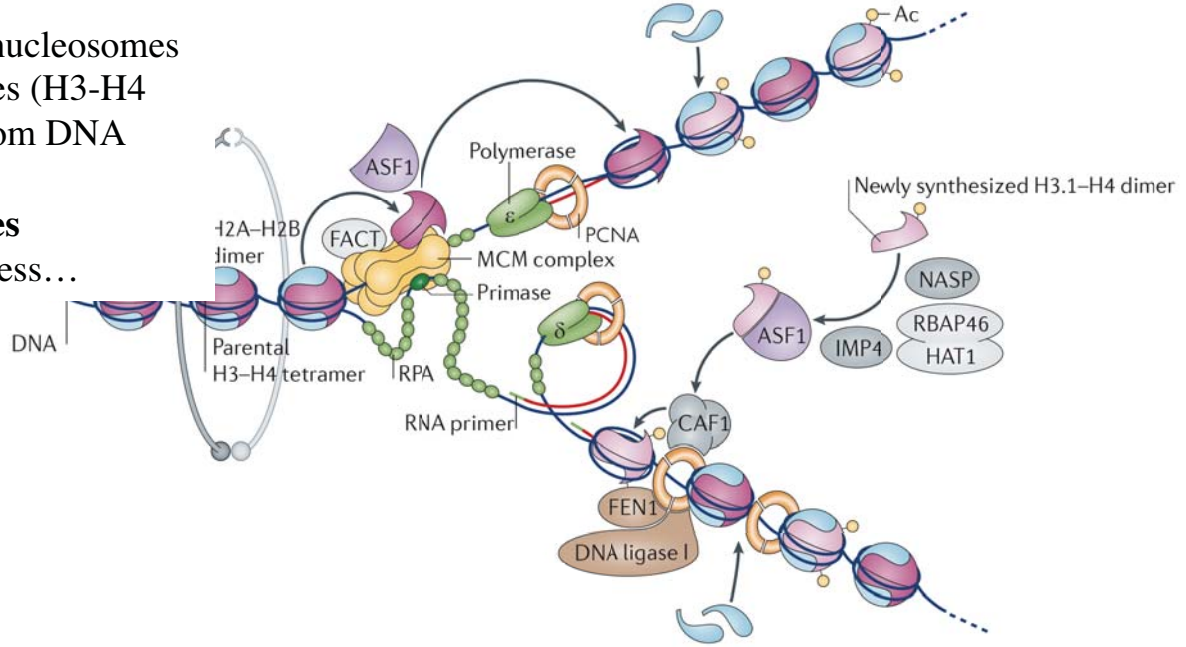
Semi-conservative DNA replication
(Meselson and Stahl, 1958)



Proliferating cell nuclear antigen (PCNA):
DNA clamp and processivity factor for DNA Pol δ, essential for DNA replication.
As a **homotrimer**, it encircles the DNA acting as a **scaffold** to recruit proteins involved in **DNA replication**, **DNA repair**, **chromatin remodeling** and **epigenetics.**

FACT loosens histones in nucleosomes and helps to remove histones (H3-H4 dimers and/or tetramers) from DNA

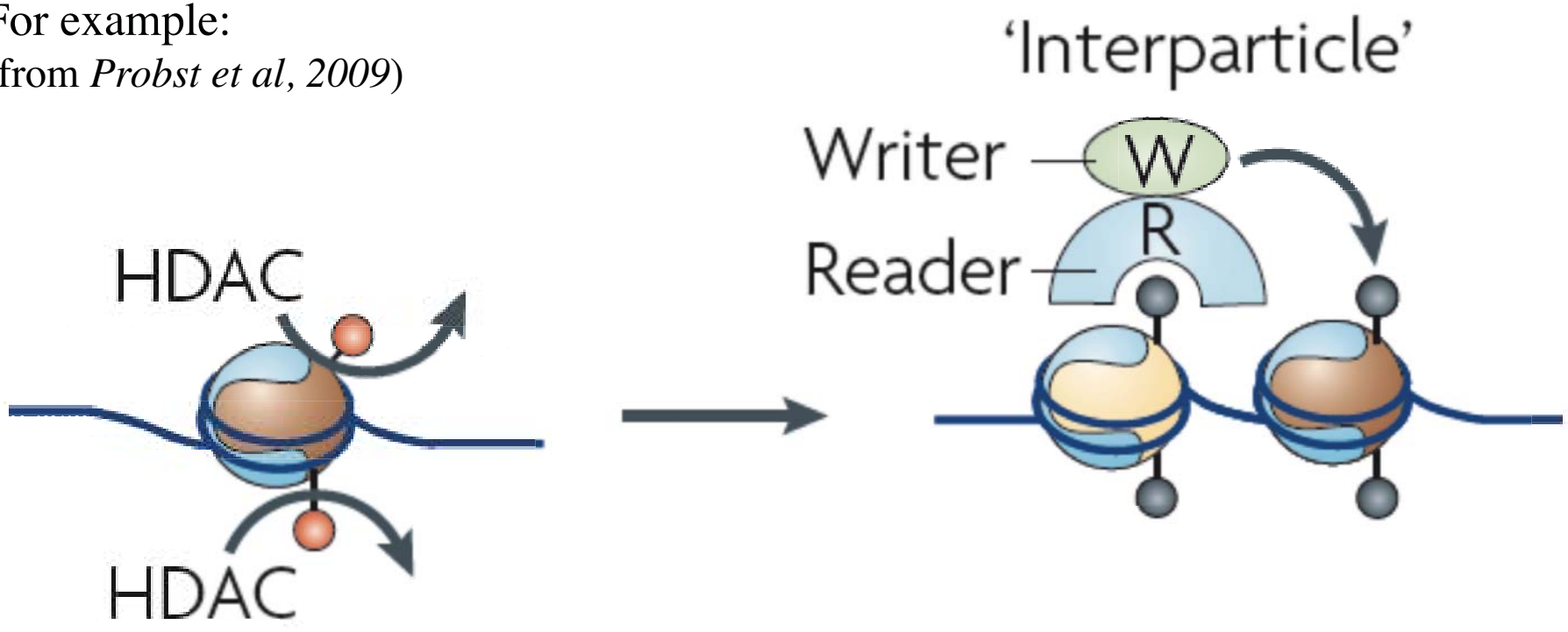
Several **Histone chaperones** accompany this whole process…



