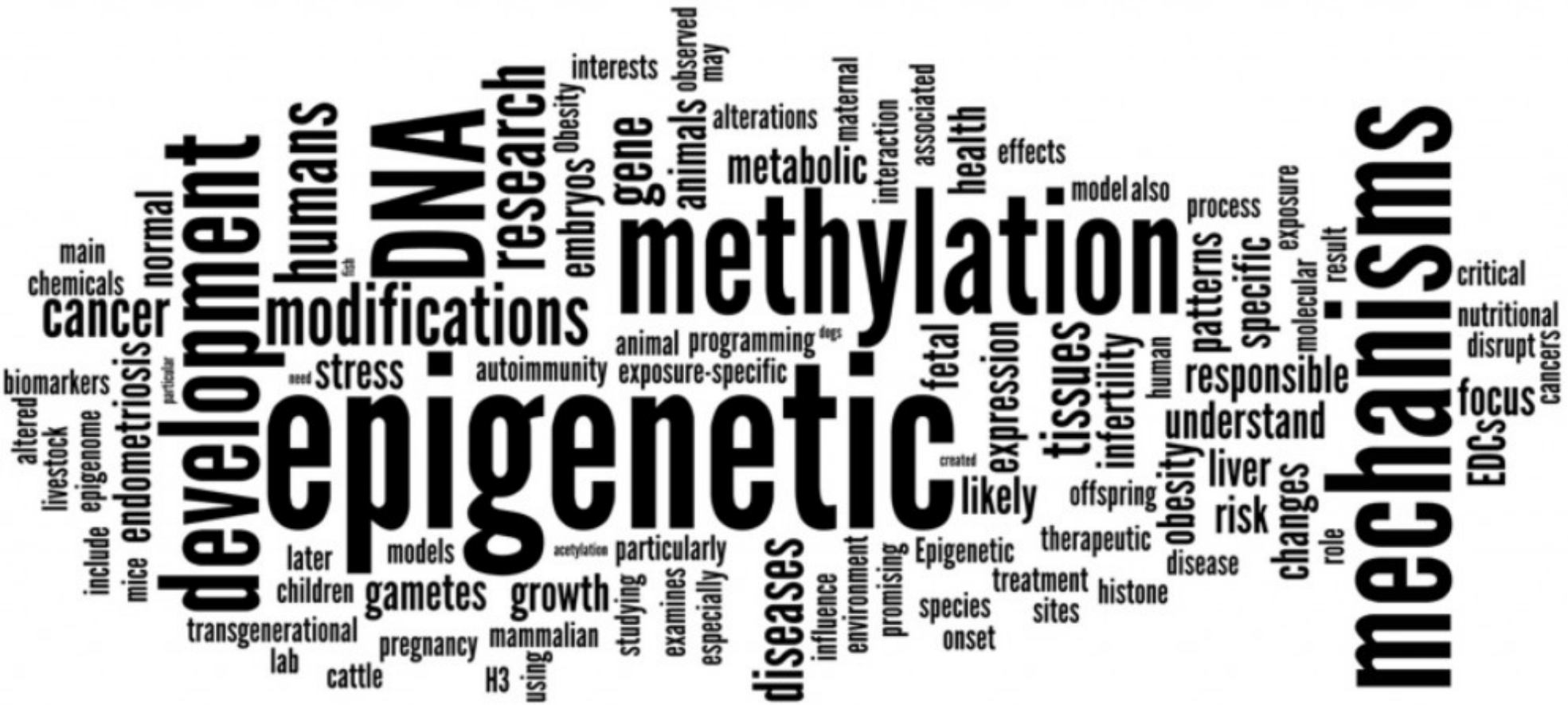
EPIGENTICS


epigenetic
methylation
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obesity
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nutritional
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cancers
focus
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Epigenetic
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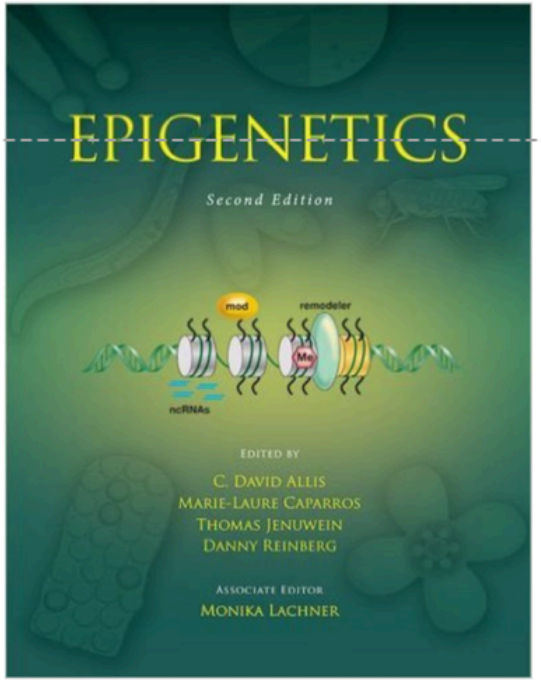
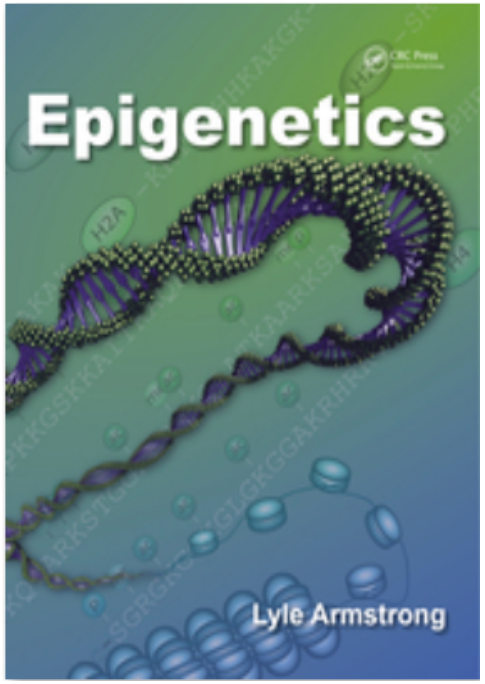
Materiale didattico AA2021-2022





1. Moodle federato

Book Chapters
PPT slides
Publications

2. MS Teams

Recording of lectures
Code for Team: x2z919k

3. Textbook

Allis et al. "Epigenetics", Second Edition CSH Press;
Prezzo: 150 Euro; Advanced Textbook
Avaialble in Library Uni TS

Leyle Armstrong. "Epigenetics", Taylor and Francis Group;
Prezzo: 70 Euro; Introduction into Epigenetics
Avaialble in Library Uni TS

Lecture Program AA2020-2021

Goal of the lecture:

- Overview on aspectes of epigenetics involving protein and RNA factors
- Detailed knowledge of selected epigentic regulatory pathays
- Integration of epigentic processes in development and disease
- Capacity to understand and interpret experimental data in scientific publications
- Capacity to expand from basic concepts to more complex scientific context

(for details check syllabus):

Lecture Program AA2019-2020

1. Introduction in epigentics and groudbreaking discoveries
2. Histone proteins and chromatin templartes
3. Gene expression control and epigenetics
4. Writers and readers of histone acetylation
5. Erasers of histone acetylation
6. Structural and functional coordination of DNA and histone mehtylation
7. RNAi and Heterochromatin assembly
8. Position Effect Variegation, heterochromatin formation and gene silencing in Drosophila
9. Polycomb and Trithorax group proteins
10. Histone Variants in Cell Physiology
11. Maintenance of Epigenetic Information
11. Epigenetics in development and reprogramming
12. Epigenetics in Human Disease

