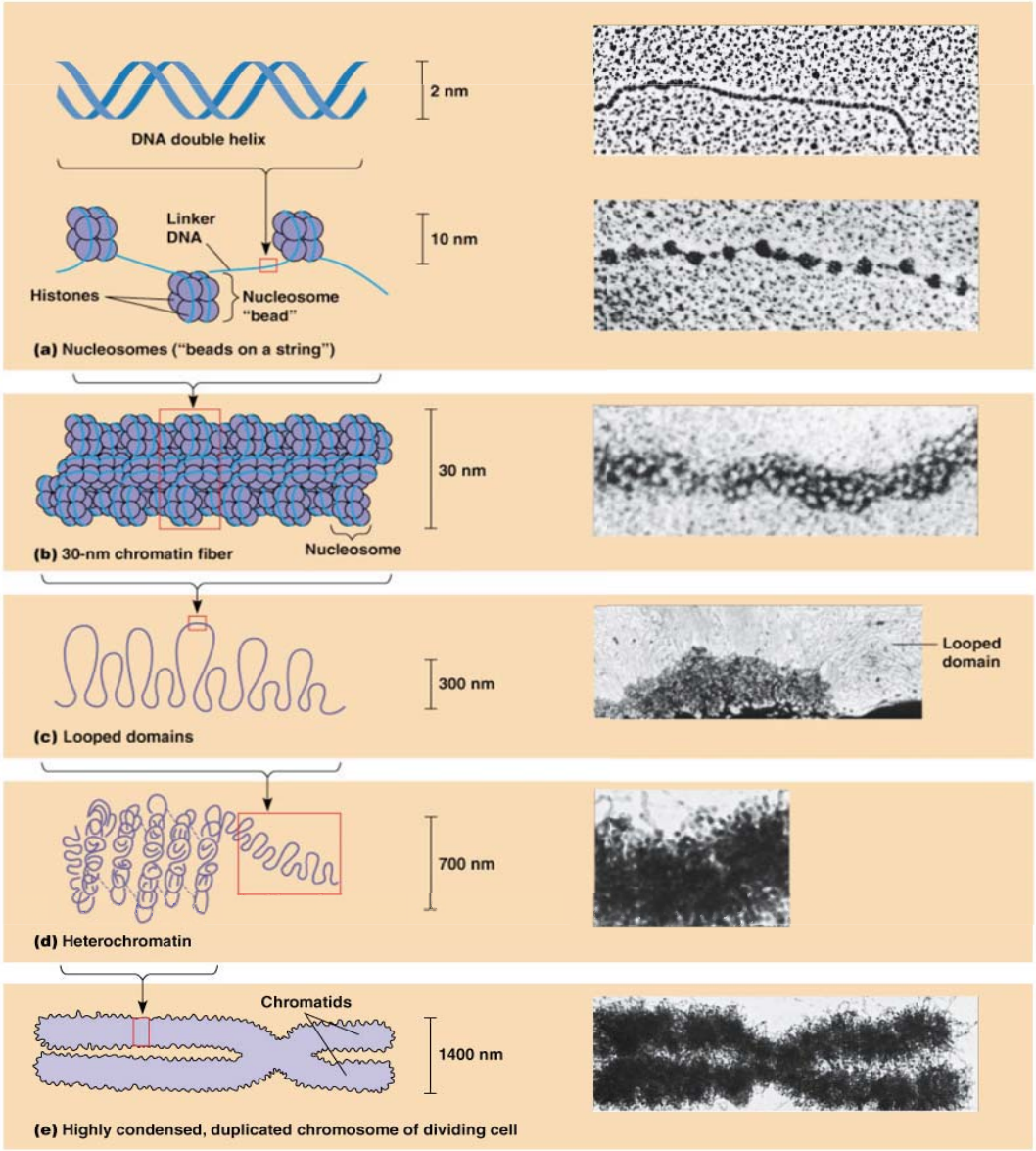
CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

2 Février, 2015

Cours I
"La chromatine et ses multiples variations"

Chromatin:
the physiological template of the genome



The History of Chromatin

F. Miescher (1871) described a strong **phosphorus-rich acid** '**nuclein**' in pus leukocytes & later in salmon sperm, which contained a protein and non-protein component

A. Kossel (1884) described the non-protein component (**nucleic acids**) and later the basic '**histon**' in acidic extracts from avian erythocyte nuclei

Nuclein from salmon sperm by Miescher

Dahn, *Dev. Biol.* 2005

(Photo Alfo
Univers
Tübin

Friedrich Miescher
(1844-1895)
Isolated nuclein and showed it was present in nuclei of all cells. He even speculated that it might have a role in the transmission of

Albrecht Kossel
(1853-1927)
Nobel Prize in 1910 for his research in cell biology, the chemical composition of the cell nucleus, and for his work in

"*If one (. . .) wants to assume that a single substance (. . .) is the specific cause of fertilization, then one should undoubtedly first and foremost consider nuclein*" (Miescher, 1874).

The Nucleic acid or the Protein?

(maybe both! Cours IV)

The History of Chromatin

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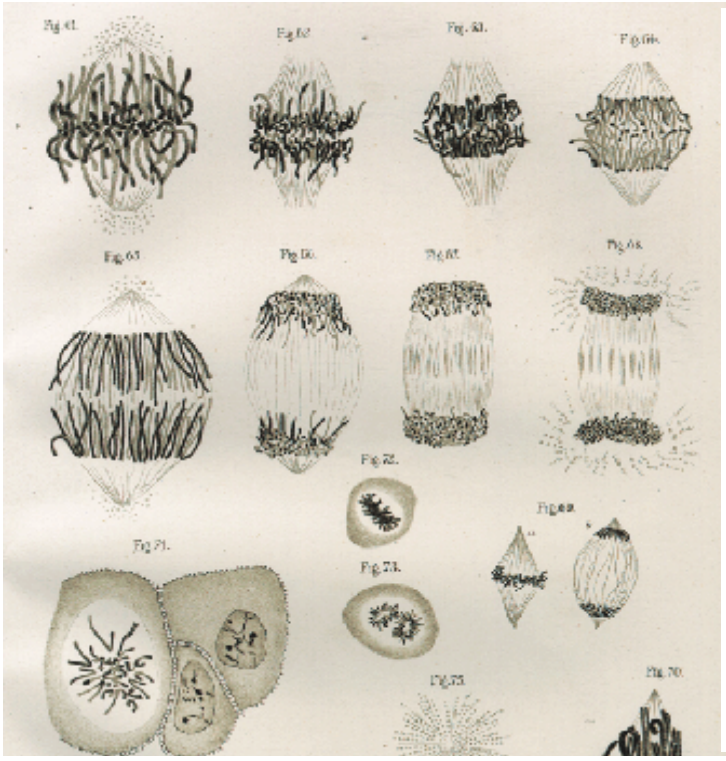
A. Kossel (1884) described the non-protein component (nucleic acid) and later the basic '**histon**' in acidic extracts from avian erythocyte nuclei

W. Flemming (1880's) coined the word **chromatin** while analysing nuclear division

The botanist **Eduard Zacharias** showed that nuclein was an integral part of chromosomes. In 1881 he was the first to combine the histological concept of chromatin with the chemical substance nuclein.



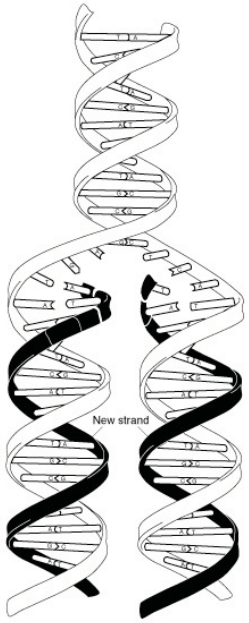
"…in view of its refractile nature, its reactions, and above all its affinity to dyes, is a substance which I have named chromatin. Possibly chromatin is identical with nuclein, but if not, it follows from Zacharias' work that one carries the other. The word chromatin may stand until its chemical nature is known, and meanwhile stands for that substance in the cell nucleus which is readily stained."
W. Flemming (1882)

By 1941, although chromatin and chromosomes were accepted to form the structural basis of heredity & genes, proteins (histones) were still postulated to be the site of genetic information.

Schultz, J. The evidence of the nucleoprotein mixture of the gene. CSH Symp Quant Biol (1941)

The Genetic Material

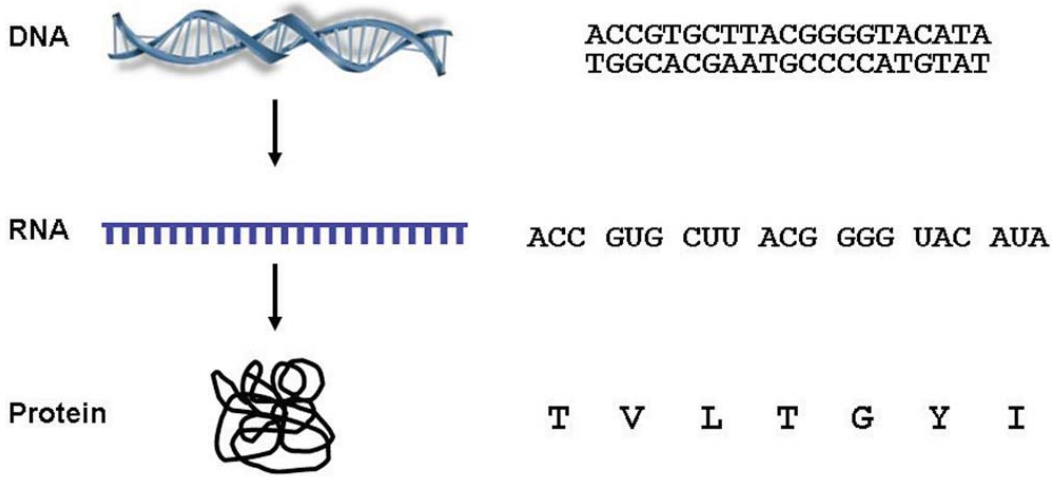
Oswald T. Avery, Colin MacLeod, and Maclyn McCarty (1944) demonstrated that **DNA** (and not protein as previously thought), could function as the **genetic material**.



The structure of the double helix

J. Watson and F. Crick (1953)

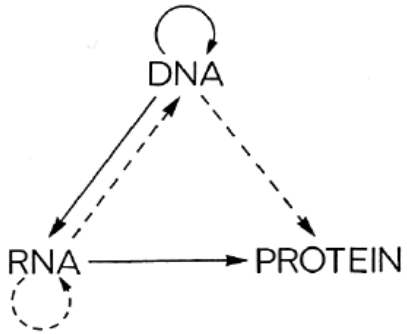
"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible **copying mechanism for the genetic material**."



Central Dogma of Molecular Biology

by
FRANCIS CRICK
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.



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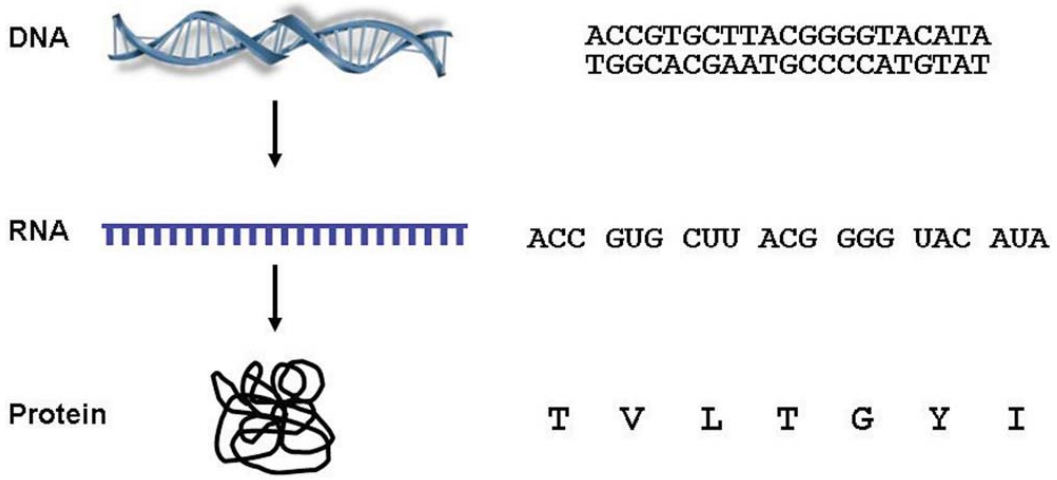
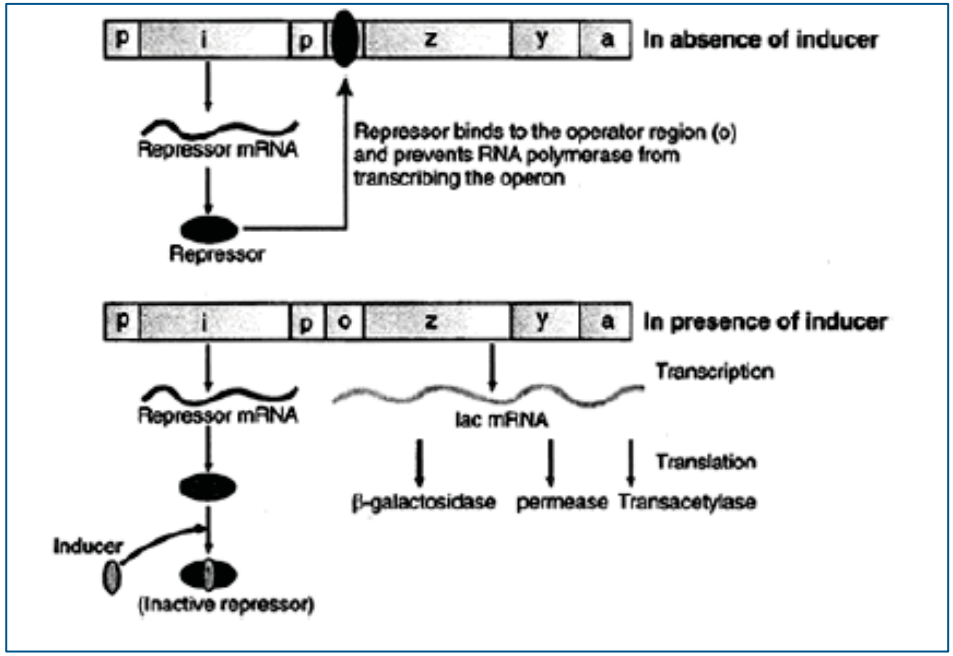
Gene regulation in bacteria:
The Jacob and Monod Lac operon model (1961)

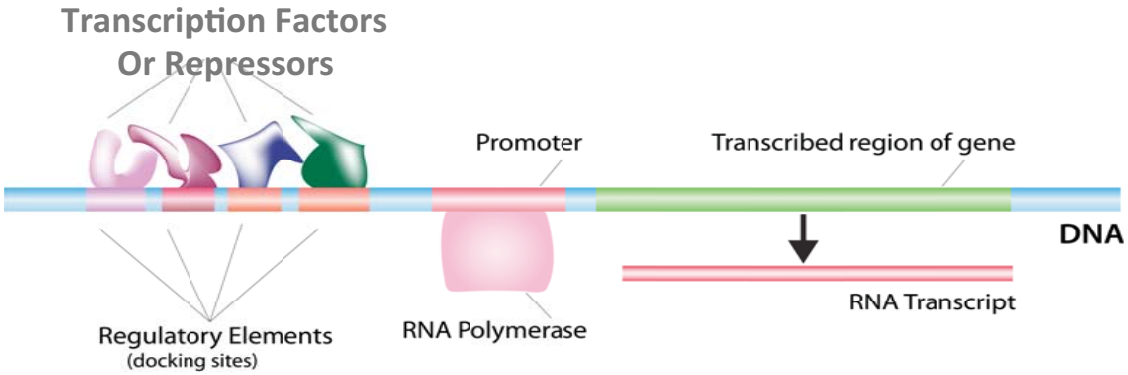
The Lac operon:
Gene control relies on specific repressors and activators and the DNA sequence elements they recognize.





Gene regulation in Eukaryotes?

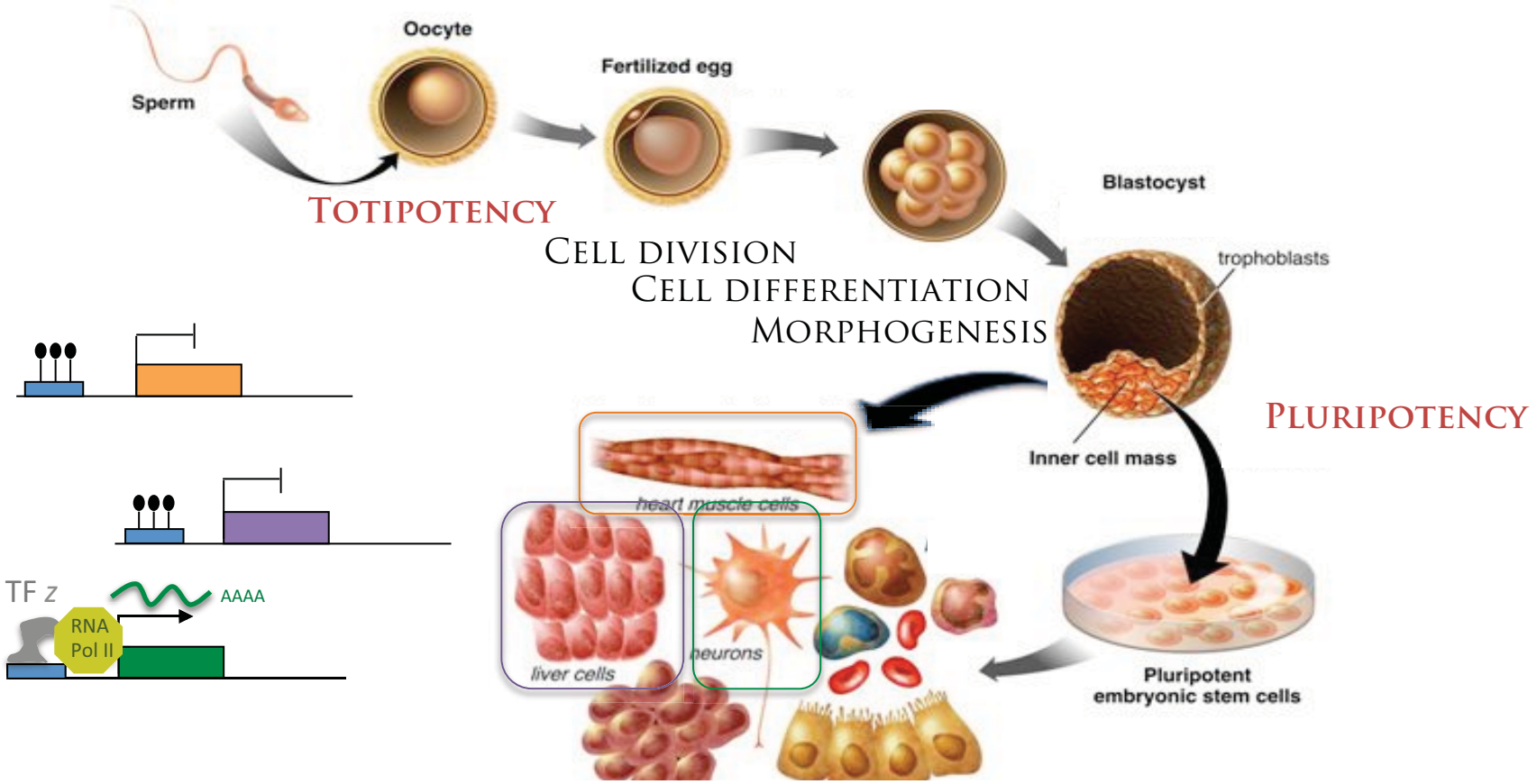
In eukaryotes – the same basic principles of gene regulation could surely apply, however they differ from prokaryotes, for example in the complexity of their genomes and of their chromatin



=> "what is true for *E. coli* is true for the elephant"

?