Chromatin replication

Deposition of parental H3 and H4 occurs (randomly)
within 400bp of pre-replication position
=> Histones and their modifications might be inherited, in theory.
However parental and newly synthesized H3-H4 tetramers
are intermixed + diluted
How can histone modifications be propagated – and are they propagators comparable to DNA methylation?

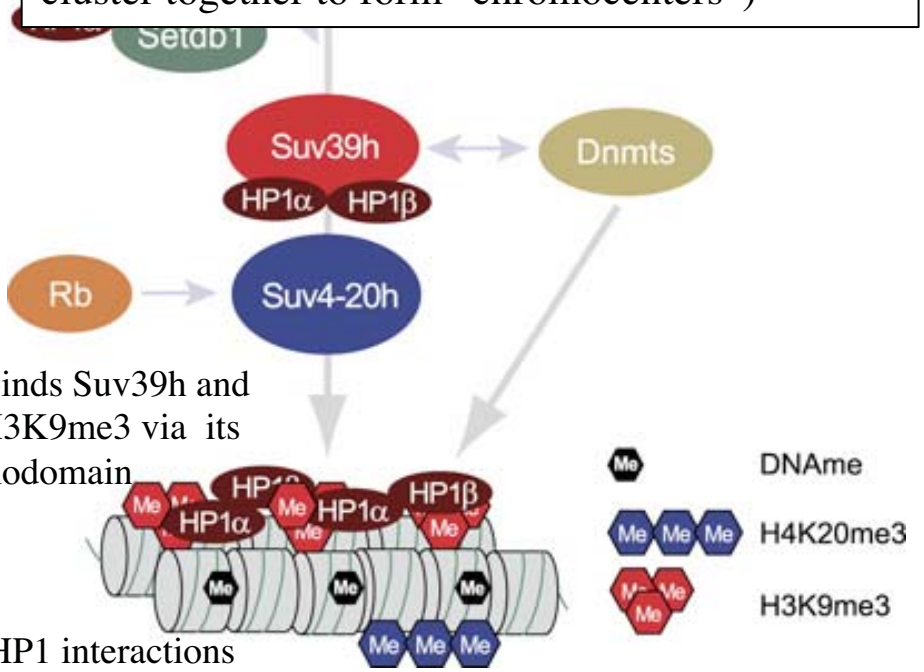
For example:
(from *Probst et al, 2009*)