Lecture Schedule

Monday *09:00 – 11:00 Aula 0B, Ed H3*

Tuesday *09:00 – 11:00 Ed.C2 Aula A*

CdL SM53 anno 1

GENOMICA FUNZIONALE
II semestre: dal 7/03/2022 al 10/06/2022

	Lunedì	Martedì	Mercoledì	Giovedì	Venerdì
8:00-9:00			Corso : 980SV Aula: **Aula C Edificio C9**	Corso : 677SM Aula: **Aula 3B Edificio H3**	Corso : 917SM Aula: **Aula C Edificio C9**
9:00-10:00	Corso : 676SM Aula: **Aula 0B Edificio H3**	Corso : 676SM Aula: Aula A **Ramponi Edificio C2**	Corso : 980SV Aula: **Aula C Edificio C9**	Corso : 677SM Aula: **Aula 3B Edificio H3**	Corso : 917SM Aula: **Aula C Edificio C9**
10:00-11:00	Corso : 676SM Aula: **Aula 0B Edificio H3**	Corso : 676SM Aula: Aula A **Ramponi Edificio C2**		Corso : 602SM Aula: **1_B Edificio D**	
11:00-12:00	Corso : 677SM Aula: **Aula 2A Edificio H3**	Corso : 602SM Aula **B Edificio A**	Corso : 917SM Aula: **Aula A Edificio A**	Corso : 602SM Aula: **1_B Edificio D**	Corso : 995SV Aula: **Aula 5A Edificio H2bis**
12:00-13:00	Corso : 677SM Aula: **Aula 2A Edificio H3**	Corso : 602SM Aula **B Edificio A**	Corso : 917SM Aula: **Aula A Edificio A**		Corso : 995SV Aula: **Aula 5A Edificio H2bis**
13:00-14:00					
14:00-15:00		Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**			Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**
15:00-16:00		Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**			Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**
16:00-17:00		Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**			Corso : 675SM Aula: **Aula Ex_Cla Edificio C1**
17:00-18:00					

Codice corso	Nome del corso	Docente titolare	
677SM	TECNOLOGIE MOLECOLARI E CELLULARI	SBLATTERO DANIELE	
980SV	COMUNICAZIONE SCIENTIFICA IN LINGUA INGLESE	RAMANI	
602SM	GENOMICA APPLICATA	GERDOL MARCO	
917SM	METODOLOGIE PER LO STUDIO DEL PROTEOMA	SGARRA RICCARDO	**laboratorio DAL 26/04 da lun. a ven. tutti i pomeriggi ore 14-18 LAB.BIOL.EDIF.C-1**
675SM	MICROSCOPIA OTTICA IN BIOLOGIA CELLULARE	BAJ Gabriele	
676SM	REGOLAZIONE EPIGENETICA	SCHOEFTNER Stefan	
995SV	SEGNALAZIONE CELLULARE	COLLESI CHIARA	

RULES ESAME "REGOLAZIONE EPIGENETICA"

1. STANDARD MODALITY OF THE EXAM:

The exam comprises a WRITTEN test that comprises two parts:

Questions:
Written in English

Answers:
In Italian or English

Short answer part:

11 Questions; 1 point/question

This part of the exam can consist: questions with multiple choice or questions with short written answers or questions that require a simple, schematic drawing/diagram as answer.

Detailed answer part:

4 Questions, 5 points/question

Questions of this part of the exam will address a general concept of molecular biology or central processes in molecular biology. Students are asked to give a detailed and focused answer (max. 1 page). A focus will be given on the use of specific scientific terms that relate to the respective topic of the question. The question can also be formulated in a manner that evaluates the logic understanding of topics addressed during the lecture.

Duration of the exam:

2 hours

What to bring:

Carta d'Identità

2 pens with different colors (useful for answers that require simple drawings)

Max. Points:
11+20 → **30** (31)