Gene Reguation during Mammalian Development



1. All cells contain same genes: cell identities depend on **which** genes are expressed/repressed.
2. Gene expression patterns established by transcription factors via signalling, cell-cell communication, positional information?
3. <u>However</u> a gene is not necessarily activated *just* by the presence of a Transcription factor
4. Also - changes in gene expression become <u>stable and heritable</u> (through mitosis) during development.

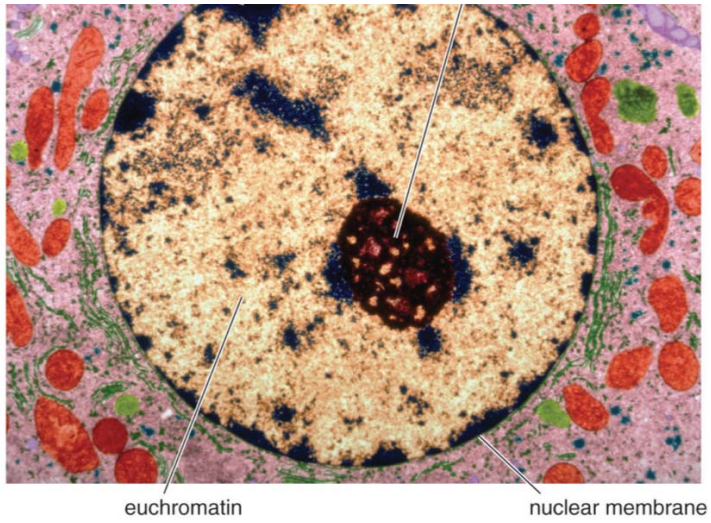
The Genetic Material in Eukaryotes?

In eukaryotes - basic principles of gene regulation could surely apply, however they differ from prokaryotes, for example in the complexity of their genomes and of their chromatin

"*Few structures in nature appear to be arranged with as much abandon as the chromatin of the interphase nucleus. And yet this random appearance may be deceiving, since any degree of order would be well hidden by the very mass of the chromatin itself.*" Comings, 1968

Is their order in this apparent disorder?
How does it relate to *genes* and their expression?
How is a copying mechanism (DNA replication) ensured ?
Is *chromatin* also copied and if so, how?

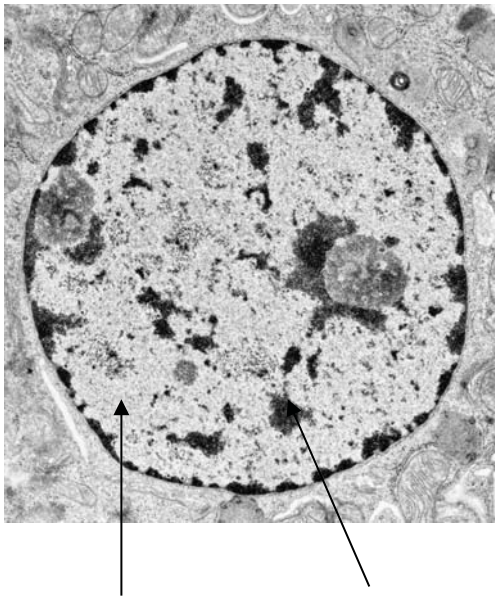
Packaging
$3x10^9$ base pairs (2 metres) of DNA packaged into a nucleus of just 10μm approximately



Staining with dyes showed that DNA is **non-uniformly** distributed

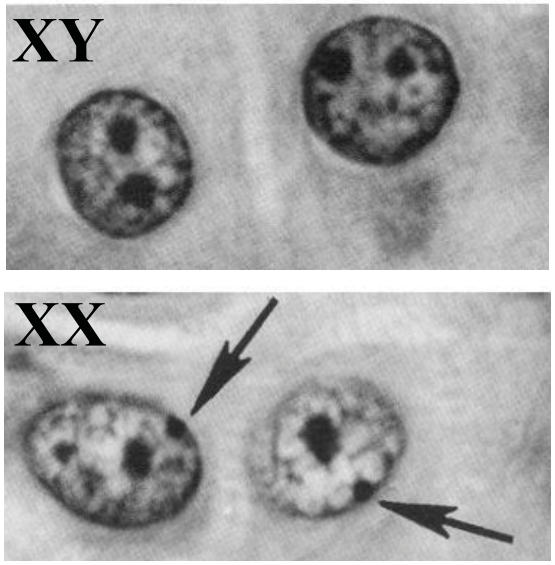
Heterochromatin and Euchromatin

Heteorchromatin and Euchromatin

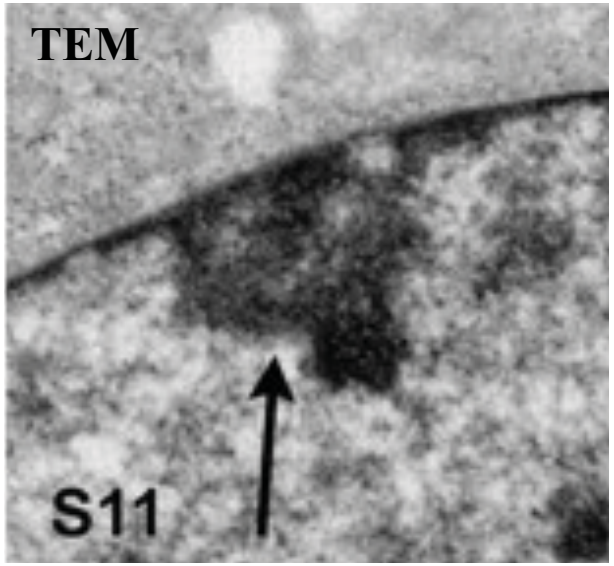


Euchromatin Heterochromatin

The Barr Body = the inactive X



Bertram et Barr, 1949



Rego et al, 2008

Heitz (1928) in "The Heterochromatin of Moss"

"*…heterochromatin refers to a part of a chromosome that remains heteropyknotic after telophase and thus behaves opposite to euchromatin*"

Constitutive heterochromatin tends to be non-coding and repetitive (Pontecorvo, 1944)
Facultative heterochromatin (protein-coding chromatin) can be either active or inactive (eg the Barr body, 1949; Lyon, 1961)

Heterochromatin and Euchromatin

From M. Lyon, 1974

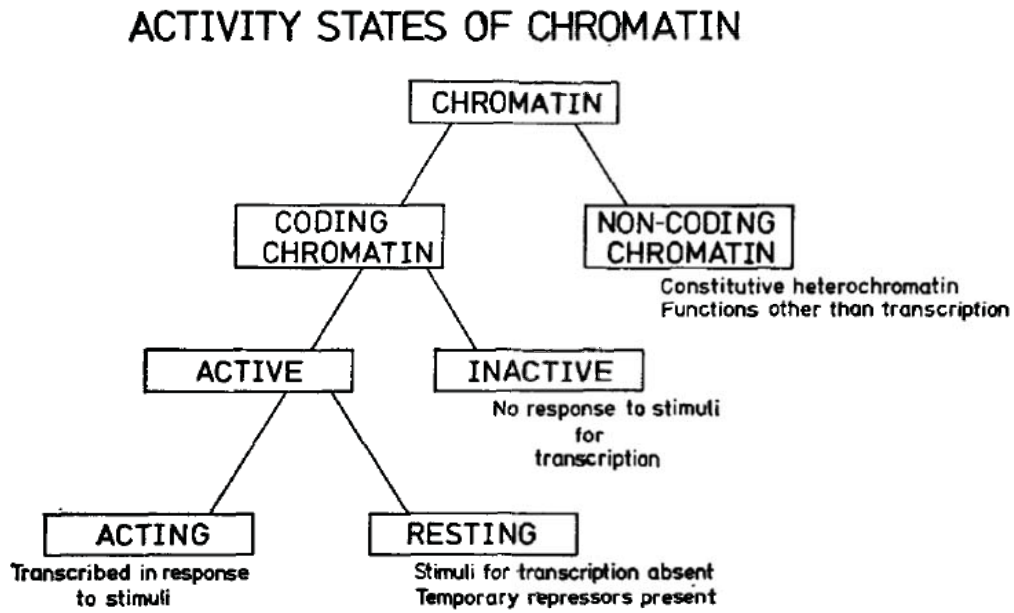
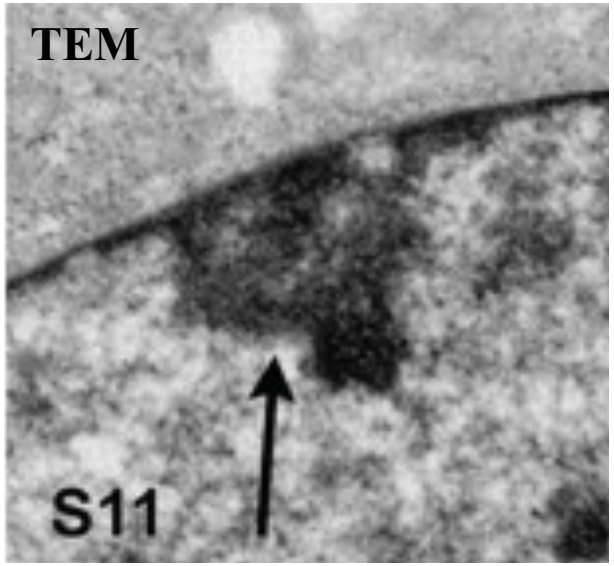


FIGURE 1.—Classification of eukaryote chromatin according to its functional state.

Genetics 78: 305–309 September, 1974.

The Barr Body = the inactive X

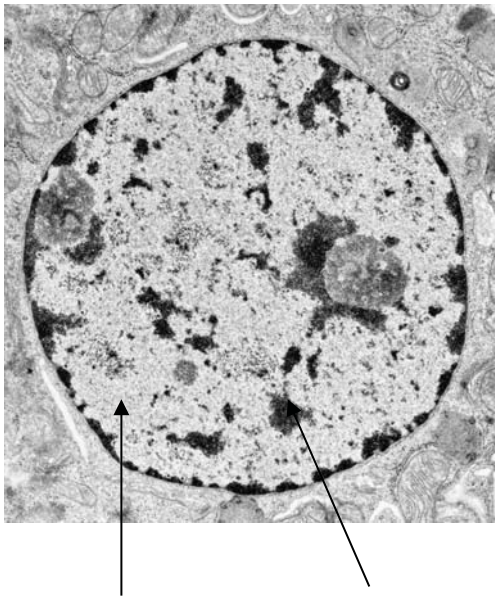


Rego et al, 2008

"*Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action*" (McClintock, 1950).

Heterochromatin and Euchromatin

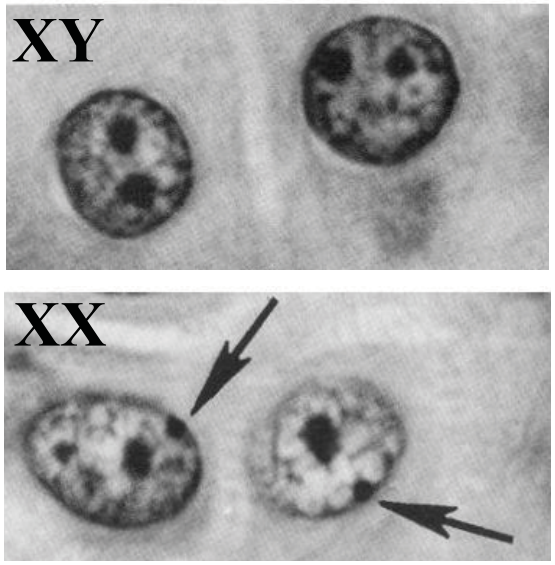
Heteorchromatin and Euchromatin
Emile Heintz, 1929



Euchromatin Heterochromatin

http://medcell.med.yale.edu/histology/

The Barr Body = the inactive X



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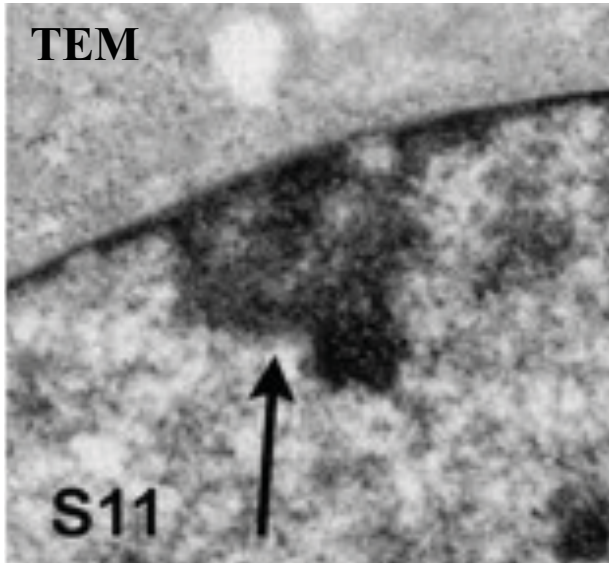
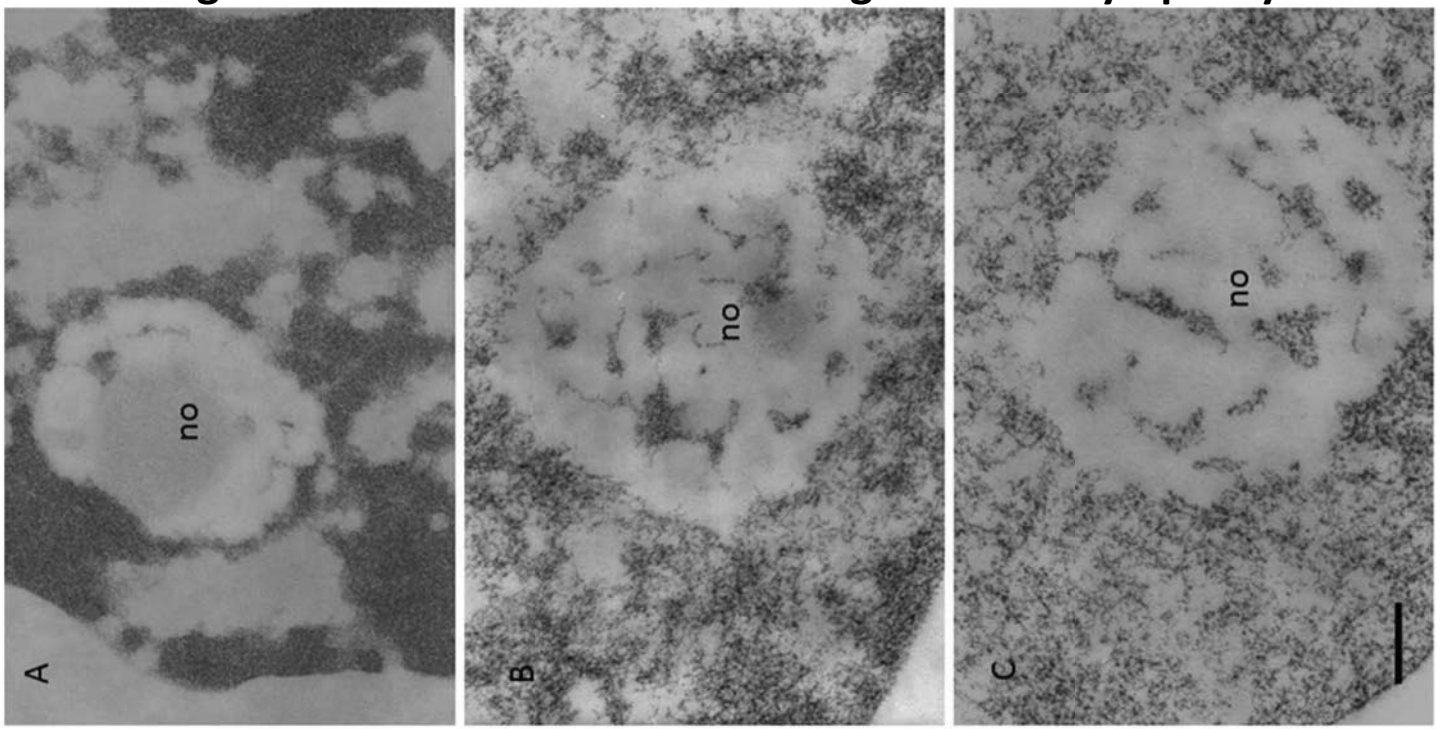


Rego et al, 2008

DNA is differentially "packaged" as chromatin in the nucleus
Different cell types show rather different chromatin distributions
Might this be a cause, or consequence, of differential gene expression?