Example of Constitutive Heterochromatin

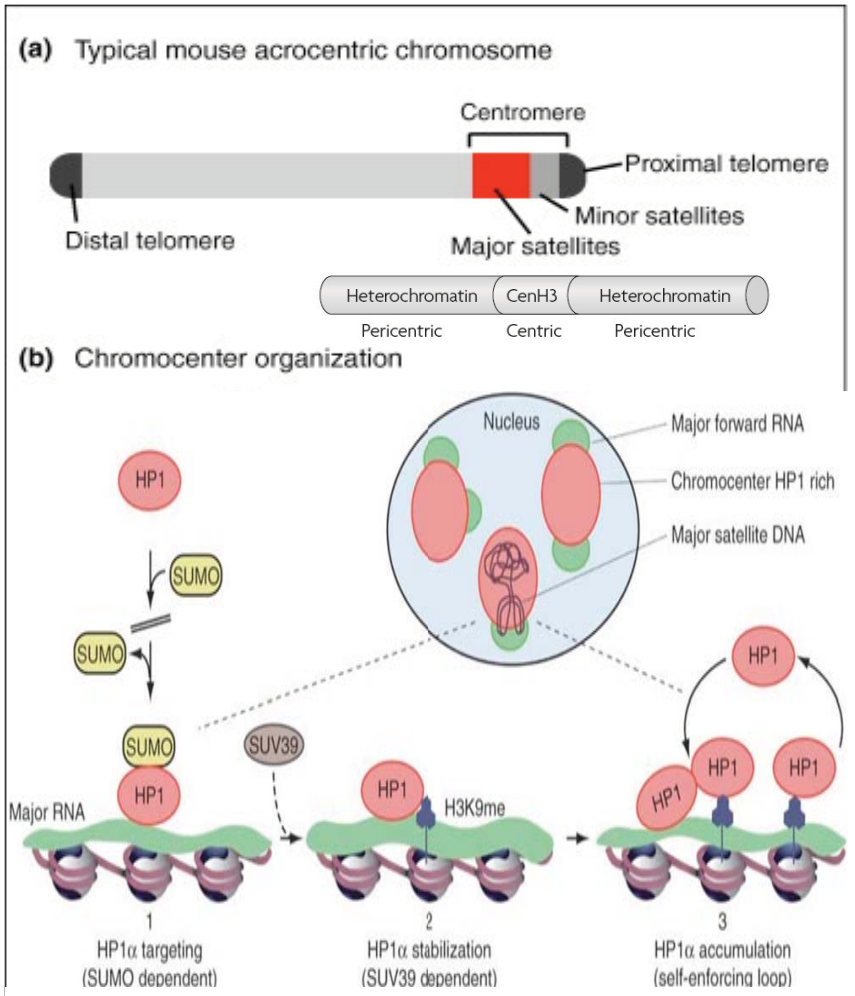
In mammalian cells, H3K9 trimethylation (H3K9me3) is a hallmark of constitutive heterochromatin (Peters et al., 2002) and is also required for transcriptional silencing of genes and retroviral elements (Magklara et al.,2011; Matsui et al., 2010; Nielsen et al., 2001).

Pericentric heterochromatin (pHC) contributes to centromere function by ensuring sister chromatid cohesion. Pericentric heterochromatin remains condensed throughout the cell cycle (in mouse, cluster together to form "chromocenters")



HP1 binds Suv39h and also H3K9me3 via its chromodomain

HP1-HP1 interactions favour compaction?



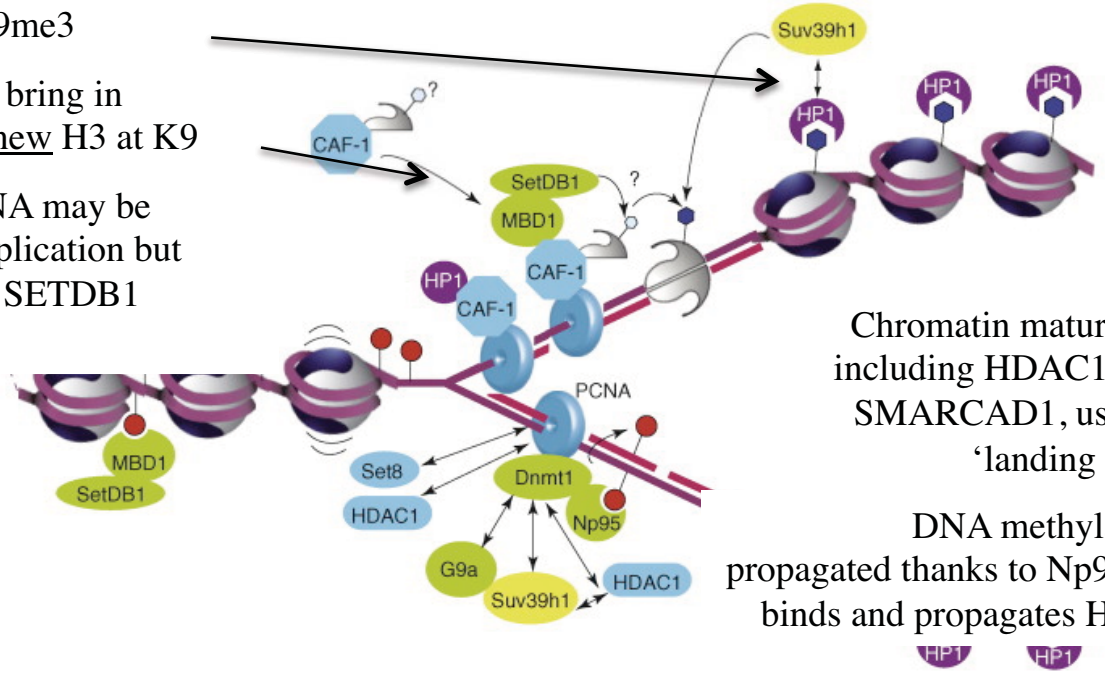
Maison et al, Nat. Genetics. 2011

Some Trans-acting Chromatin Modifiers are recruited at the Replication Fork

HP1 is recruited together with the SUV39H to <u>parental</u> histones carrying H3K9me3