2. RESULTS OF THE WRITTEN EXAM:

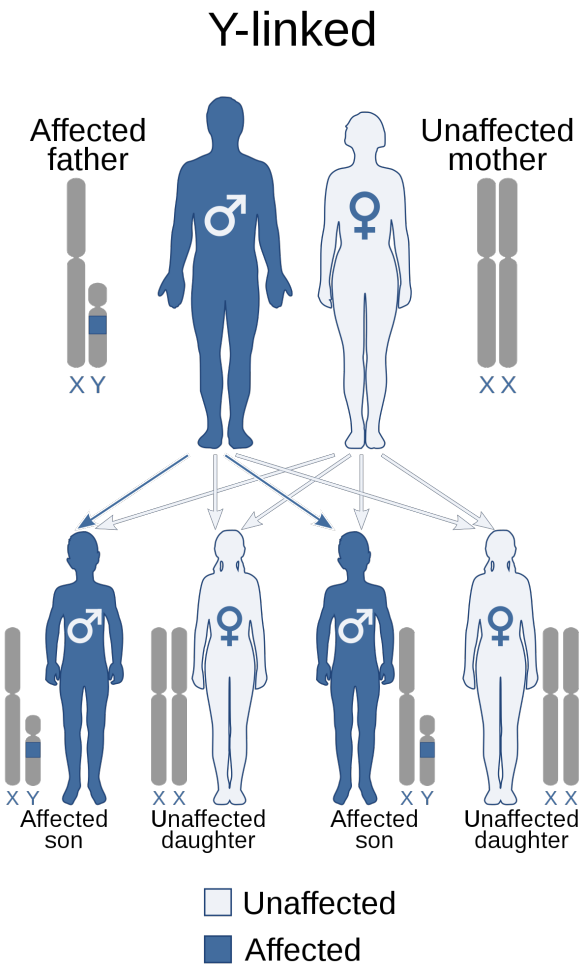
- **Students that participated in a call/Appello will be informed via e-mail when the results of the exam have been published on ESSE3**
- Student representatives can ask for an occasion to have a look at the respectives tests
- Students need to accept the result within one week of the publication of the result.
- Instructions will be sent per e-mail to all students.

1. Introduction in epigentics and groudbreaking discoveries

GENETICS ← → EPIGENETICS

GENETICS: the study of heredity and the variation of inherited characteristics. DNA based