Heterochromatin and Euchromatin

Feulgen-like osmium-ammine staining of human lymphocytes



Scale bar: 0.2 μm.

Resting lymphocyte

Lymphocyte stimulated to proliferate and increase RNA PolII Activity (*24 h or 48 h phytohemagglutinin exposure)*

Derenzini et al, 2014

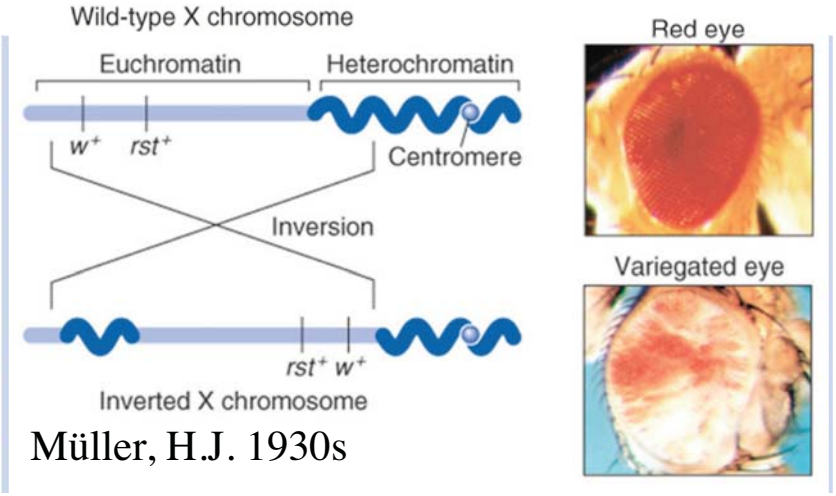
Chromosomes during interphase are highly plastic structures.

The relationship between chromatin and the interchromatin space is highly variable depending upon RNA transcription and cell cycle phases

Heterochromatin and variegated gene silencing

Genetics: genotype -> phenotype

However variation in phenotype (gene expression) could sometimes be observed within a single individual = clonal, alternating activity states linked to heterochromatin



Müller, H.J. 1930s

Such non-mendelian phenomena provided new insights into "Epigenetics" and a shift in its definition

They also laid the foundations for the discovery of epigenetic mechanisms and the factors involved

Fro

E. Hea

Insights into "Epigenetics" and a shift in its definition

In Waddington's definition of Epigenetics, changes in gene regulation and activity during development were implicit; the notion of heritability less so.

In the 1970's-80's a major shift took place in the use of the word, to include the notion of *transmission* or *heritability* of gene expression states.

WHY?

1. **Observations from cultured cells** raised the question of **somatic inheritance** : how could replicating cells "remember" their differentiation state with such high fidelity?

2. **Stem cell differentiation**: what caused **switches in gene activity**? The realization that some specialized genes, which determine the phenotype of differentiated cells are permanently turned on, and other genes—active in some other cell type—are permanently turned off. Some of these controls must be **mitotically heritable** – how?

3. **X-chromosome inactivation (XCI)**: how is one of the 2 X chromosomes stably shut down during development – what triggers the **switch in gene activity** and how is it subsequently made **somatically heritable**?

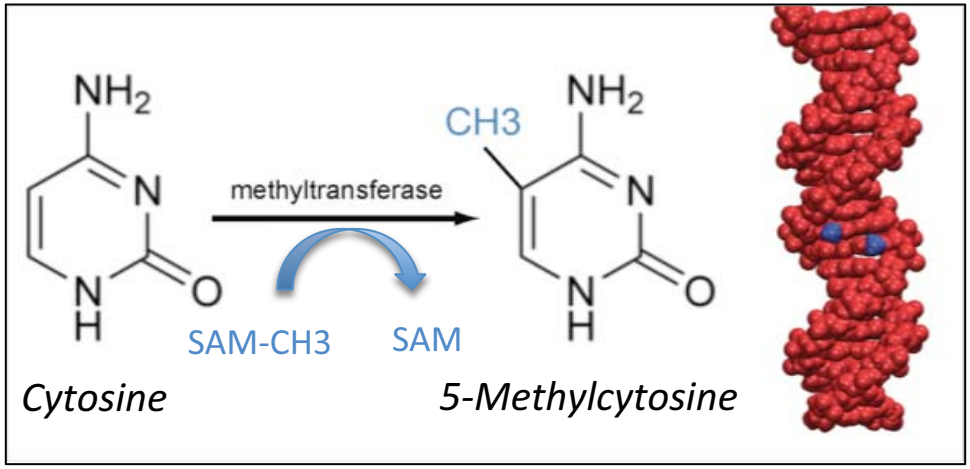
4. Phenomena with **unusual (non-Mendelian) inheritance** eg XCI, Paramutation, imprinting etc.

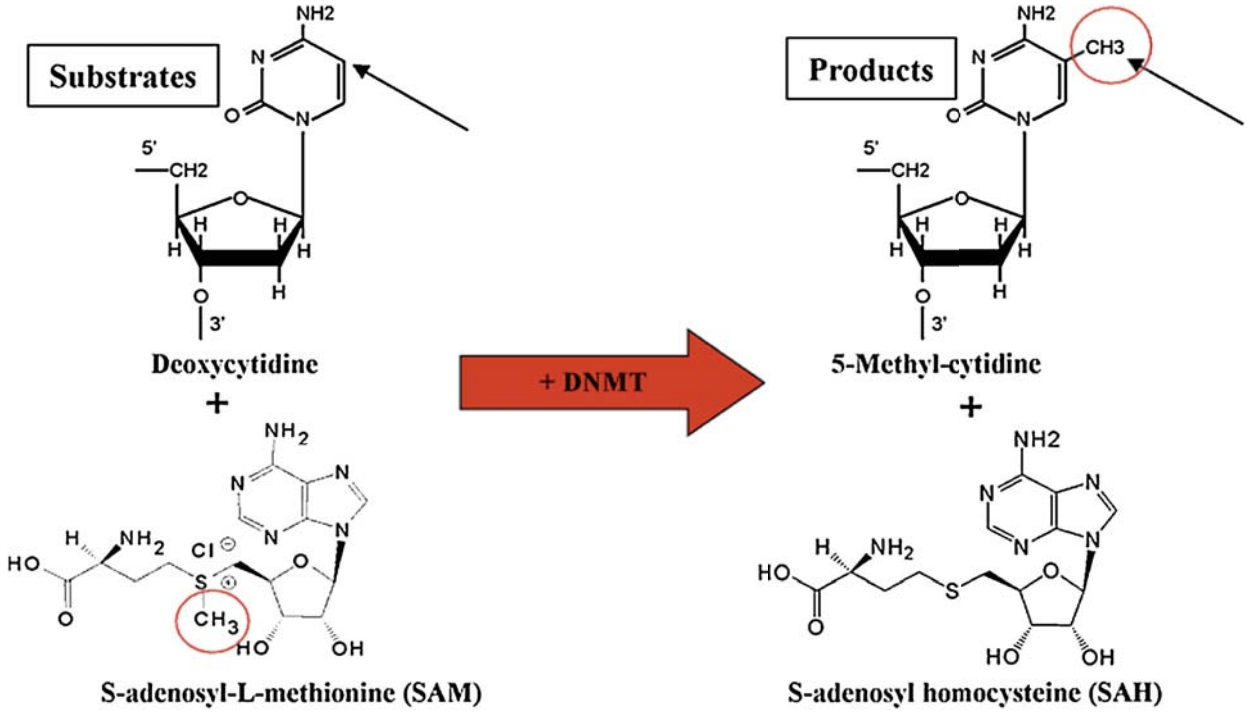
HOW might heterochromatin (and euchromatin) underlie such heritable epigenetic phenomena?

DNA Methylation – one of the first "epigenetic" marks

Work of A. Riggs and R. Holliday in the 1970's and 80's and Bestor, Bird, Jones and others in 90's

(see lectures – COURS 2013)





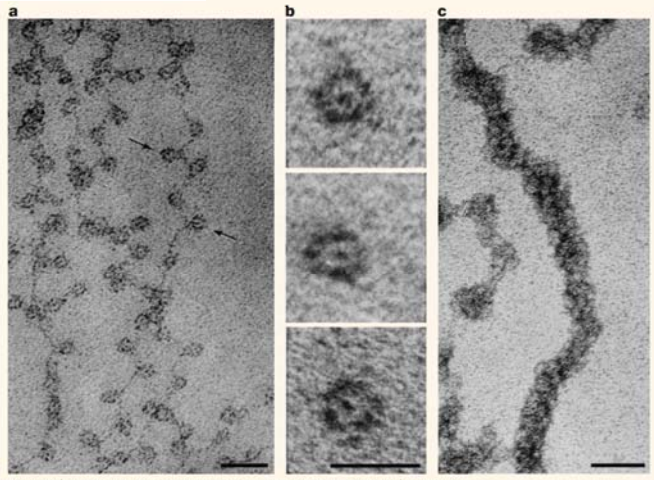
http://www.ks.uiuc.edu/Research/methylation/

The Nature of Chromatin - its Basic Repeat Unit

Almost a century after its discovery, the structure of chromatin was found to consist of repeating units (Olin and Olin, 1974; Kornberg, 1974), the **nucleosomes**, made up of **histones** and **DNA.**

⇒ DNA was no longer seen as being "coated" by histones <u>but coiled around histones</u> and thus <u>accessible</u> to other binding factors

Electron microscopy studies and studies with isolated chromatin subjected to treatment with detergents, different ionic strength solutions and metal cation concentrations, as well as to nuclease digestion….



Ultrastructurally, nucleosomes are flat cylinders with a diameter of 11 nm &with a height of 5.5 nm. (Feulgen-like osmium-ammine staining - only DNA is stained) (review Olins & Olins, 2003)

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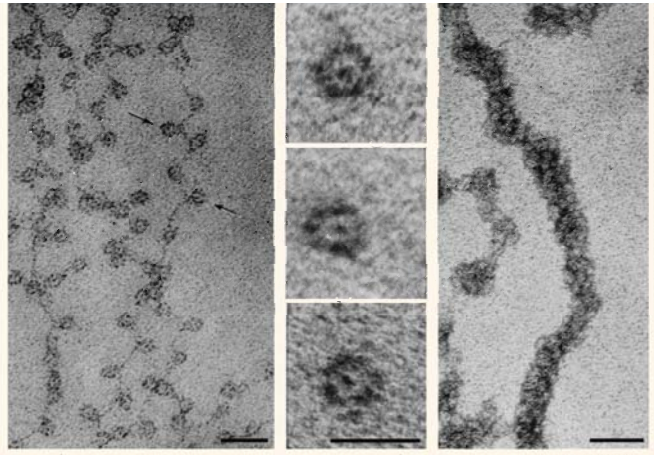
A new era of understanding of the structural organization of DNA in chromatin and of the mechanisms controlling gene expression.

How nucleosomal arrays with the 5th linker histone (H1) then fold this chromatin fiber into increasingly more compacted filaments leading to defined higher order structures still no clear.



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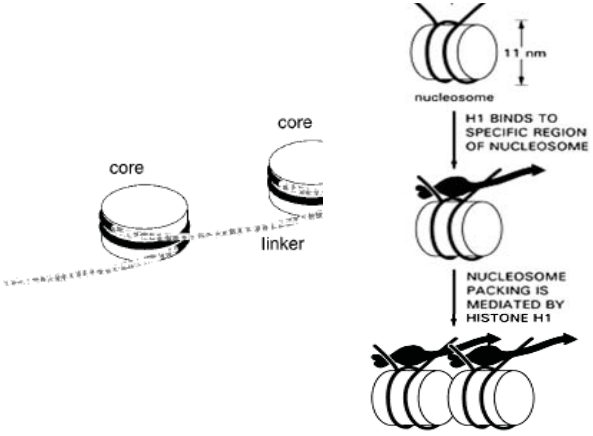
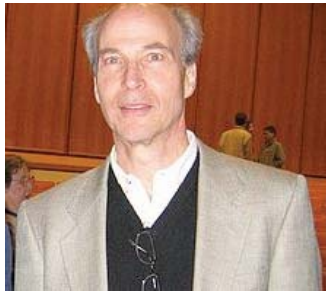




Roger David Kornberg (born 1947) , Biochemist, Stanford University School of Medicine

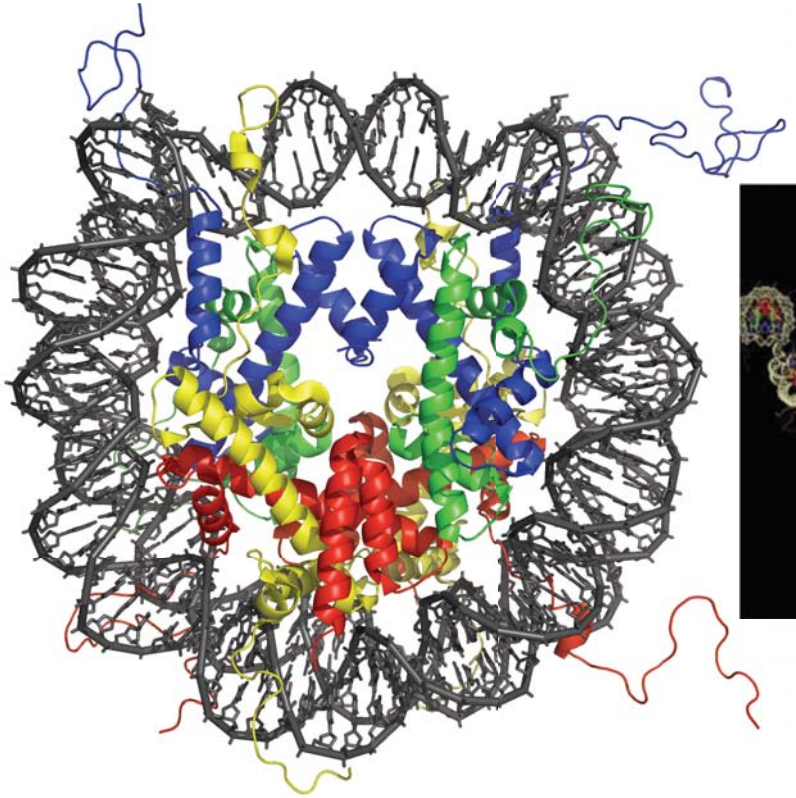
The nucleosome is composed by a histone octamer, two each of histones H2A, H2B, H3 and H4, which constitute a protein core around which **147 base pairs of DNA** are wrapped **in 1.7 left-handed super-helical turns**; histone H1 is attached to the complex.

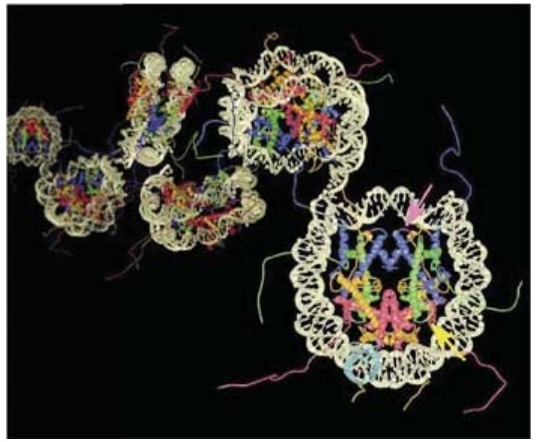
Crystal Structure of the Nucleosome

14 DNA–histone interactions within the core particle, positioned at each minor groove (Luger et al. 1997). Twelve of these interactions are mediated by histone fold motifs of all four histone proteins, and the remaining two involve residues in the histone H3 αN helix at the DNA entry and exit points.

Majority of these interactions are mediated by nonspecific electrostatics between the DNA and protein backbones, but important positively charged histone side chains have also been shown to play a role (Luger and Richmond 1998).

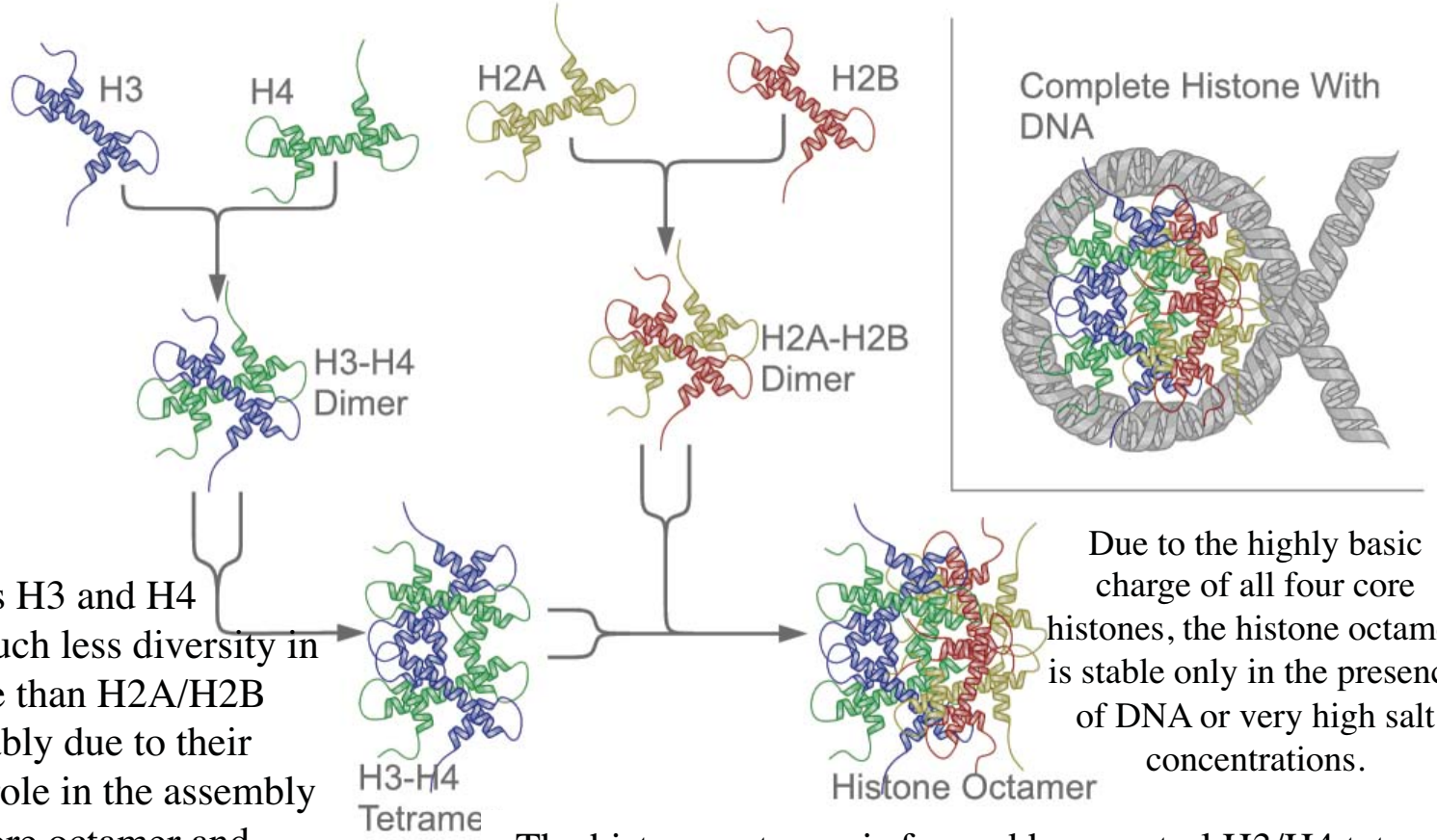
Histones are small basic proteins consisting of a globular domain and a more flexible and charged NH2-terminus (histone "tail") that protrudes from the nucleosome.