CAF-1 histone chaperone helps bring in (SETDB1) to **monomethylate** <u>new</u> H3 at K9

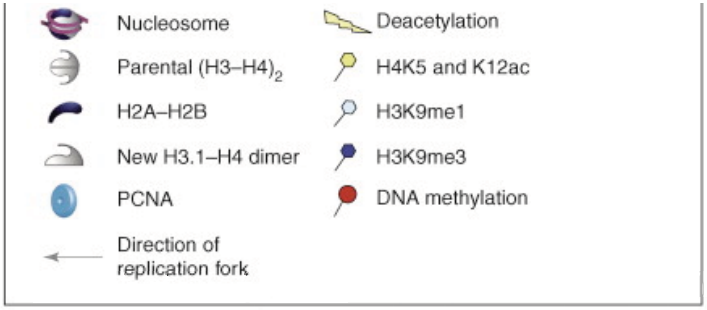
MBD1 bound to methylated DNA may be temporarily displaced during replication but brought back in with its partner SETDB1 thanks to CAF-1



Chromatin maturation factors, including HDAC1, DNMT1 and SMARCAD1, use PCNA as a 'landing pad'.

DNA methylation is propagated thanks to Np95/Uhrf1 which also binds and propagates H3K9 methylation

SMARCAD1 nucleosome remodeller complex with HDAC1, HDAC2, KAP1 and G9A integrate **nucleosome spacing** with **histone deacetylation** and **H3K9 methylation.**



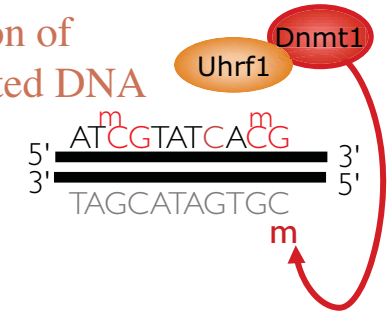
Replication of DNA Methylation

- DNA methylation found on up to 80% of CpGs in mammalian genome
- Roles in pericentric heterochromatin, XCI, imprinting…
- Dnmt1 and DNA methylation are essential for mammalian development.

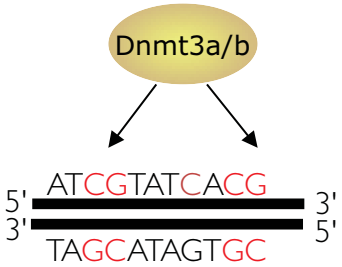
Li E, Bestor TH, Jaenisch R. (1992) *Cell* 69, 915-26.

Maintenance
DNA Methyltransferase

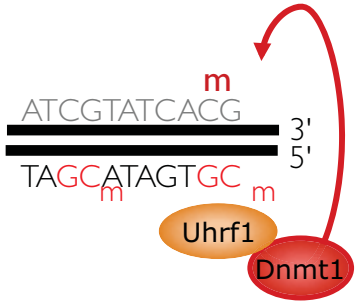
Recognition of
Hemi-methylated DNA



De novo DNA Methyltransferase



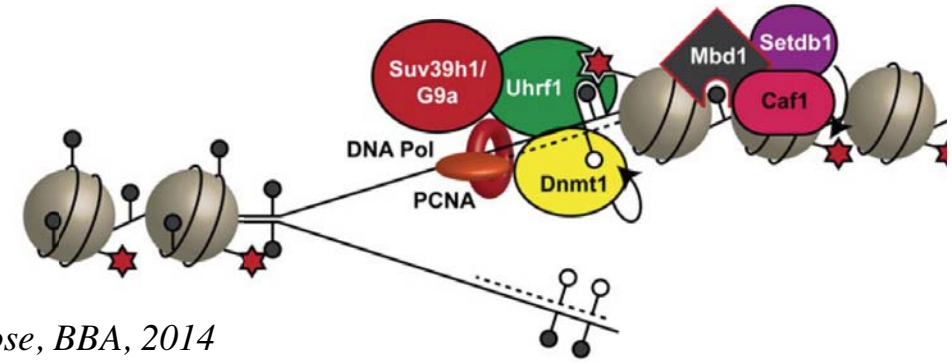
UHRF1 protein senses the presence of hemi-methylated DNA (cytosine on one strand only) and recruits DNMT1, which methylates the cytosine on the newly synthesized DNA (the preferred substrate of DNMT1 is hemi-methylated DNA)