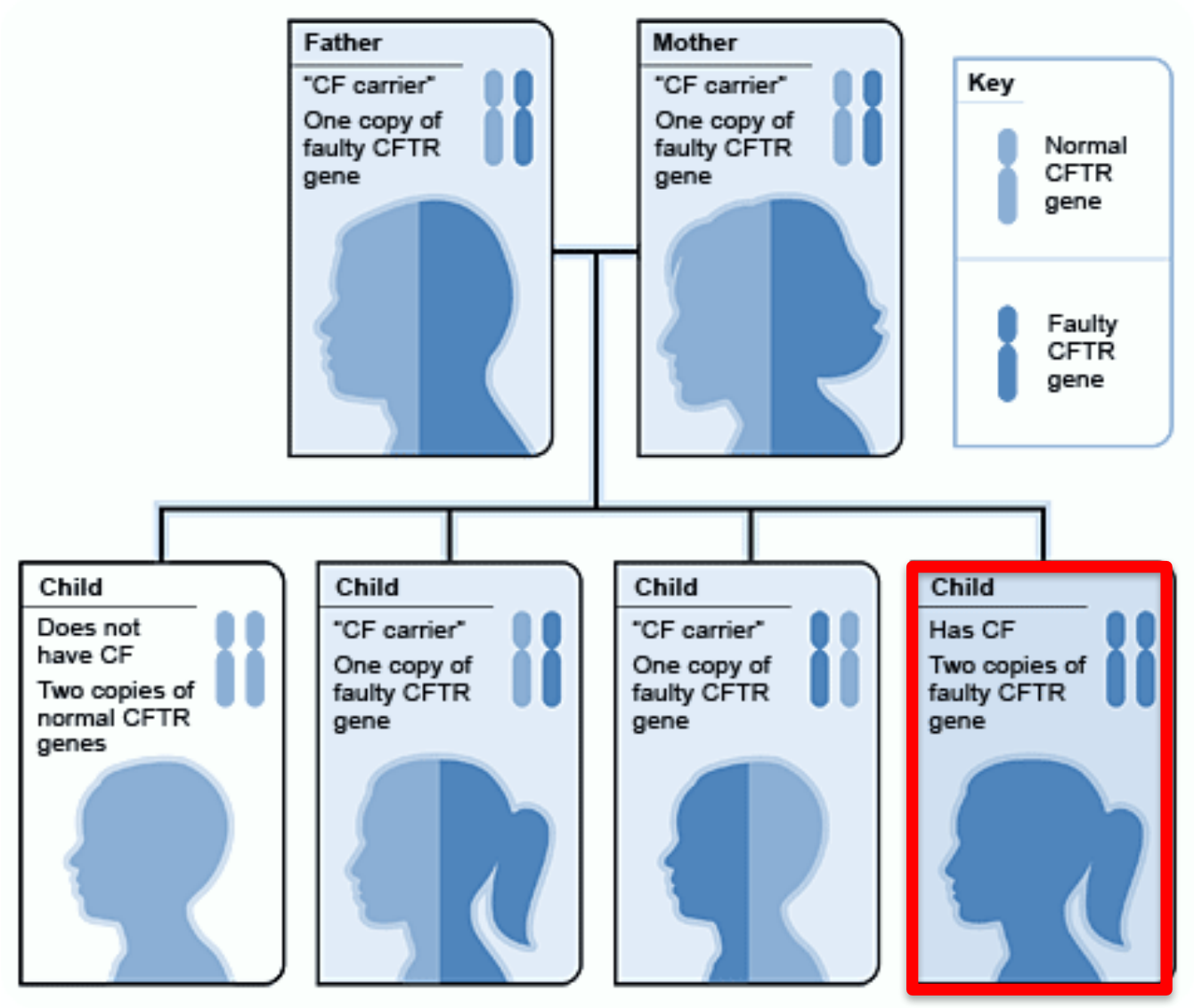
Genetic inheritance



Genetic disease

GENETICS ← → EPIGENTICS

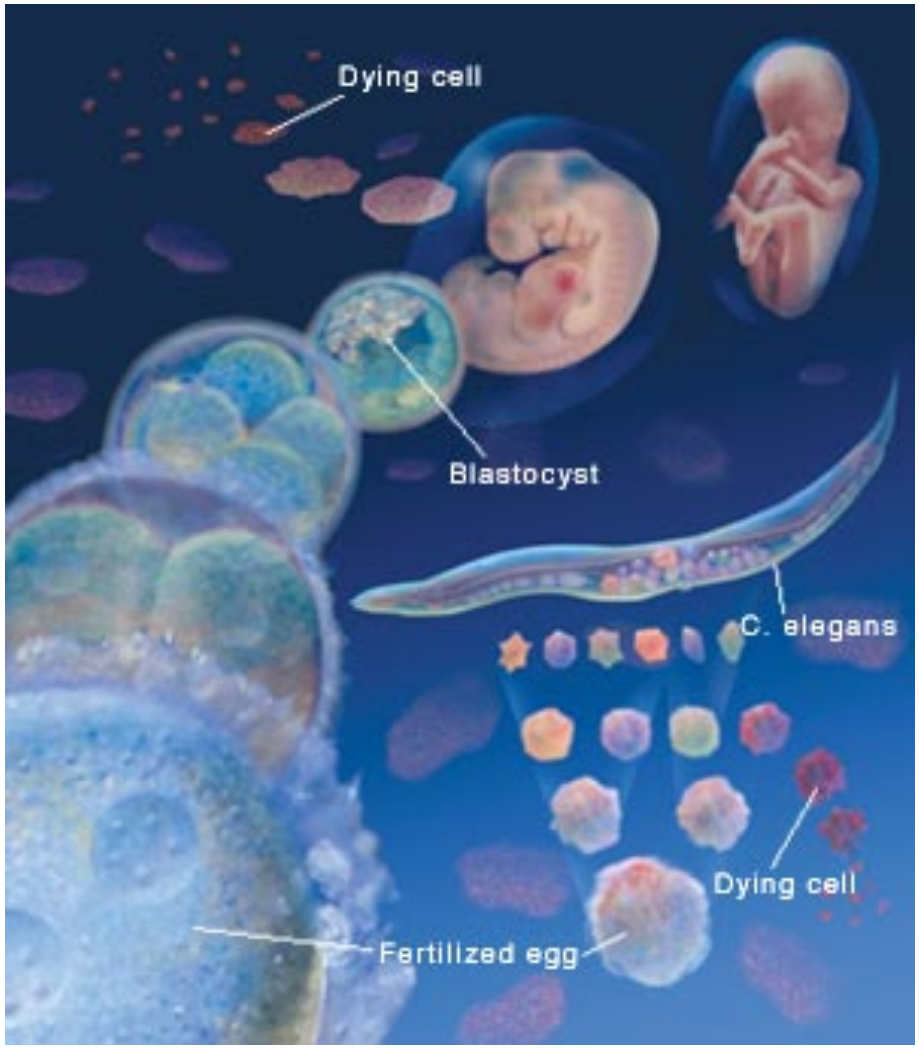
Gentic inheritance of Cystic fibrosis



Recessive CTCF mutation is inherited

GENETICS ← → EPIGENETICS

What is Epigenetics?….a scientific term in evolution



A developed organism with specialized tissues and cell types derives from a single cell; the zygote