Crystal Structure of the Nucleosome

The core histone proteins contain a "histone fold," = 3 alpha-helices separated by 2 loops.
In solution, the histones form H2A-H2B heterodimers and H3-H4 heterotetramers.
Histones dimerise about their long α2 helices in an anti-parallel orientation.
In the case of H3 and H4, 2 dimers form a 4-helix bundle stabilised by H3-H3' interactions.

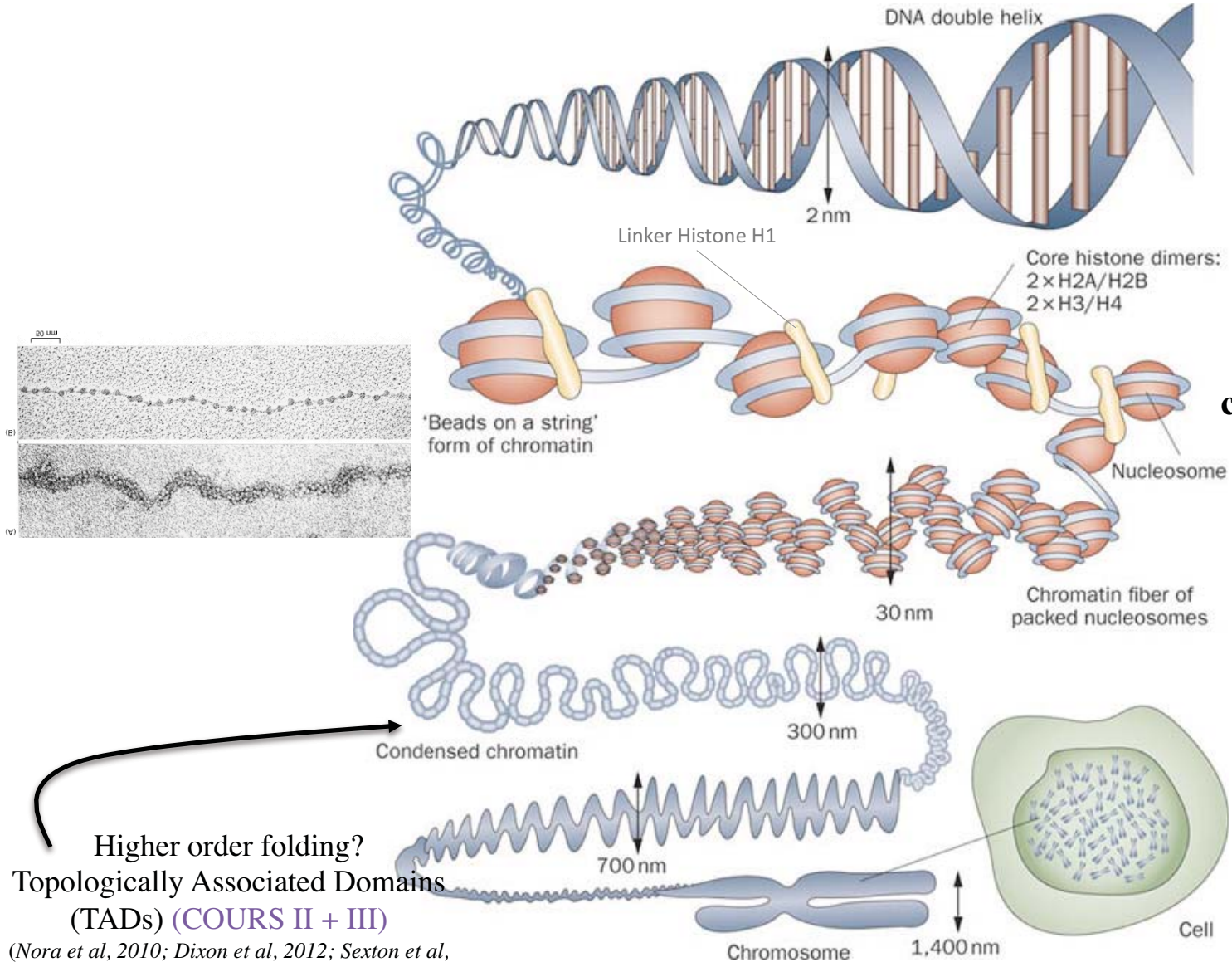


Histones H3 and H4 show much less diversity in structure than H2A/H2B presumably due to their critical role in the assembly of the core octamer and nucleosomes….

Due to the highly basic charge of all four core histones, the histone octamer is stable only in the presence of DNA or very high salt concentrations.

The histone octamer is formed by cenntral H3/H4 tetramer sandwiched between two H2A/H2B dimers

From the Nucleosome Fibre to Higher Order Folding?



Nucleoeosomes alone (together with H1) cannot account for the DNA compaction in the nucleus (eg up to 10 000-fold in heterochromatin)

Other factors (proteins, RNAs) via interactions with nucleosomes (DNA and/or histones)?

Higher order folding?
Topologically Associated Domains (TADs) (COURS II + III)

(*Nora et al, 2010; Dixon et al, 2012; Sexton et al, 2012; Nora et al, Bioessays, 2013*)

E. Heard, February 2nd, 2015

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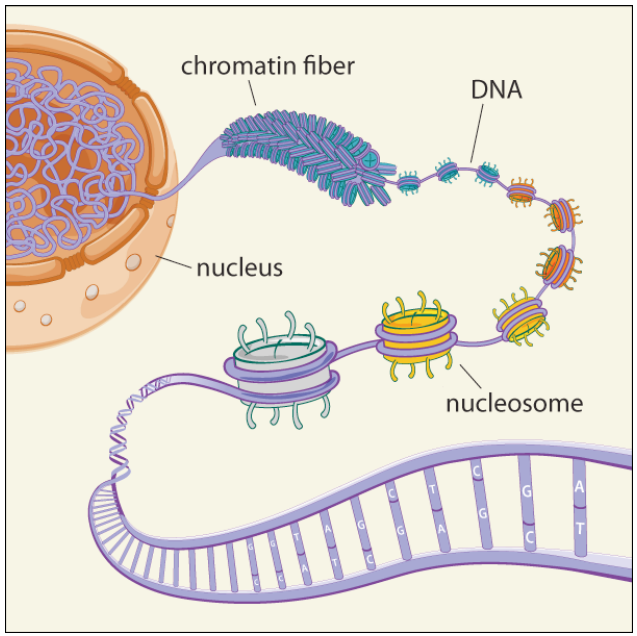
Chromatin Variations

Chromatin is the physiological template of the eukaryotic genome.
Yet **nucleosomes <u>repress</u> transcription** based on:
in vitro transcription assays (Knezetic and Luse, 1986; Lorch et al, 1987)
in vivo genetic assays in yeast (Han and Grunstein, 1988)

⇒ Elaborate mechanisms have evolved to introduce meaningful variation into chromatin to enable and alter gene expression and other important biological processes.

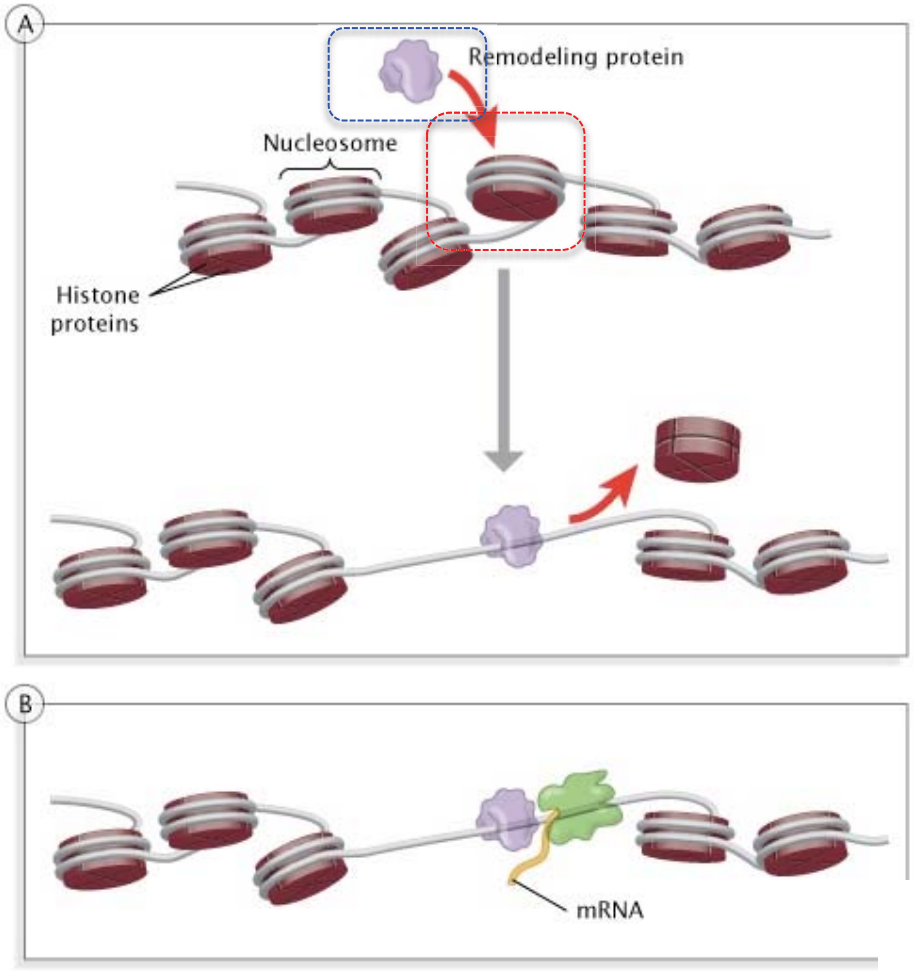
- **Histone Modifications**
- **Histone Variants**
- **Chromatin Remodeling Complexes**
- **DNA methylation**
- **Non-coding RNAs**

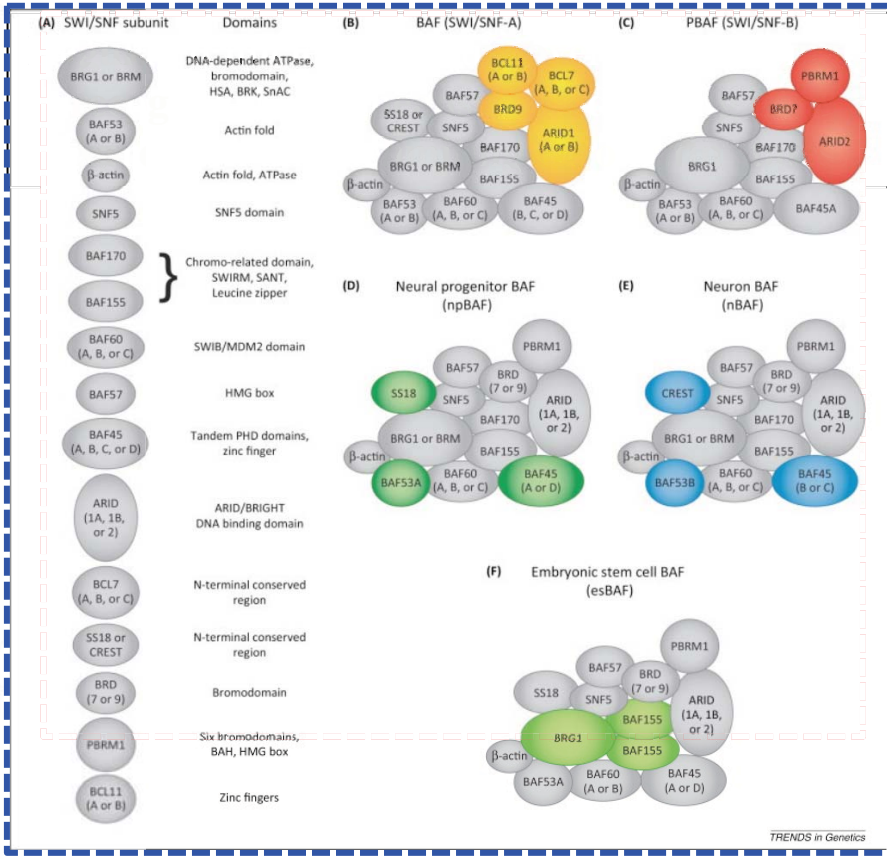


Chromatin Remodeling

Nucleosomes can be barriers or facilitators of gene expression

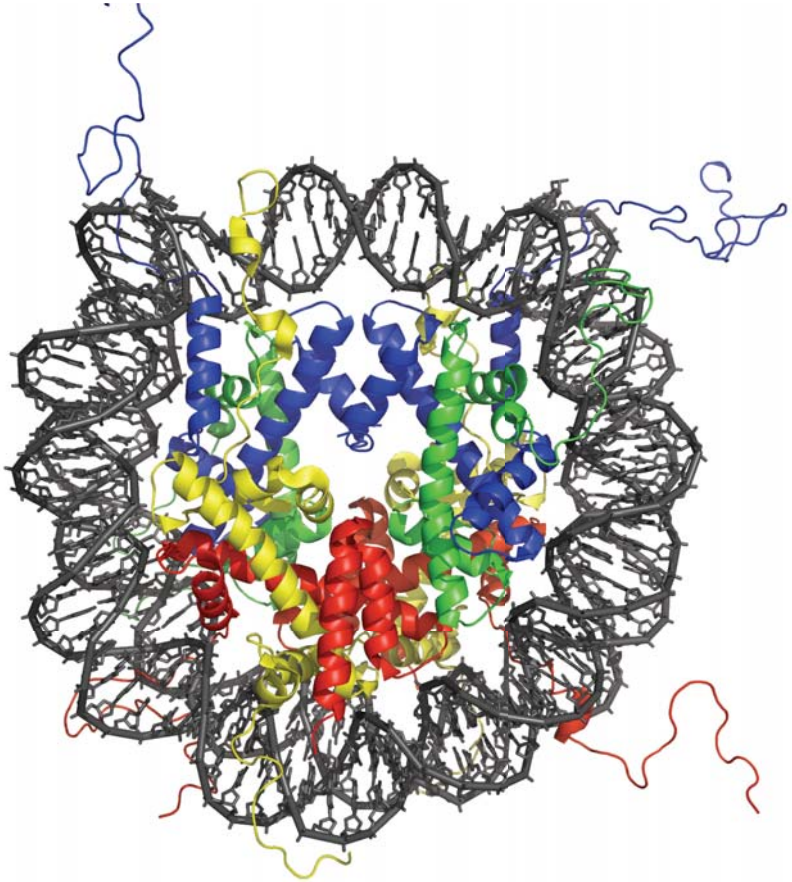
- **How is TF accessibility to DNA achieved in a chromatin context?**
- **Large, multiprotein complexes that use the energy of ATP hydrolysis to mobilize and restructure nucleosomes.**

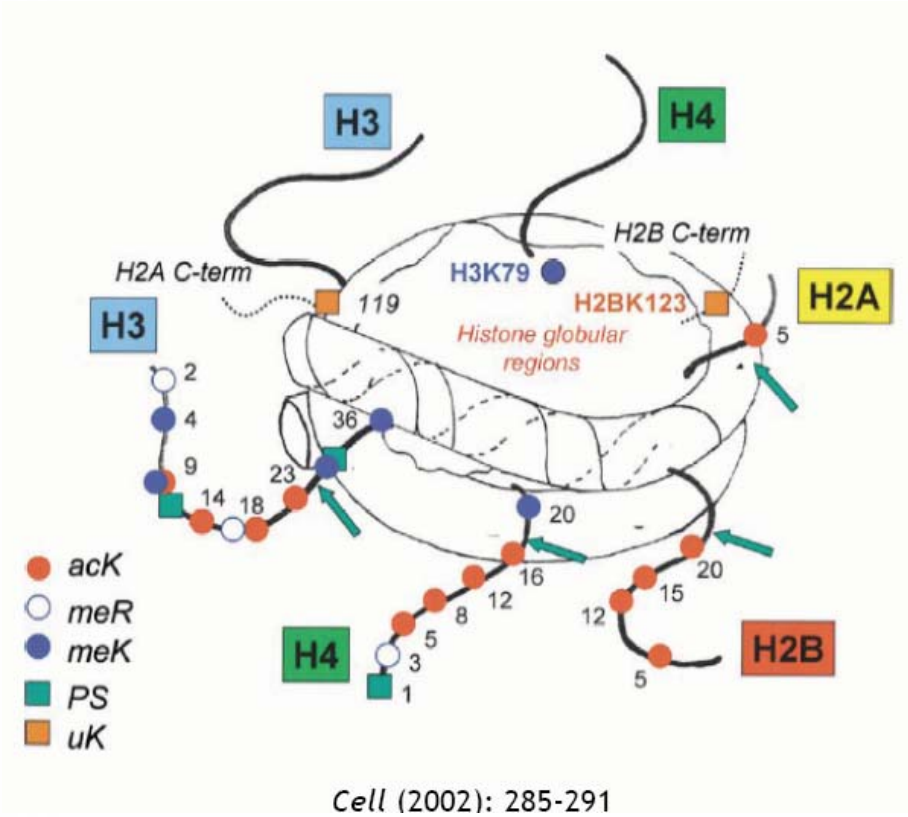




Remodelers are partitioned into several families, each with specialized biological roles. All contain a subunit with a conserved ATPase domain. Each remodeler complex also possesses unique proteins that specialize it for its unique biological role.