One of the first "epigenetic" heritable marks:

Holliday, R., Pugh, J. E. (1975) *Science 187*, 226-232.
Razin, A., Riggs, A. D. (1980) *Science 210*, 604-610
Bestor, V.M. Ingram. (1983) *Proc. Natl. Acad. Sci. USA*, 80, 5559–5563
Leonhardt H. et al. (1992). *Cell*, 71, 865–873
Spada F. et al (2007) *J. Cell. Biol.,* 176, 565–571
Bostick, M. et al (2007) *Science* ;317, 1760-4.
Sharif, J. et al (2007) *Nature* 450, 908-12.

UHRF1 enables and integrates both DNA and Histone Methylation during DNA replication (and in G2/M)

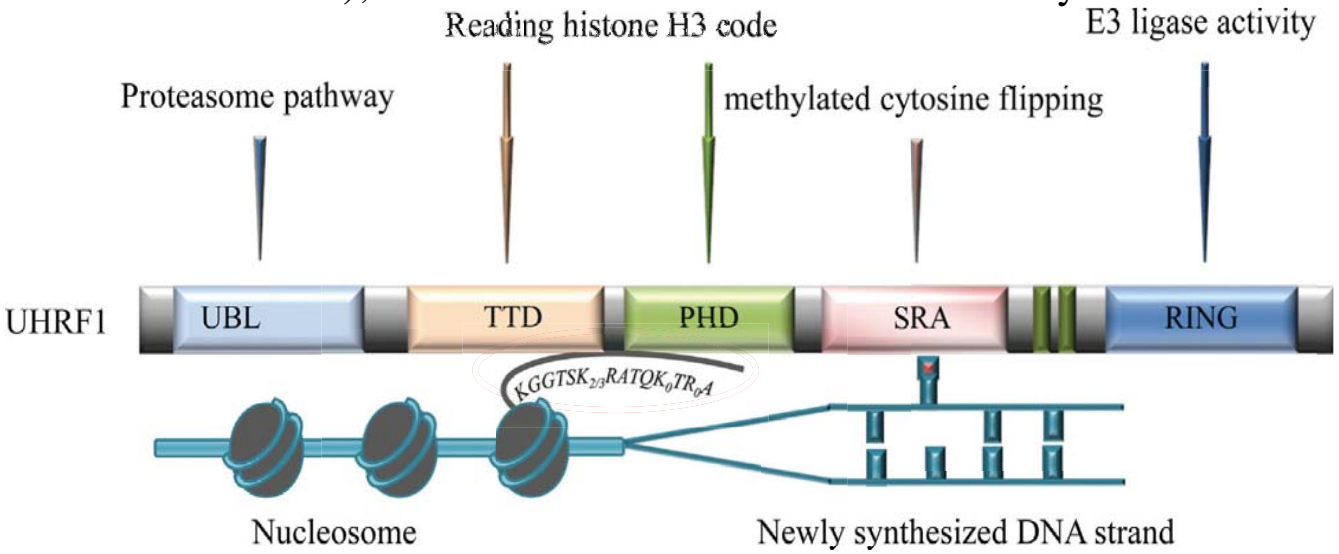


Mbd1 also recruits Setdb1 to newly synthesised chromatin by interactions with CAF1 and methylated DNA, where it assists in maintainingH3K9methylation

From: Rose and Close, BBA, 2014

Maintenance of DNA methylation and histone methylation at replication forks

Uhrf1 localises to replication forks through interactions with **PCNA**, **H3K9me3/me2** (and unmodified H3R2, K4 - via its PHD + Tandem TUDOR domain) and **hemi-methylated DNA** via the SRA (SET and RING finger Associated domain), where it recruits Dnmt1 and H3K9methyltransferases to add DNA methylation and



UHRF1 (Ubiquitin-like, containing PHD and RING finger domains, 1) is a "hub protein" involved in epigenetic information integration, eg it can sense the presence of a methyl group on both DNA & histones – more affinity for H3K9me3 than H3K4me3