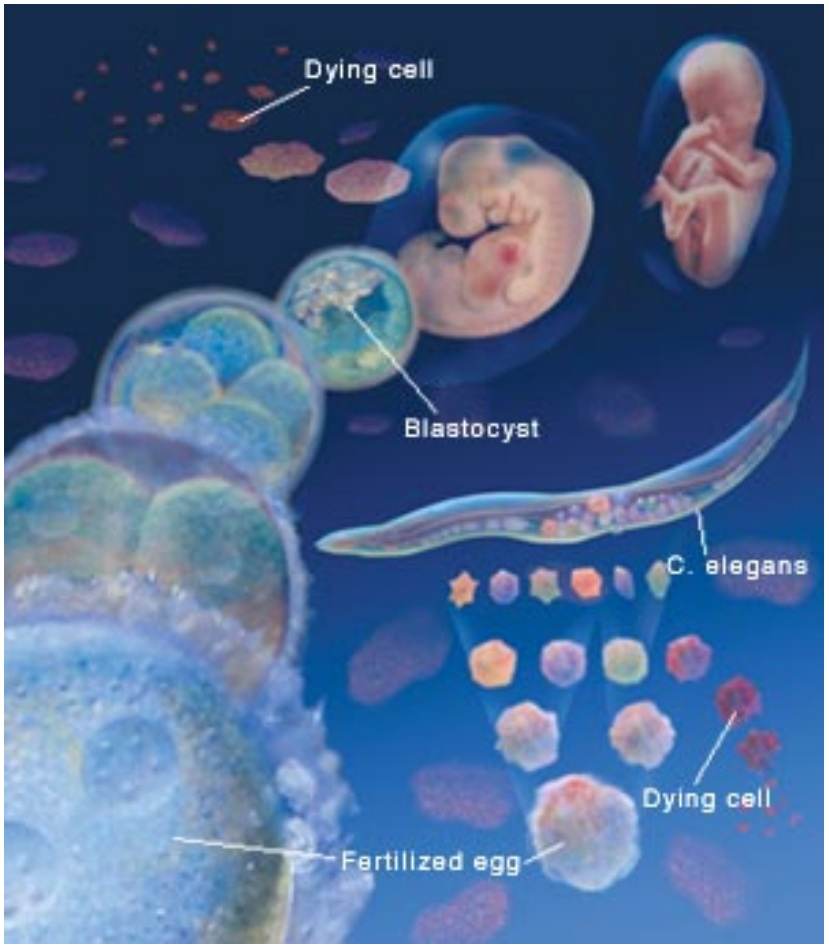
All cells of an organism contain idential genetic information = DNA

KEY QUESTION IN EARLY 1900

What molecules within the chromosomes carry the genetic information, how do they direct the developmental program, and how is the information transmitted during cell division?

GENETICS ← → EPIGENTICS

What is Epigenetics?....a scientific term in evolution



Conrad Hall Waddington:
British embryologist; 1950

...Epigenetics is the study of processes that categorize all of the developmental events leading from the fertilized oozyte to the mature organism – that is, all of the regulated processes that, beginning with the genetic material, shape the final product