Histone Modifications





The **histone tail extensions** constitute up to 30% by mass of histones, but are not visible in the crystal structures of nucleosomes due to their high intrinsic flexibility, and have been thought to be largely unstructured.

Discovery of Histone Modifications

Vincent Allfrey. Photo courtesy of the Rockefeller Archive Center.

1921-2002

*ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS**

Incubated nuclei from the calf thymus with ^{14}C-labeled sodium acetate, histones were then separated and analyzed chromatographically, and their amino acid components were quantified. Only two histone fractions, H3 and H4, had significant levels of acetyllysine residues.

Allfrey predicted that histone acetylation could be linked to chromatin decompaction and gene expression:

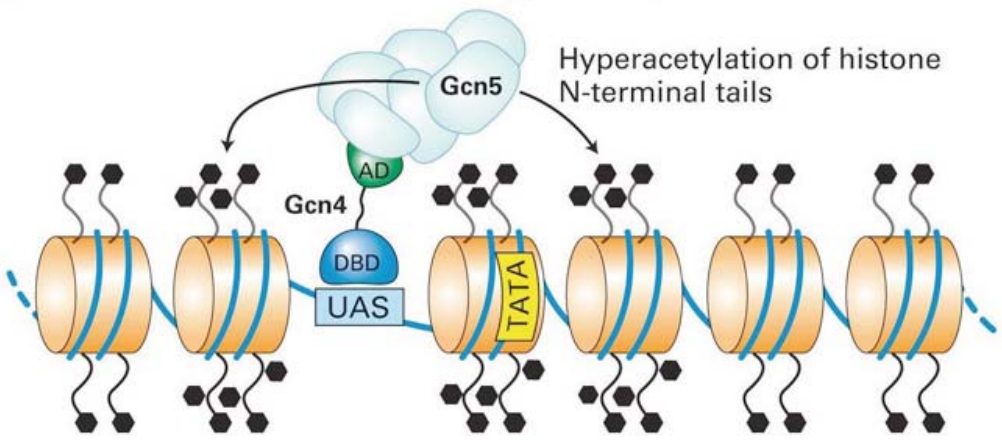
"*As a charge neutralization mechanism, acetylation of the histones would be expected to modify DNA-histone interactions, and this may offer a molecular basis for the pronounced changes in histone acetylation and RNA synthesis during the course of gene activation in many cell types.*"

Structural Modifications of Histones and their Possible Role in the Regulation of RNA Synthesis

Histone Acetylation: 30 years later
First evidence for a Regulatory role of the Nucleosome

• C.D. Allis purified the first type A histone acetyltransferase (HAT) from macronuclei of the protozoan *Tetrahymena thermophila*. Purification and cloning of the gene encoding this protein revealed that it was the orthologue of **Gcn5**, a yeast **transcription regulator**.

• Schreiber and colleagues used an affinity matrix based on the HDAC inhibitor trapoxin to purify a 46-kDa bovine thymus protein. Microsequencing of the trapoxin-bound protein revealed a bovine orthologue of the yeast **transcription repressor Rpd3 =>** human histone deacetylase 1 (HDAC1) cloned and found to exhibit HDAC activity in vitro.

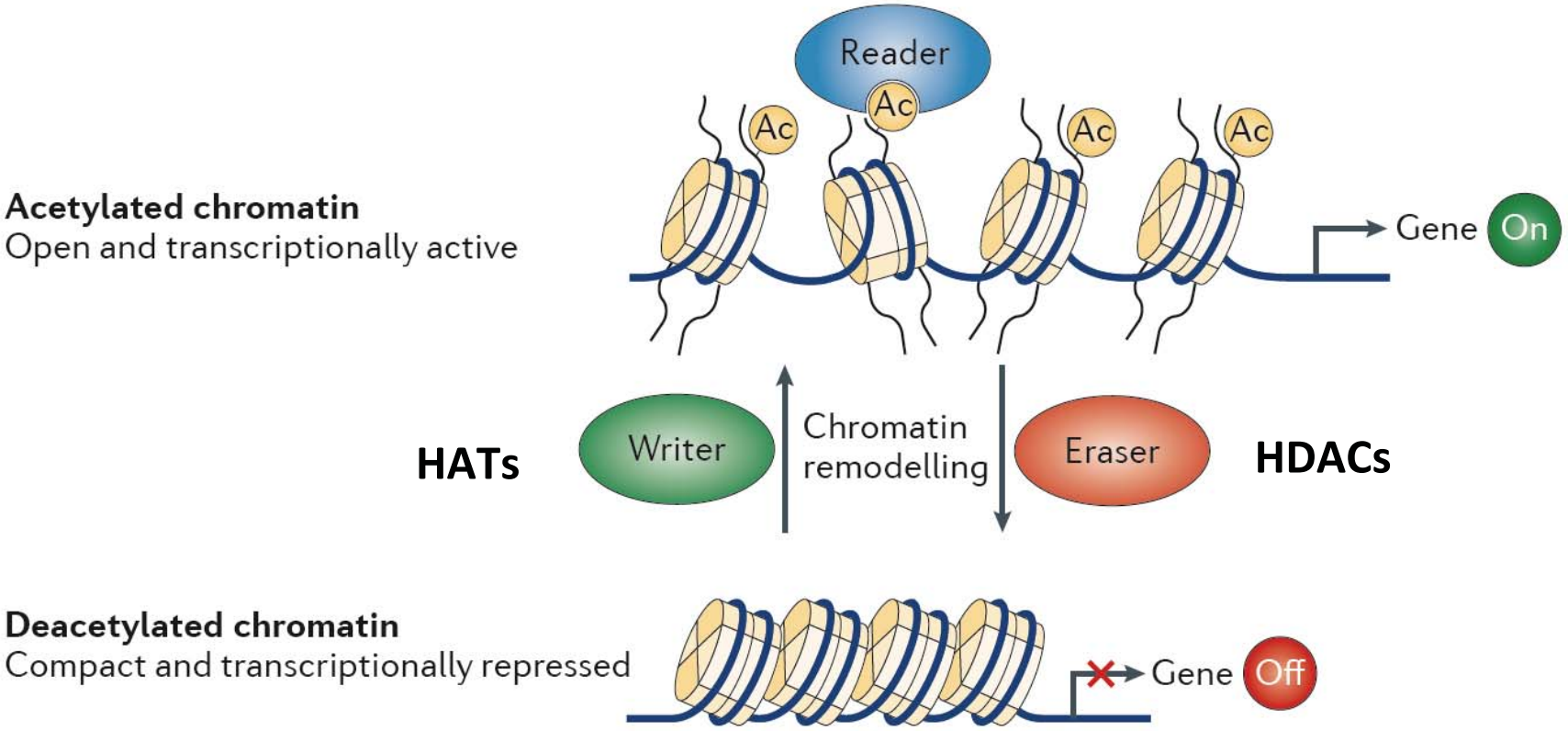


Histone acetylation is a reversible modification of lysines in the N-termini of the core histones leading to :
• reduced binding to DNA
• destabilization of chromatin
• a mark that can be recognised by other factors (eg **bromo-domain** factors)

Numerous HATs and HDACs identified, located in large complexes, that interact with DNA binding proteins, and other proteins that can bind chromatin and remodel it.

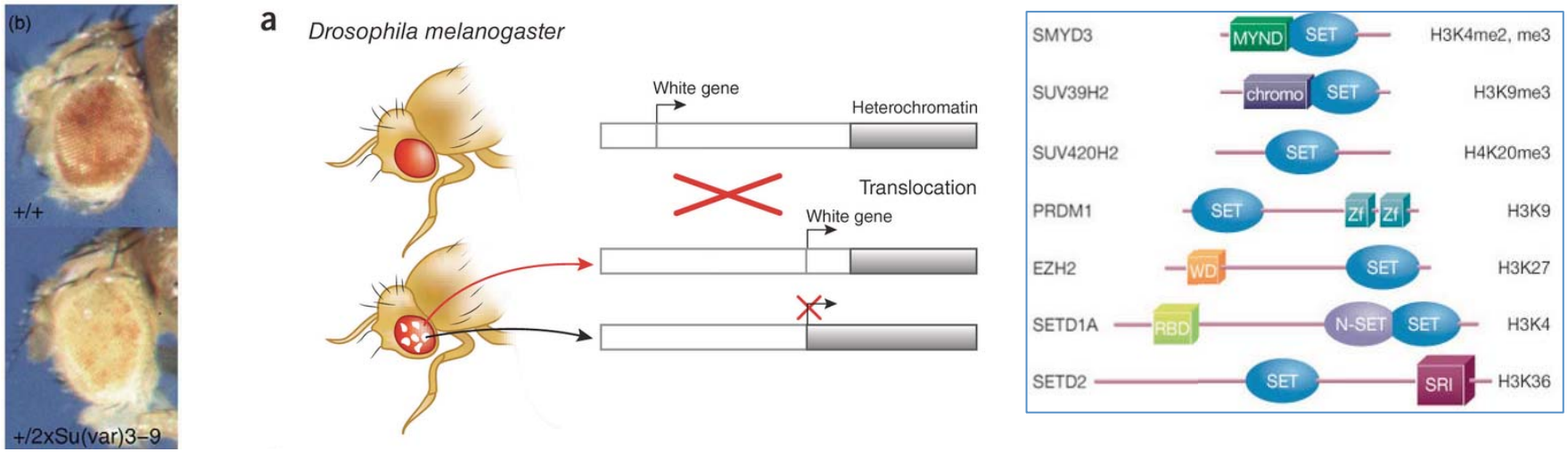
Brownell, J. E. *et al*. Tetrahymena histone acetyltransferase A: a homo
acetylation to gene activation. *Cell* **84**, 843–851 (1996).
Taunton, J., Hassig, C. A. & Schreiber, S. L. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 272, 408–411 (1996).

Histone Acetylation: 30 years later
First evidence for a Regulatory role of the Nucleosome



Histone Modifying Enzymes with Functional Roles

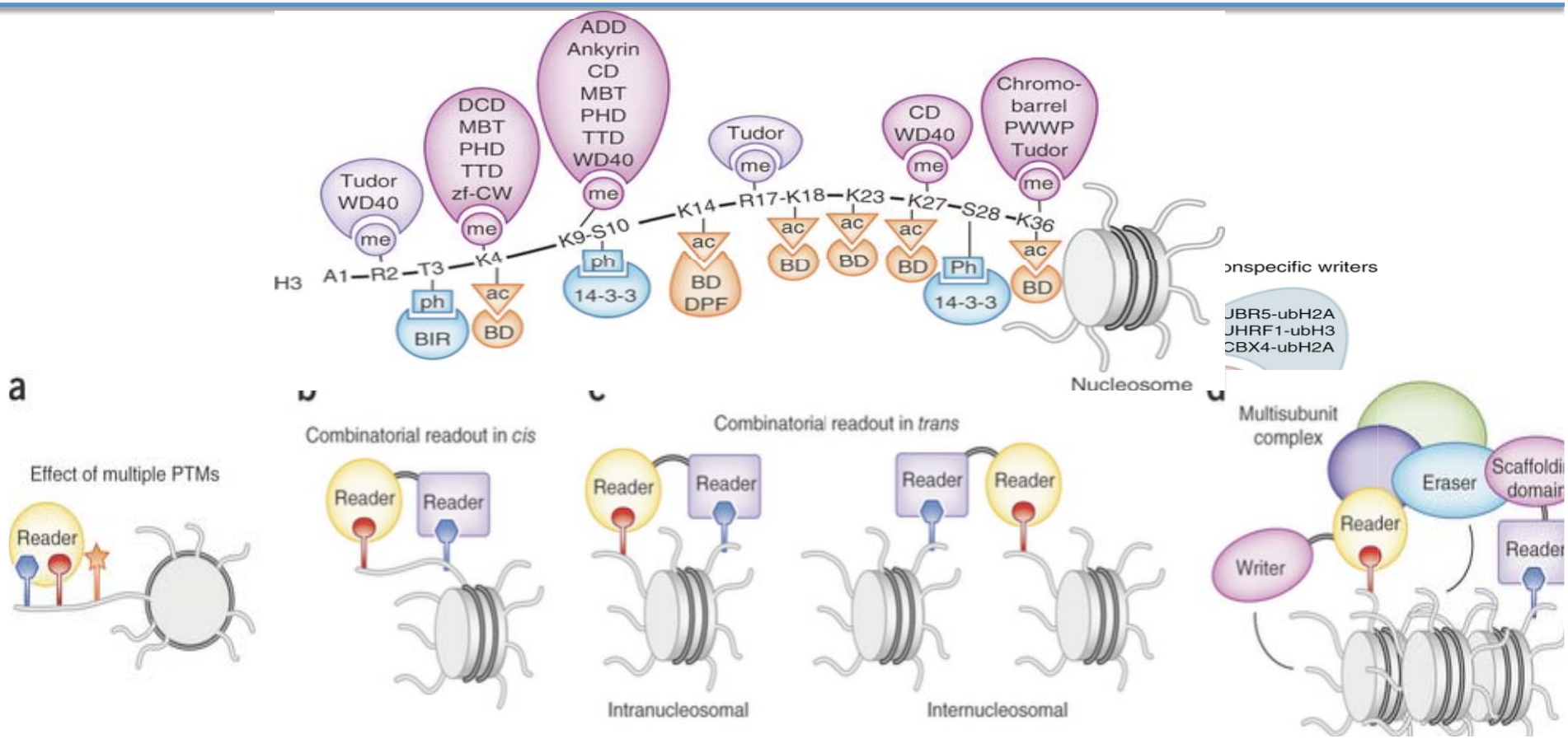
Position-effect variegation (PEV), allowed the development of genetic screens in *Drosophila* and *S. pombe* that identified multiple loci involved in modifying PEV: Suppressors Su(var) and Enhancers (E(var) of PEV). Mating-type switching in budding and fission yeast represents another paradigm for a variegating mechanism.



Several *Su(var)* loci identified & shown to encode heterochromatin associated proteins. From these, the first evolutionary **conserved histone H3 lysine 9 methyltransferase SU(VAR)3-9** to be discovered was found to play a central role in heterochromatic gene silencing. It contains both a **SET domain** (methyltransferase) and **chromodomain** (capable of binding H3K9me3) => **Involved in Propagation +Memory of silent states (COURS II)**

Rea S et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature, 2000
Nakayama J et al. Role ofhistone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science, 2001.
Schotta G et al. Central role of *Drosophila* SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. EMBO J, 2002.

Histone Modifications: their "writers", "readers", "erasers"



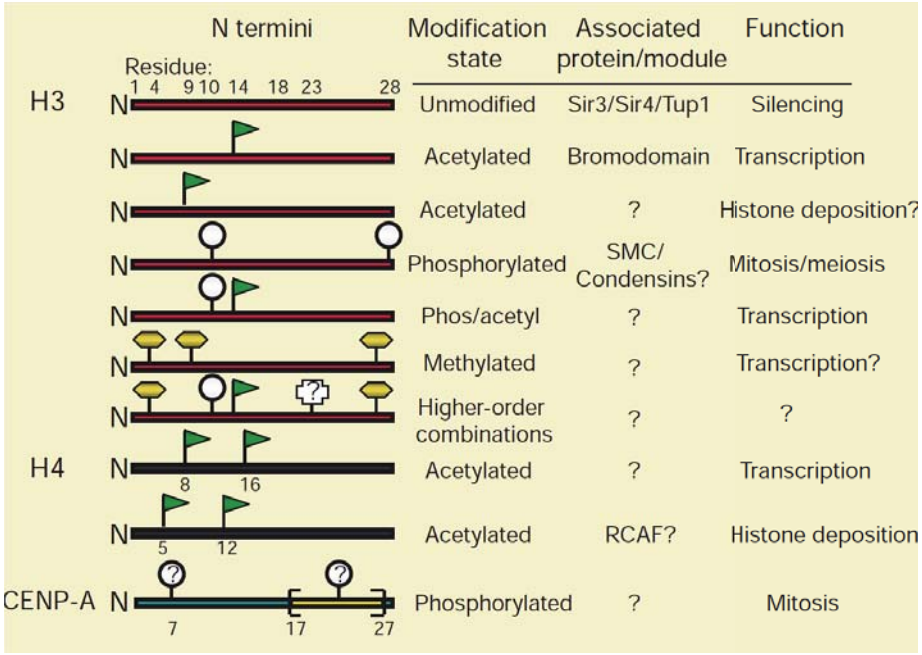
Among these writer / reader / eraser histone modifying proteins members of the Polycomb and Trithorax complexes previously identified in flies to play a role in maintaining gene activity states (Cours III)

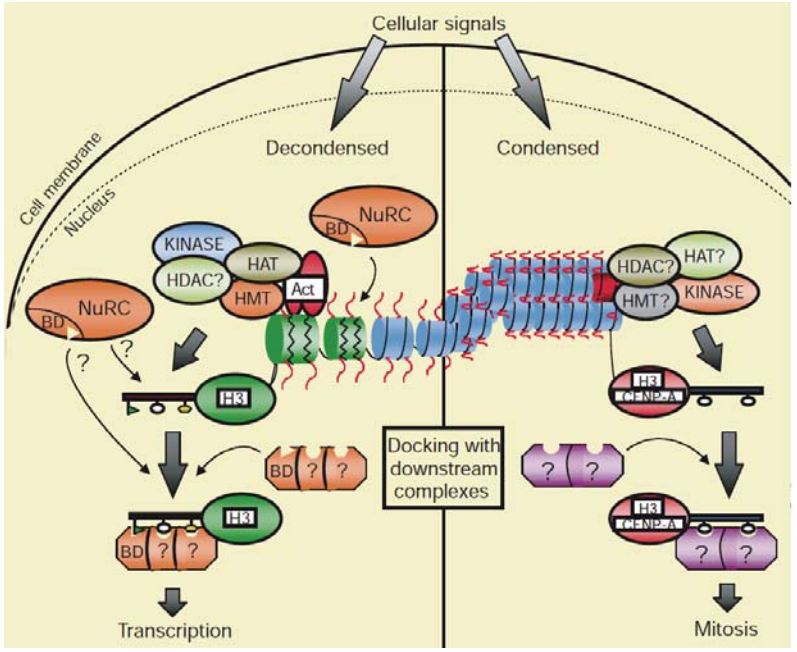
The Histone "Code" Hypothesis

The language of covalent histone modifications

Brian D. Strahl & C. David Allis

Department of Biochemistry and Molecular Genetics, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA





Some histone modifications may function in combinations to recruit factors or facilitate / mediate their roles?