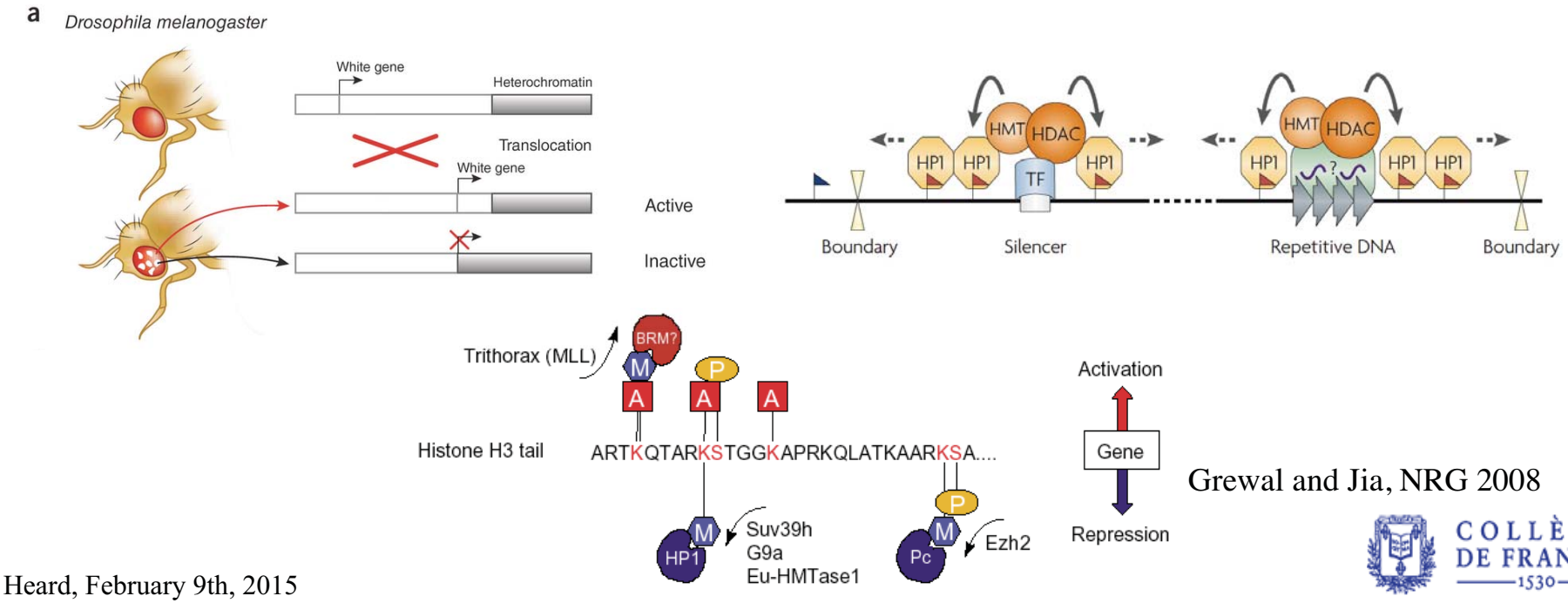
Xie S, Jakoncic J, Qian C. (2012) UHRF1 double tudor domain and the adjacent PHD finger act together to recognize K9me3-containing histone H3 tail. JMB 415:318–28.

Are histones carriers of cellular memory?

Deposition of parental nucleosomes on both nascent strands may be sufficient to "seed" propagation of histone modifications…

Histone modifications can target modification enzymes
⇒ maintenance by spreading in *cis*?

Classic example is H3K9me3 - bound by HP1 which recruits Suv39 HMTase and by oligomerising can spread the modification across a domain



Grewal and Jia, NRG 2008

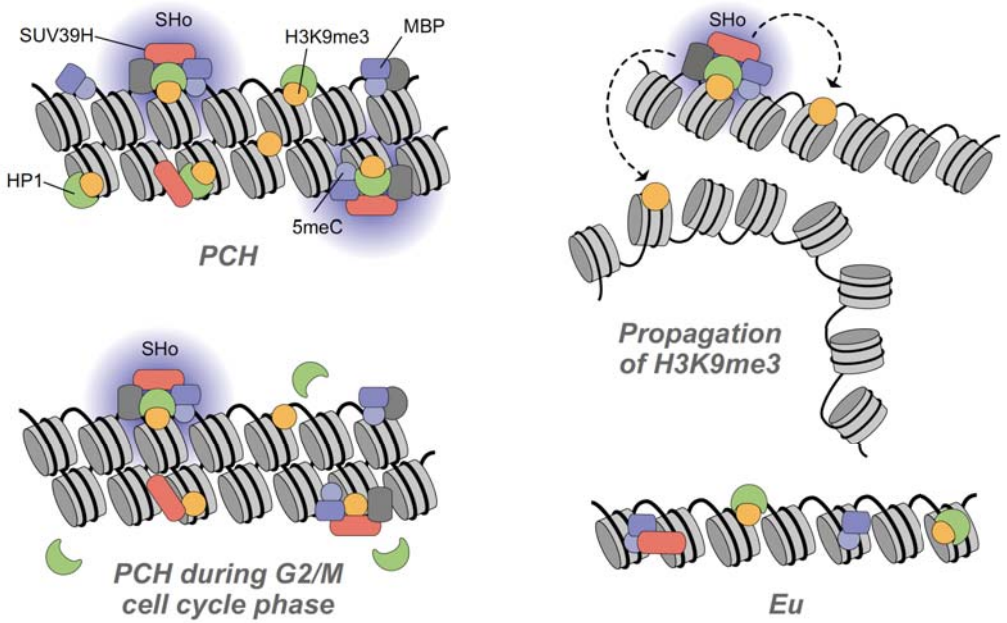
Is H3K9 methylation a propagator of cellular memory?

Deposition of parental nucleosomes on both nascent strands may be sufficient to "seed" propagation of histone modifications…

Histone modifications can target modification enzymes
⇒ maintenance by spreading in *cis*?

Mathematical models suggest thaat this can only work if domains spanning several kilobases are marked…

Müller-Ott et al, 2014



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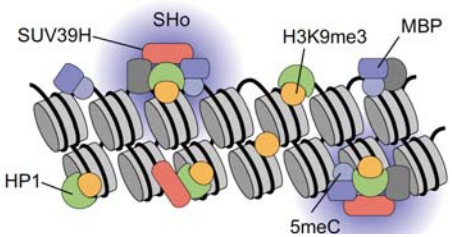
Is H3K9 methylation a propagator of cellular memory?

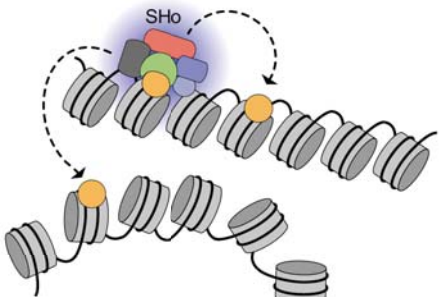
"Nucleation and looping" model - propagation of H3K9me3 in pericentric heterochromatin:

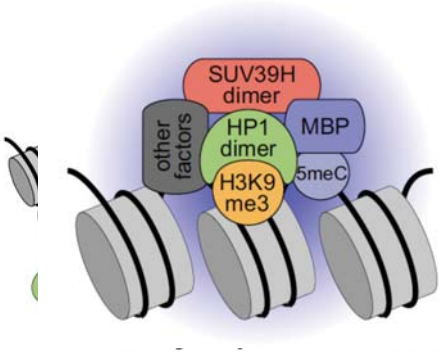
- High-affinity binding, immobilized HP1 and SUV39H = **SUV39H nucleation complex** ("Sho"), at PCH
- Low-affinity binding - single protein factors throughout the whole nucleus
- **SUV39H nucleation complex** provides a **high local concentration of the enzyme** => catalytically productive collisions in PCH (>> than soluble SUV39H)
- Via **chromatin looping**, SUV39H complexes can methylate adjacent chromatin in 3D

The persistence of stably chromatin-bound SUV39H throughout the cell cycle (including mitosis) sustains the H3K9me3 modification.



Müller-Ott et al, 2014

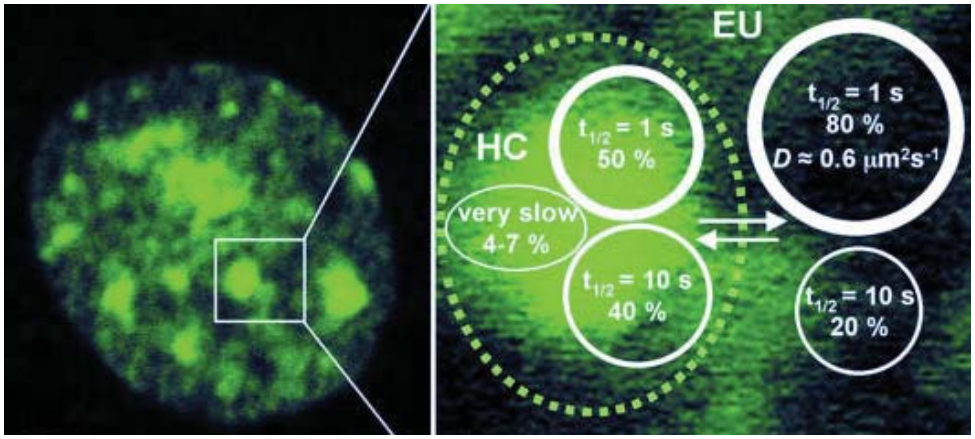




Cooperativity between marks and their readers and writers likely stabilise states

Chromatin dynamics and memory?

Chromatin is highly dynamic in interphase :
Even heterochromatin-associated proteins bind with residence times of <1min
though higher residence time in heterochromatic foci
(Phair et al. 2004; Schmiedeberg et al, 2004).



CAN HISTONE MARKS and CHROMATIN PROTEINS TRULY TRANSMIT *EPIGENETIC INFORMATION* GIVEN SUCH DYNAMICS?