(not really equivalent to genetic "code"!)

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Tools for detecting Specific Histone Modifications

In the 1990's, highly specific **antibodies** raised - discriminating between chemically modified histones at specific amino acids, => histone modifications could be detected by **immunofluorescence** (IF) and

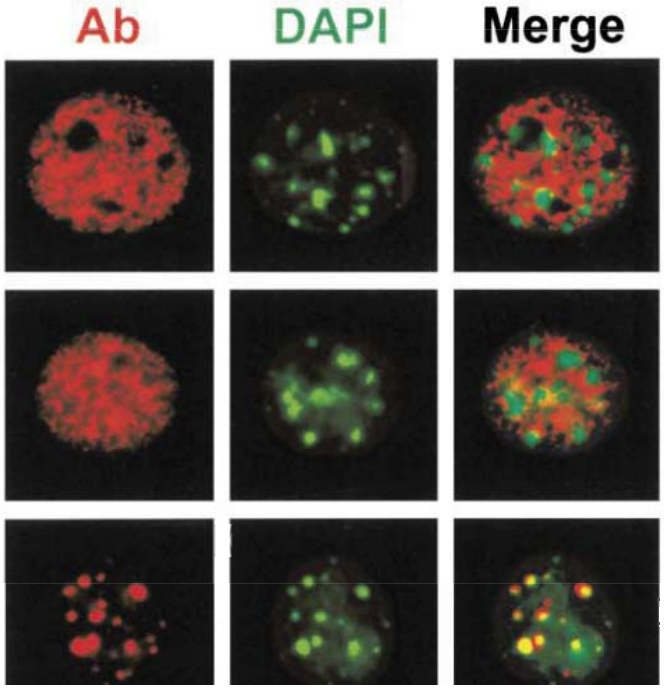
in in ecitipa

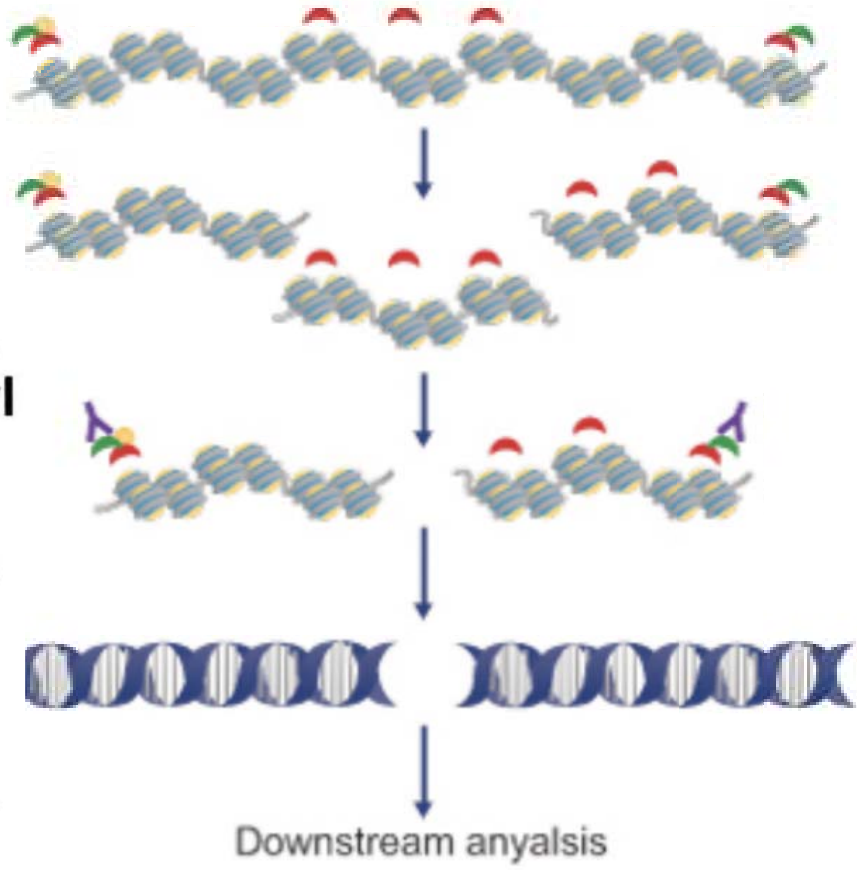
and c

Bryan Turner

C. David Allis

Thomas Jenuwein





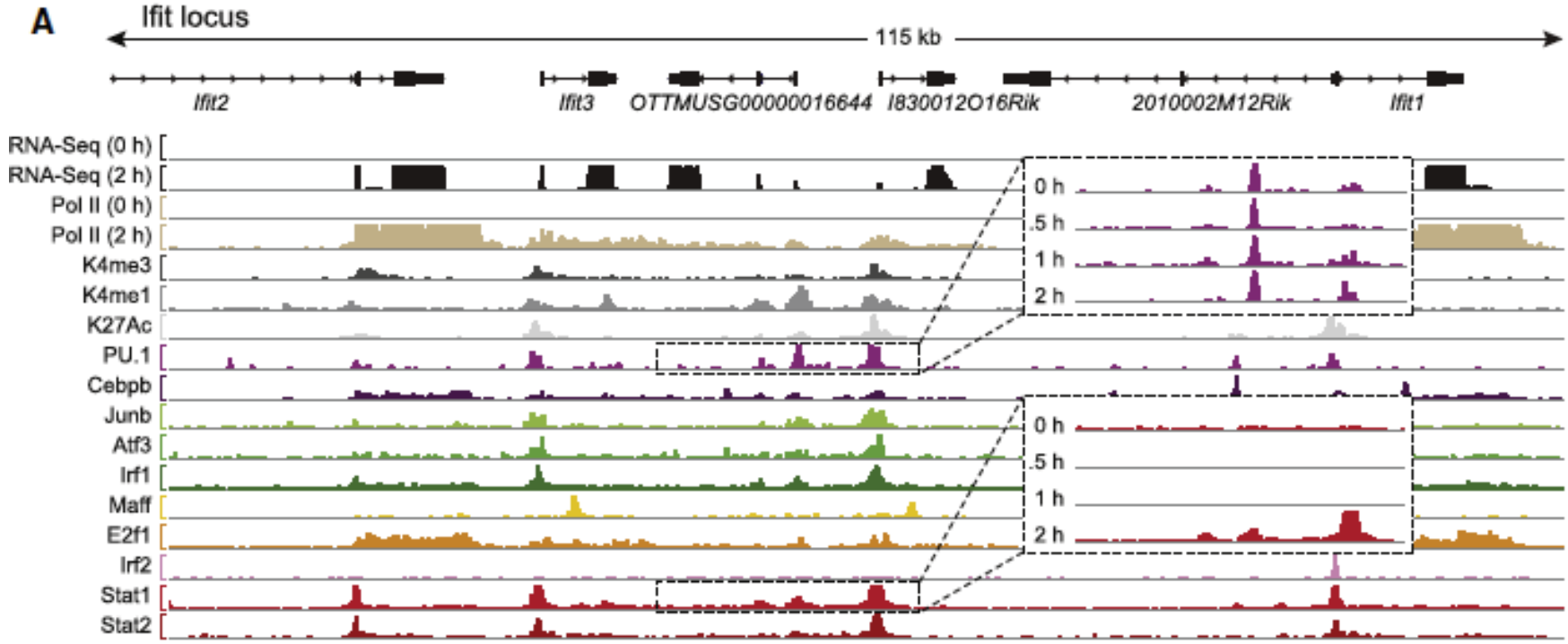
Unique tools to explore the differential states of chromatin and generating Epigenomic maps

E.

Dynamic Epigenomic Landscapes

Gene expression and epigenomic dynamics

Epigenomic landscape of dendritic cells at four different time-points after a pathogenic stimulus:



- Overlaying ChIP-seq data on gene-expression dynamics reveals that many TF/DNA interactions are established *prior* to the stimuli
- "Pioneer" factors potentiate binding by opening previously inaccessible sites
- "Primer" TFs (eg Jun-b) prime the response and set basal expression levels of many genes
- Other TFs dynamically bind subsets of genes of a shared biological process

Garber et al (2012) "A High-Throughput Chromatin Immunoprecipitation Approach Reveals Principles of Dynamic Gene Regulation in Mammals" Mol. Cell 47, 810–822

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Differential Chromatin Signatures = Different Activity States

The Modern View

Arabidopsis

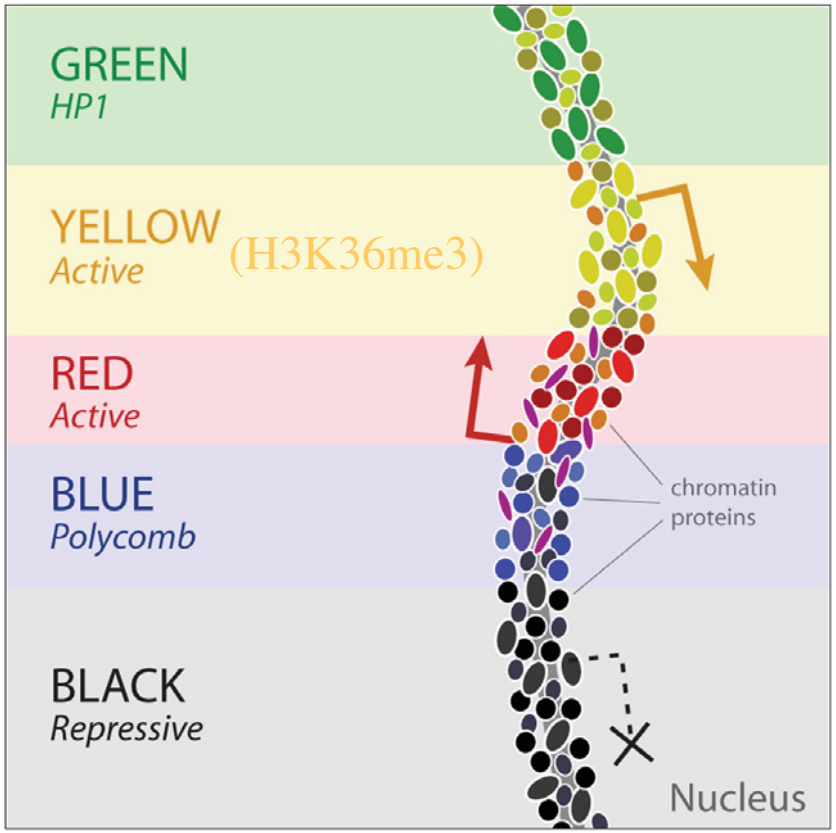
Drosophila

CS3
Heterochromatin

CS1
Expressed genes

CS2
PcG-mediated gene repression

CS4
Silent euchromatin



Roudier et al., *EMBO J* (2011)

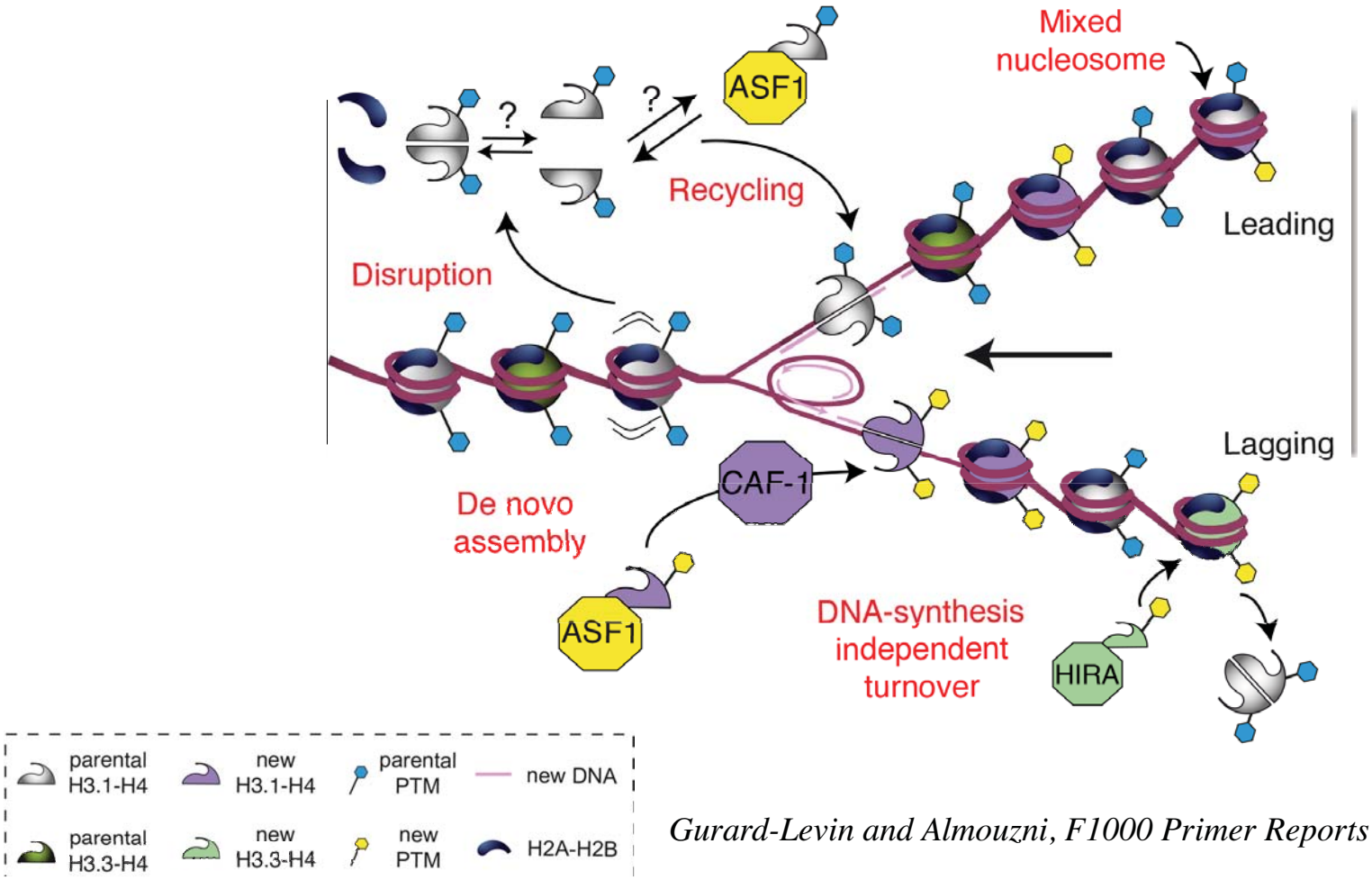
Filion et al., *Cell* (2010)

Systematic Protein Location Mapping Reveals Principal Chromatin Types

Histone Variants

Most histones are synthesized at S phase for rapid deposition behind replication forks to fill in gaps resulting from the distribution of pre-existing histones.

Canonical S- phase histones can be replaced by variants, independent of replication, and can potentially differentiate chromatin.



Gurard-Levin and Almouzni, F1000 Primer Reports

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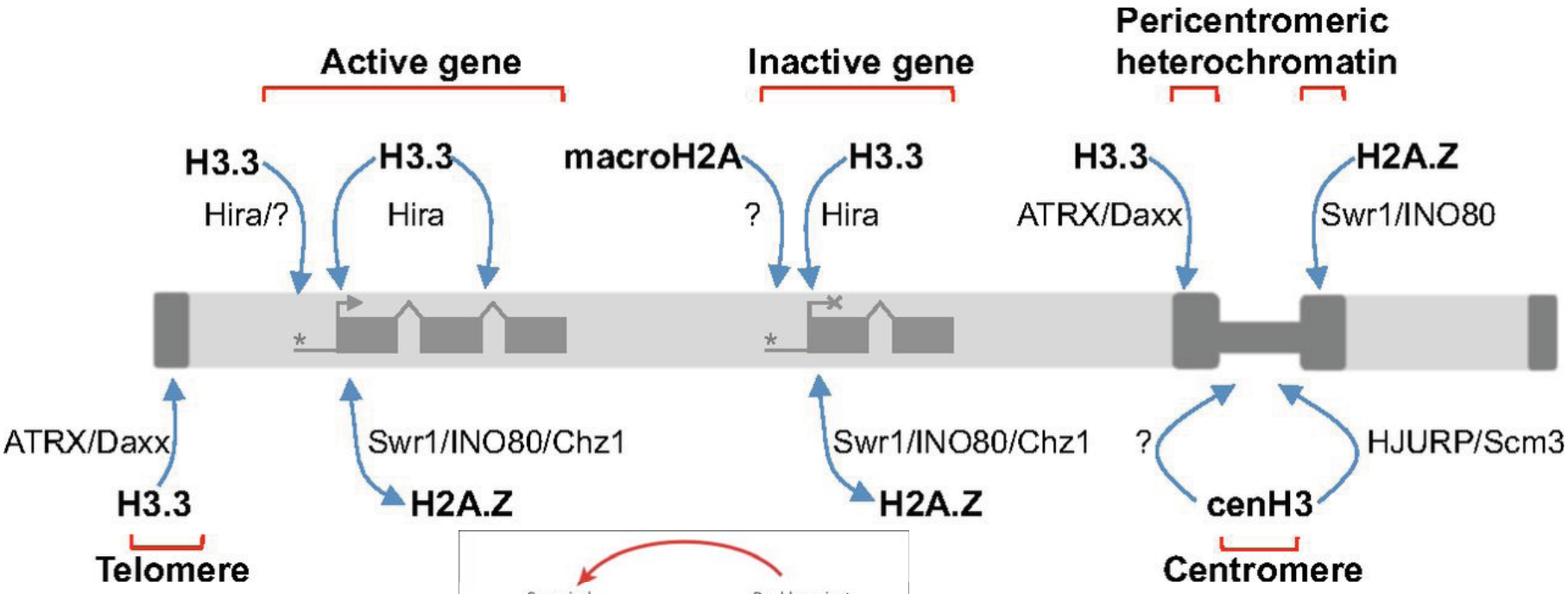

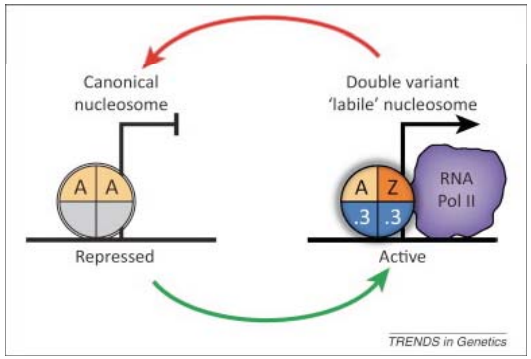

Skene P J , and Henikoff S
Development
2013;140:2513-2524

Nucleosomes with <u>double</u> Histone variants may lead to more "labile" nucleosomes – facilitating transcription…?

E. Heard, February 2nd, 2015

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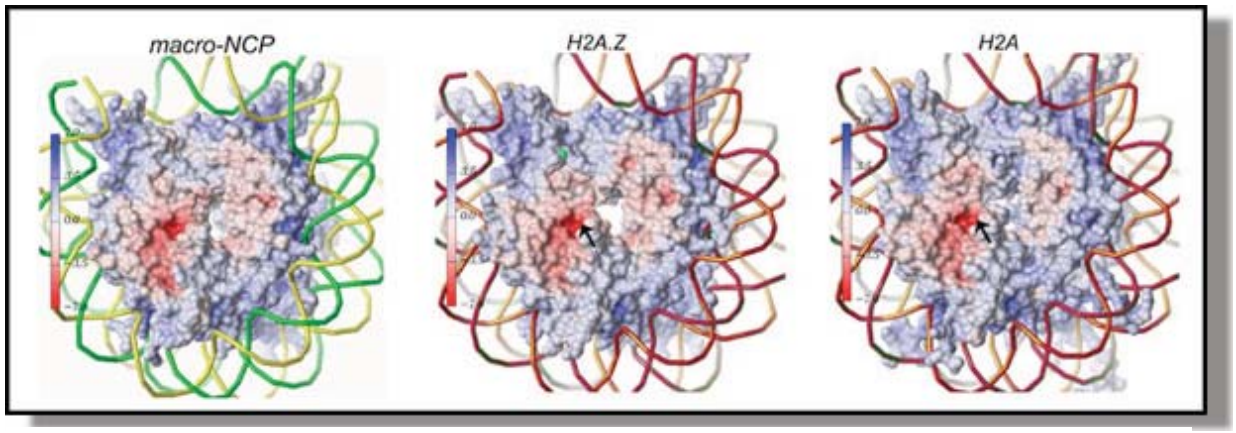
Histone Variants



Surface representation of variant nucleosomes.
From Cold Spring Harb. Symp. Quant. Biol. 69, 227-234, 2004

- Histones are remarkably conserved throughout evolution but many variant forms exist.
- Histone variants confer unique properties on chromatin structure by promoting differential interactions with associated proteins, including chaperones and chromatin remodelling factors
- H2A can be replaced by H2AZ (leads to reduced nucleosome stability), H2AX (associated with DNA repair and T cell differentiation), macroH2A (enriched on inactive X).
- H3 can exist in several forms (very few αα differences): H3.1 is only deposited during DNA replication; H3.3 is incorporated independently of DNA replication - correlates with activate genes /regulatory elements; in centromeres H3 is replaced by CENPA.

Histone Variants: emerging roles from functional studies

Key players in gene regulation during development, differentiation and disease, as well as in genome replication and stability

Histone	Number of gene copies	Cell-cycle expression	Location	Function	Knockout phenotype
H2A	15	RD	Throughout the genome	Core histone	ND
H2A.X	1	RI	Throughout the genome	DNA repair (mediated by the phosphorylated form γH2A.X) and genome integrity	Male infertility (that is, defects in sperm meiosis) in mice
H2A.Z	2	RI	Throughout the genome	Gene activation, gene silencing and chromosome segregation	Embryonic lethality in H2A.Z.1-knockout mice at E4.5–E7.5
MacroH2A	2	Possibly RI	Inactive X chromosome	Gene silencing	Brain malformation in zebrafish
H2A.Bbd	3	RI	Active X chromosome and euchromatin	Active transcription	ND
H2B	17	RD	Throughout the genome	Core histone	ND
TSH2B	1	Possibly RI	Throughout the sperm genome and in telomeres of somatic cells	Chromatin-to-nucleoprotamine transition	ND
H2BFWT	1	Possibly RI	Primate telomeres in sperm	ND	ND, as there is no gene product in mice
H2BE	1	RI	Throughout the genome of olfactory neurons	ND	Overexpression of olfactory receptor in mice
H3.1	10	RD	Throughout the genome	Core histone	ND
H3.2	3	RD	Throughout the genome	Core histone	ND
H3.3	2	RD and RI	Throughout the genome	Gene activation, silencing and chromosome segregation	ND; adult infertility in H3.3A-gene-trap and H3.3B-knockout mice
H3.4	1	RI	Sperm genome and nucleolus of somatic cells	ND	ND
H3.5	1	Possibly RI	Euchromatin in hominid testis	ND	ND, as there is no gene product in mice
H3.X and H3.Y	1 of each	Possibly RI	Euchromatin in primates	ND	ND, as there is no gene product in mice

Technical challenge – compared to histone modifications: no antibodies could distinguish between many of these histone variants (Eg H3.1, H3.2, H3.3 …) due to high degree of similarity…

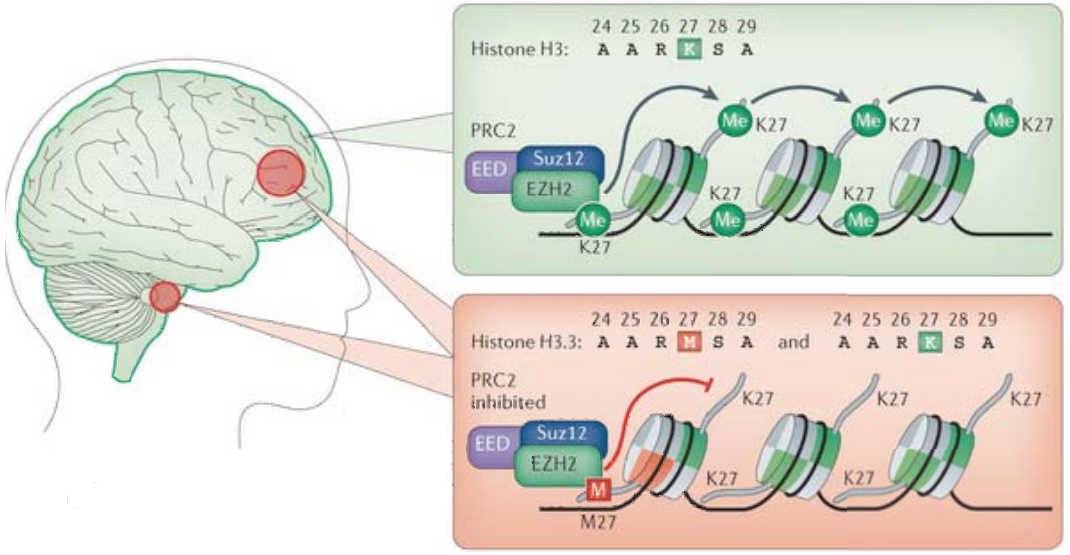
E. He

Histone Variants and Modifications implicated in Disease

Histone	Number of gene copies	Cell-cycle expression	Mutation and expression pattern	Tumorigenic consequences
H2A.X	1	RI	Reduced expression	Increased cancer progression in p53-knockout mice
H2A.Z	2	RI	Over-expression; oncogene	Numerous cancers
MacroH2A	2	Possibly RI	Reduced expression; tumour suppressor	Melanoma and other cancers
H3.1	10	RD	K27M in H3.1B	Adult and paediatric gliomas, including GBMs and DIPGs, respectively
H3.3	2	RD and RI	K27M, G34R and G34V in H3.3A	Adult and paediatric gliomas, including GBMs and DIPGs, respectively
			K36M in H3.3B	Chondroblastoma
			G34W and G34L in H3.3A	Giant cell tumours in bone
CENP-A	1	RI	Over-expression; oncogene	Numerous cancers

From Maze et al, NRG, 2014

H3.3 Lys 27-to-methionine (K27M) mutation in one of two alleles leads to very specific gliomas. This mutation reprograms epigenetic landscape and gene expression: see genome wide loss in H3K27me3 but specific aberrant enrichment at several hundred genes. This may drive tumorigenesis.
Chan et al, Genes Dev , 2013

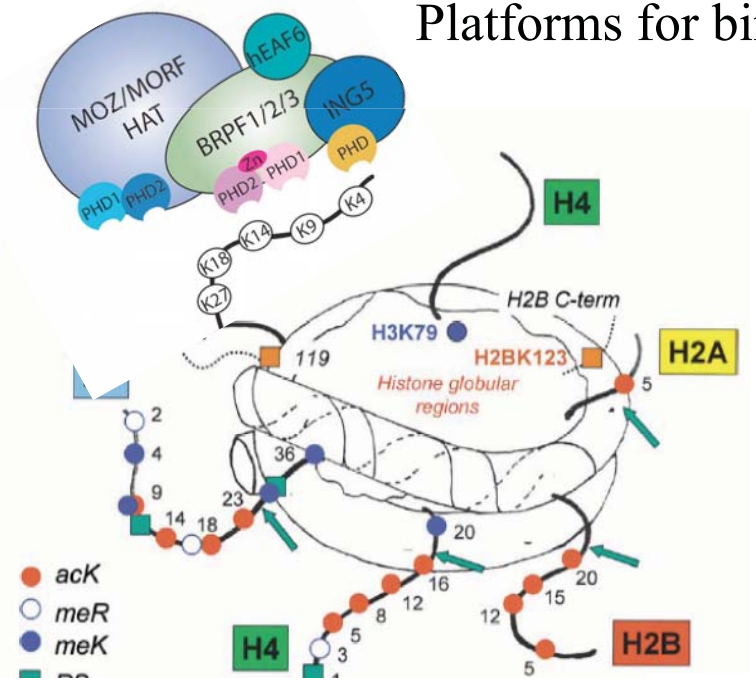


Chromatin-based States and Partners

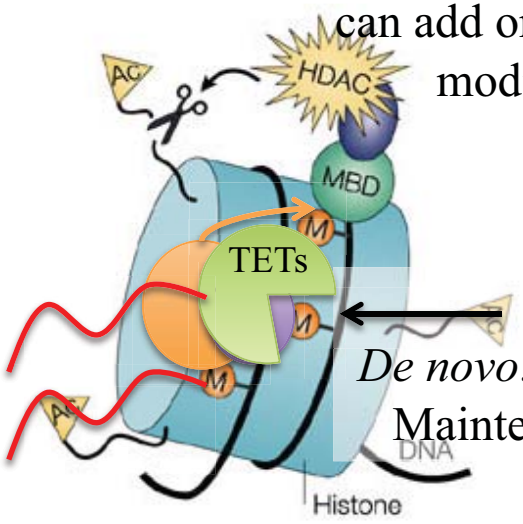
Histone Variants and Histone Modifications are:

Mediators of chromatin accessibility

Platforms for binding proteins



Histone modifying enzymes can add or remove these modifications



Dnmts

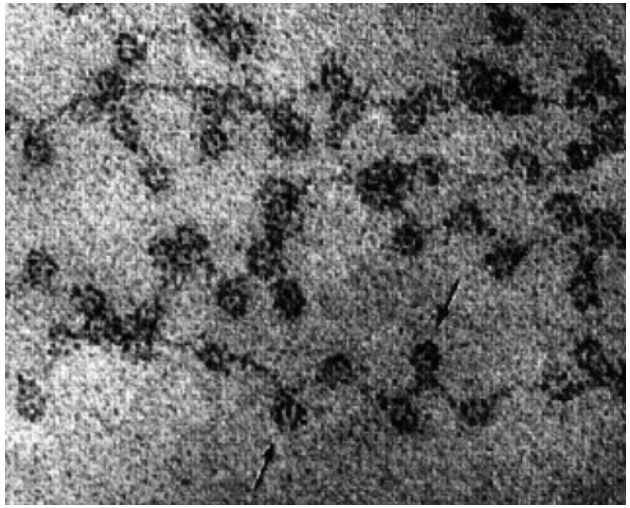
De novo: Dnmt3a,3b, 3L

Maintenance: Dnmt1

RNAs are also important players:
lncRNAs or small interfering RNAs
(in heterochromatin)
(COURS II + III)

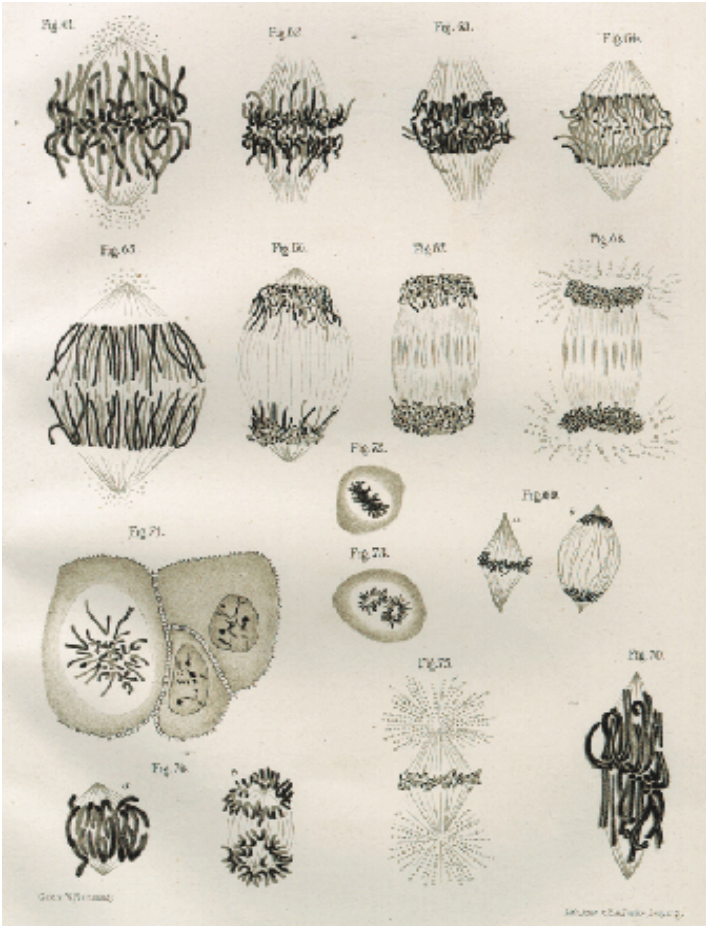
DNA methylation associated with repressed state of some genes, repeats:
Self-templating, stable - but can be removed
(**actively** eg Tet-induced conversion to 5hme;
passively during DNA replication)

What are the Roles of Chromatin?

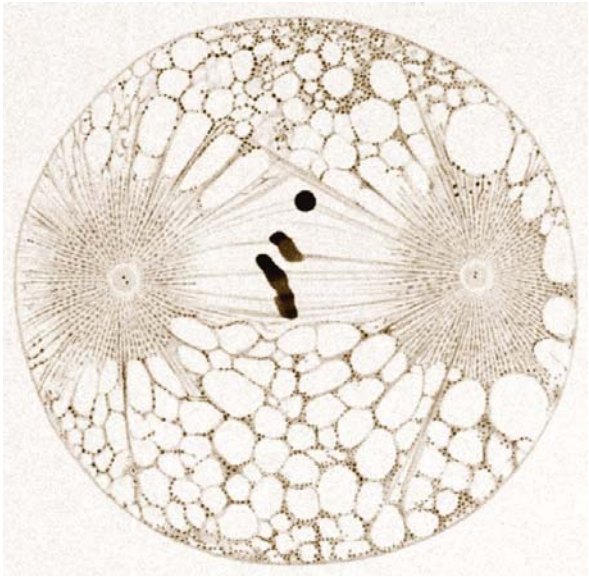


- Packaging of the the genome in interphase and during mitosis
- Barrier to aberrant gene expression and reprogramming
- Facilitator of gene regulation and genome function
- Integration of environmental signals
- Carrier of cellular memory…cell cycle, mitosis and meiosis

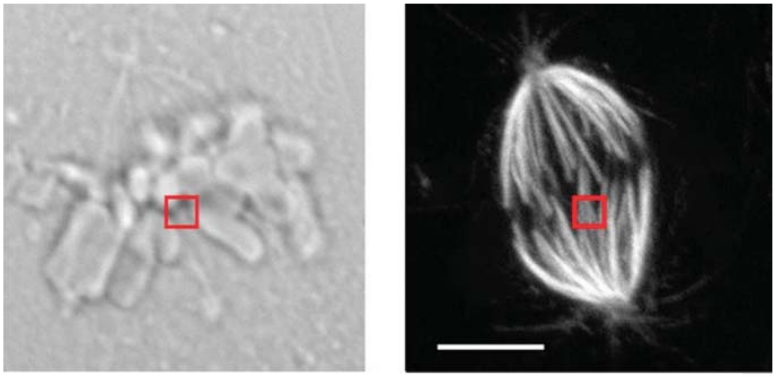
Packaging of the the genome in interphase and protecting it during mitosis and meiosis



Chromatin plays a role in reinforcing (protecting) the DNA macromolecule during mitosis



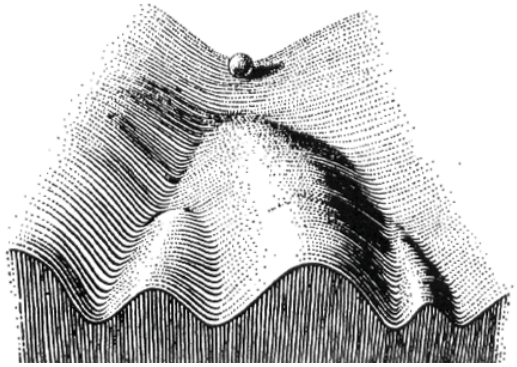
Cell division in Ascaris megalocephala bivalens, as drawn by Boveri in 1901.