Deal et al, 2010 explored genome-wide profiling of steady-state amounts of H3.3 from Drosophila S2 cells indicated that extensive nucleosome replacement occurs - most prominently across transcribed regions of active genes and at promoters and binding sites of trithorax group (trxG) and polycomb group (PcG) proteins (turnover faster than cell cycle..!)

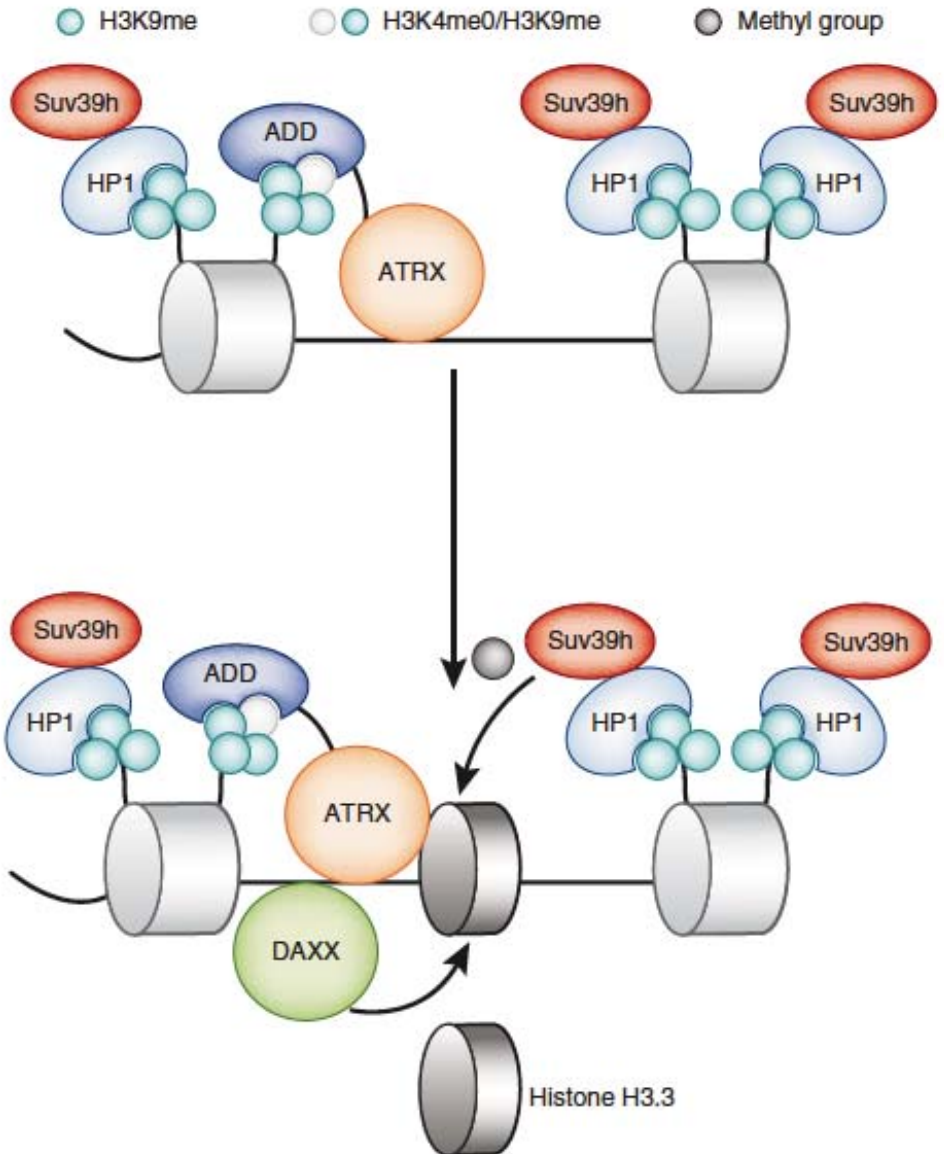
Maintenance of H3K9 methylation in a dynamic chromatin context

Model for maintenance of a histone modification through replication-independent nucleosome turnover.

The ATRX binds a site where a nucleosome has been lost; its interaction with chromatin is stabilized via the binding of its ADD domain to a histone tail bearing H3K9me3 without concurrent H3K4 methylation.

HP1 binding to H3K9me3-marked nucleosomes around this site increases the local concentration of Suv39h. ATRX recruits the H3.3-specific DAXX histone chaperone complex and facilitates deposition of a new, histone H3.3–containing nucleosome.

The high local concentration of Suv39h then facilitates H3K9 methylation of the deposited nucleosome, ensuring continuity of the mark.



From Zentner and Henikoff, NSMB, 2013

Maintaining Heterochromatin?

Design functional tests to see whether H3K9me3 can be maintained independently of an initiating signal

Hathaway et al, 2014
Ragunathan et al, 2014
R. Allshire (in press)