Ferraro-Gideon et al (MBoC, 2013) using optical traps calculated that the forces applied during mitosis are **~2-10 pN** (100x less than studies by Nicklas, 1983)

Barrier to aberrant gene expression and reprogramming

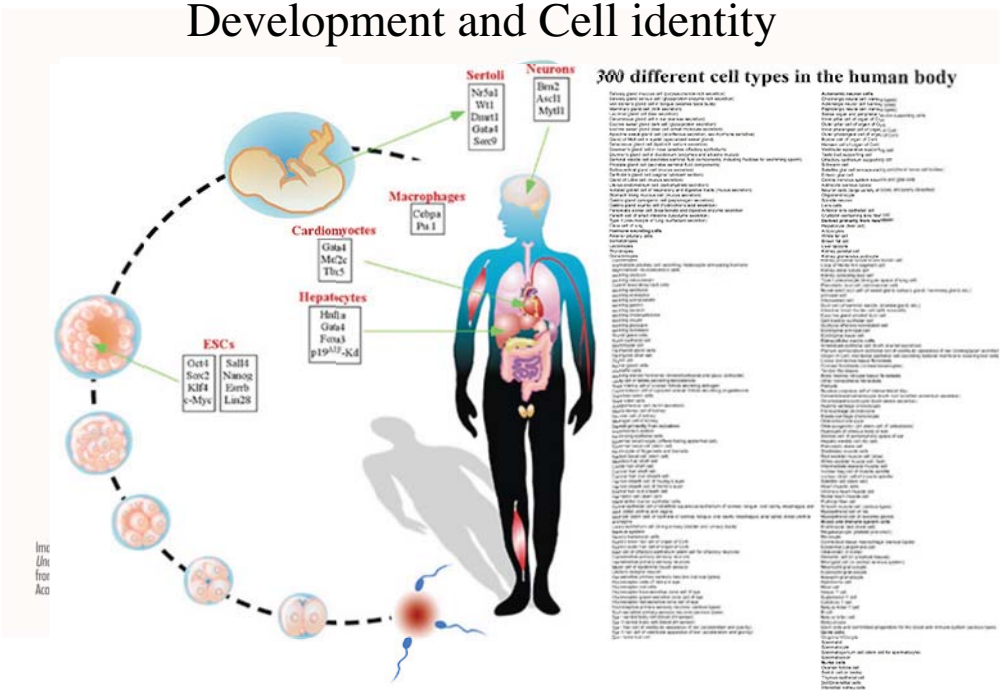
Development and Cell identity



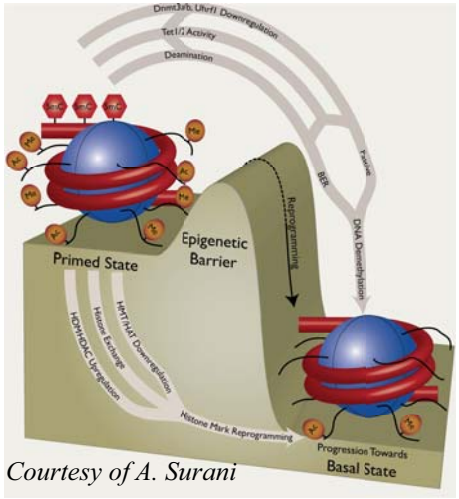
cf C.H. Waddington

Barrier to aberrant gene expression and reprogramming

Development and Cell identity

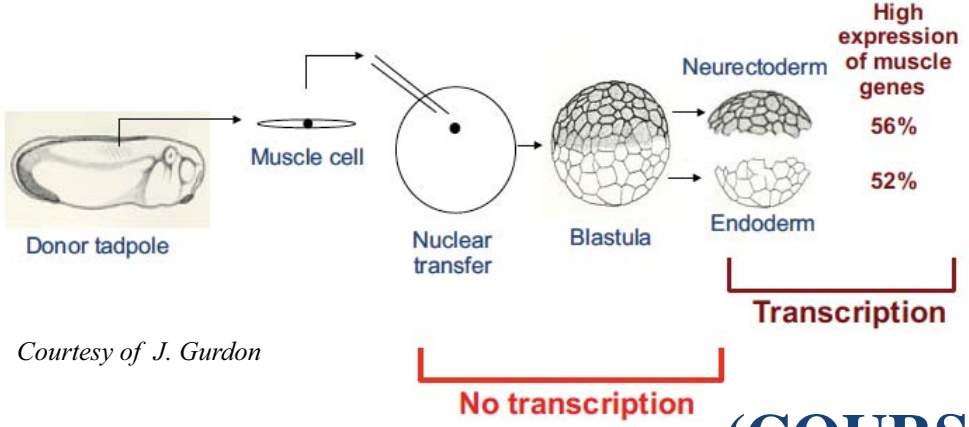


Germ line



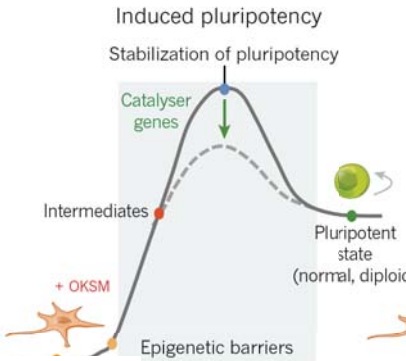
Courtesy of A. Surani

Nuclear Transfer

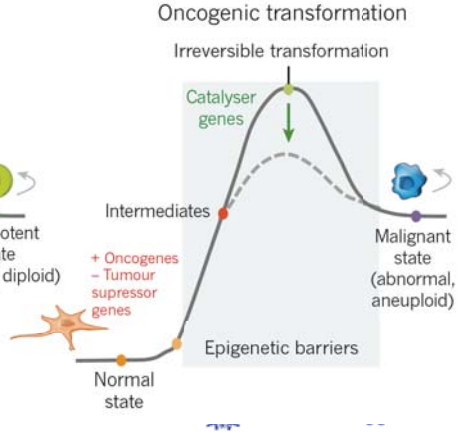


Courtesy of J. Gurdon

Induced Pluripotency



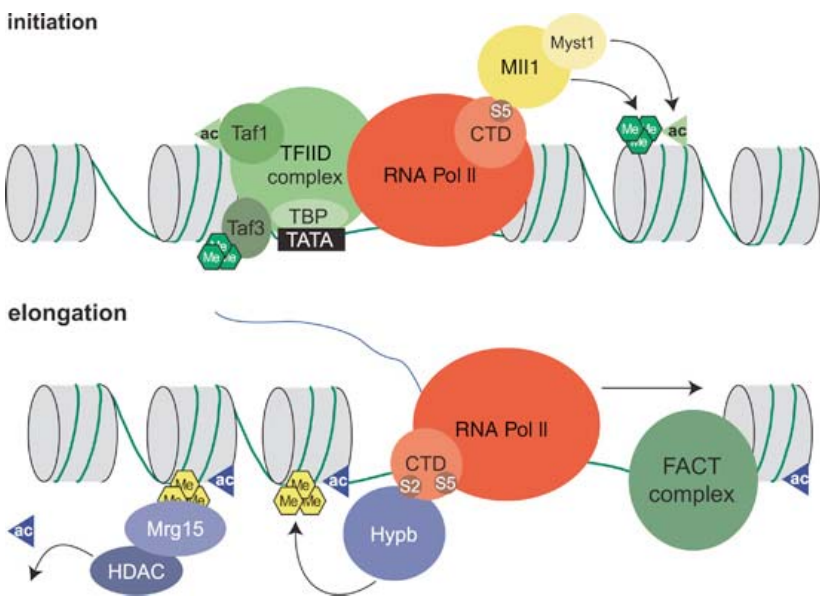
Cancer

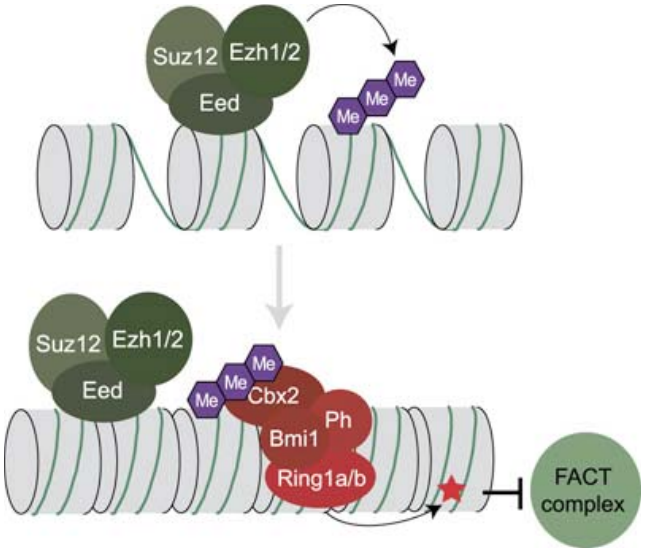


(COURS 2014)

Chromatin as a facilitator Transcription and Repression

Stable vs Dynamic…





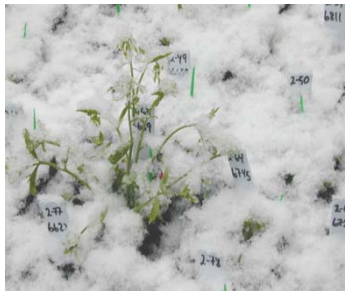
Gene expression

Gene repression

Chromatin as an Integrator of Environmental Signals?

Environmentally programmed changes

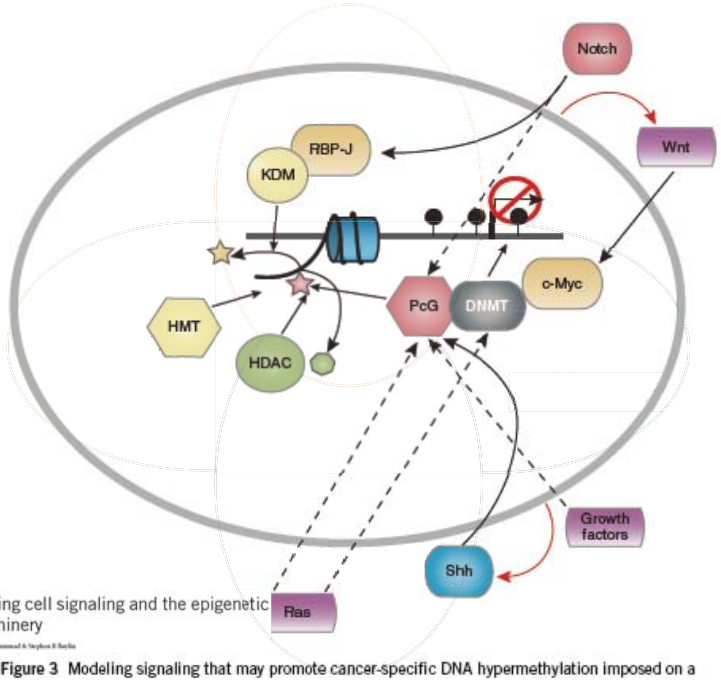
Vernalisation (Polycomb)



Courtesy of C. Dean

Honey Bees (DNA methylation?)

Environmentally induced changes….?



Linking cell signaling and the epigenetic machinery

Figure 3 Modeling signaling that may promote cancer-specific DNA hypermethylation imposed on a

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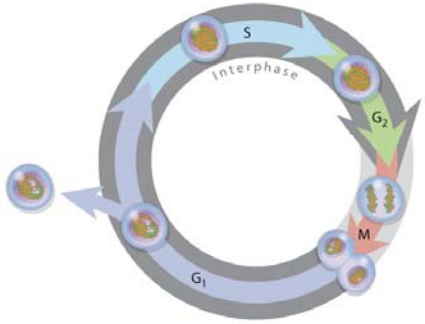
Chromatin: as a carrier of cellular memory

The Dilemma: Chromatin is Highly Dynamic

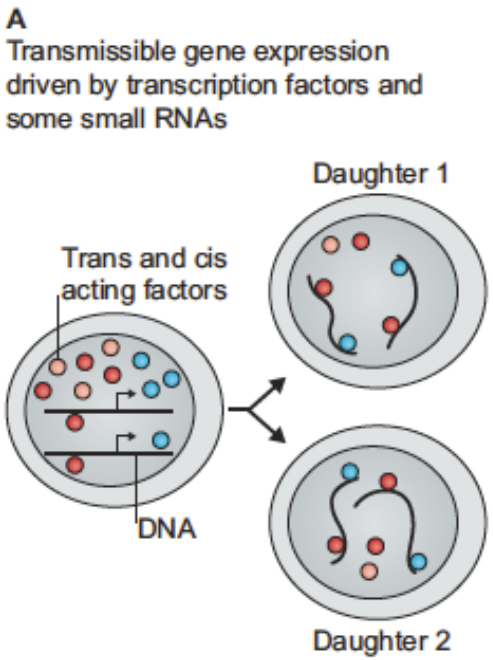
Despite chromatin being a barrier to transcription, it is highly dynamic: even heterochromatin-associated proteins bind with residence times of <1min (Phair et al. 2004).

The chromatin of actively transcribed genes is in constant flux, characterized by continual histone replacement (Dion et al. 2007).

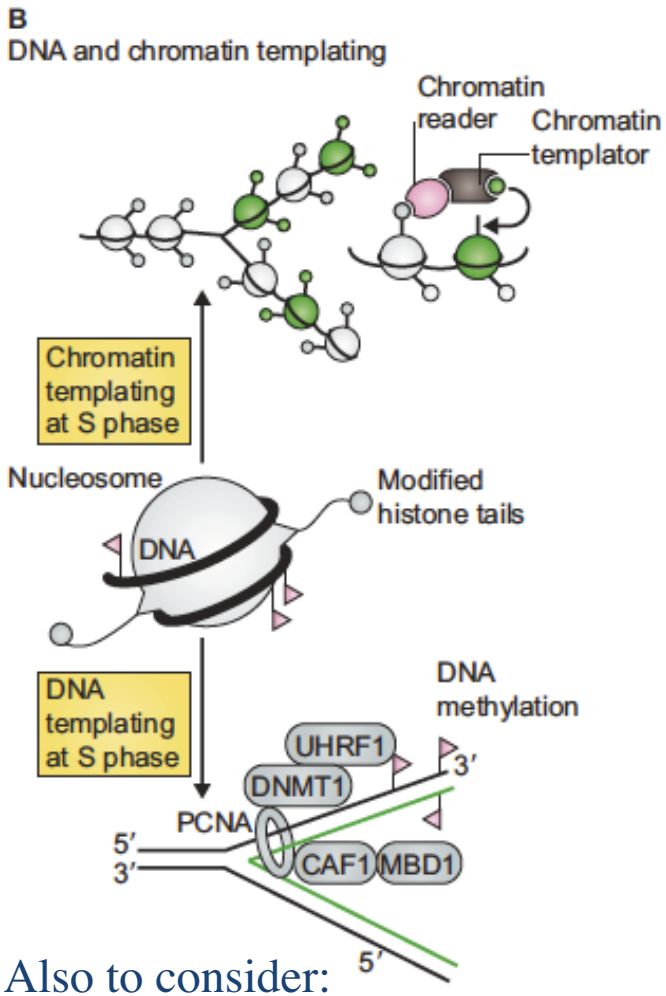
How can chromatin be a carrier of cellular memory
– in the face of such dynamic turnover
(histones exchange, chromatin remodelling, erasure of modifications)
– during the cell cycle, including S-phase
(dilution/removal of memory marks)
- across generations
(potent germ-line reprogramming in many species)

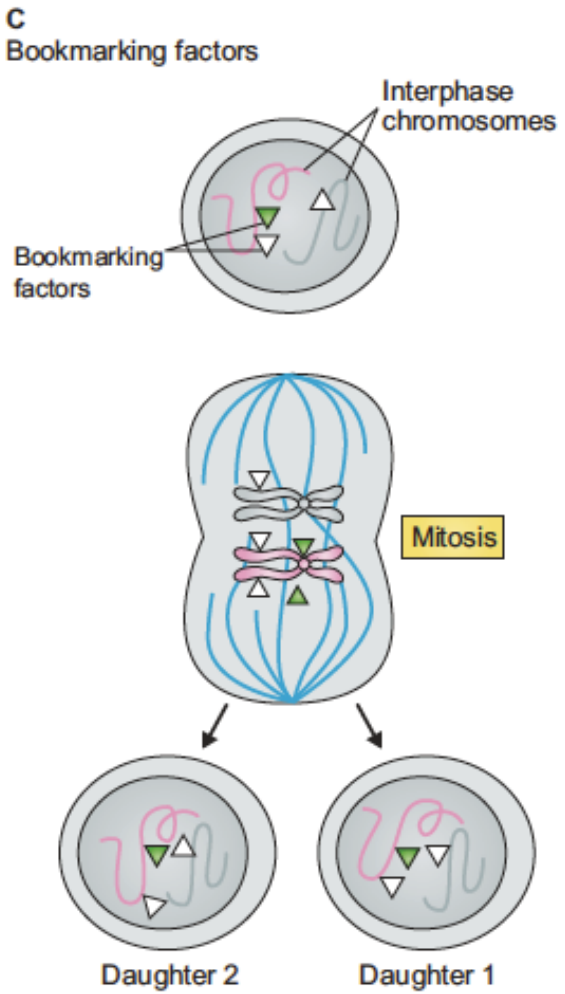


Chromatin: as a carrier of cellular memory



A. Fisher andN. Brockdorff, 2013





Also to consider:

- Long range chromosomal interactions
- Nuclear positioning (lamina)
- Replication timing