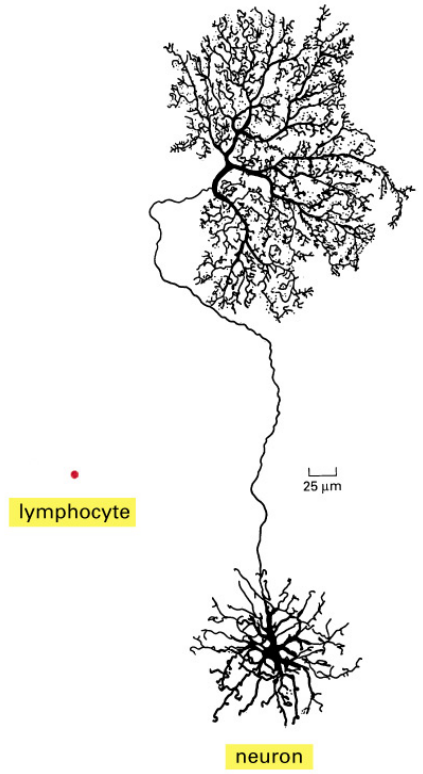
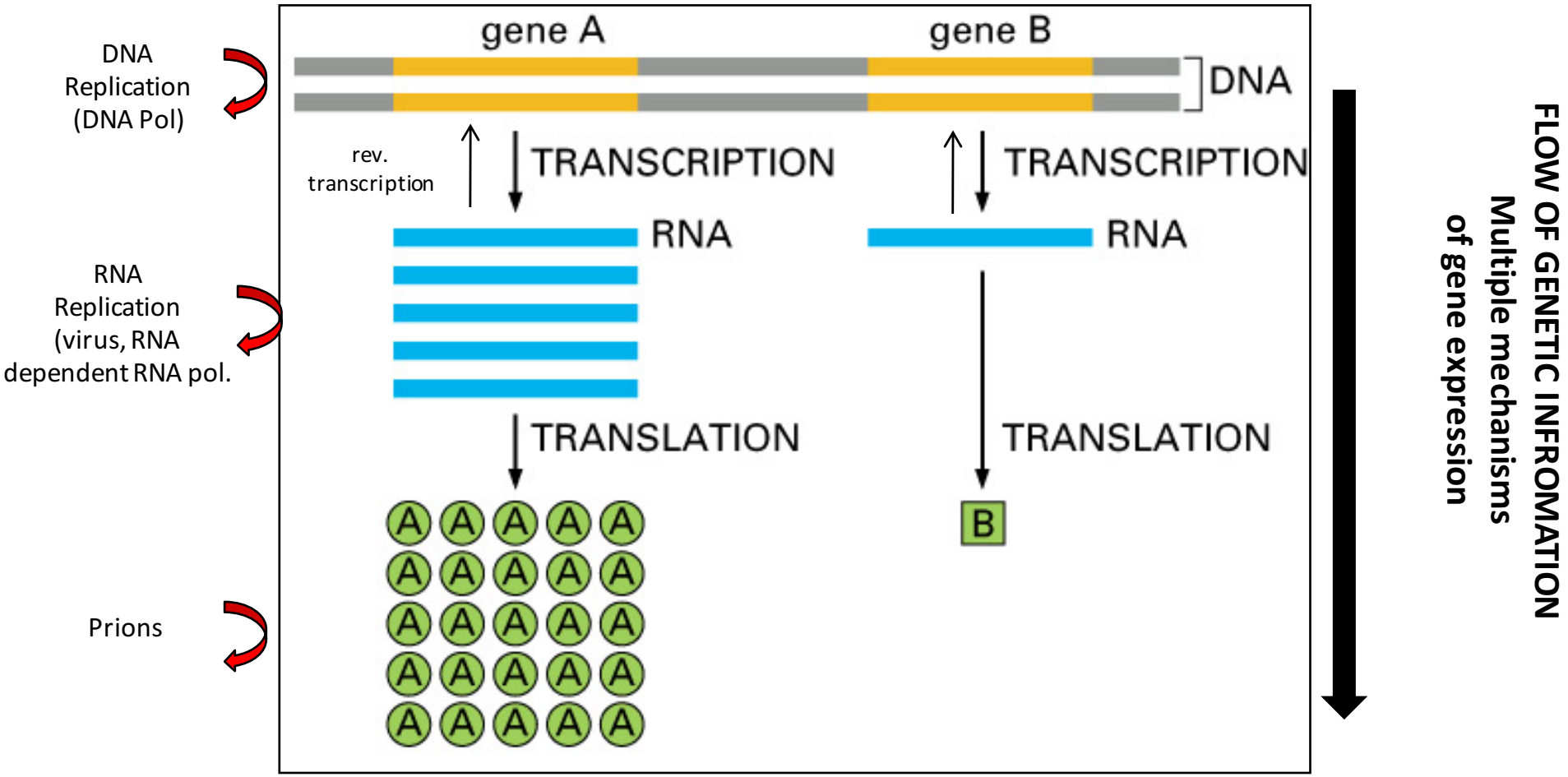


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

Genetics, Epigentics and the central dogma

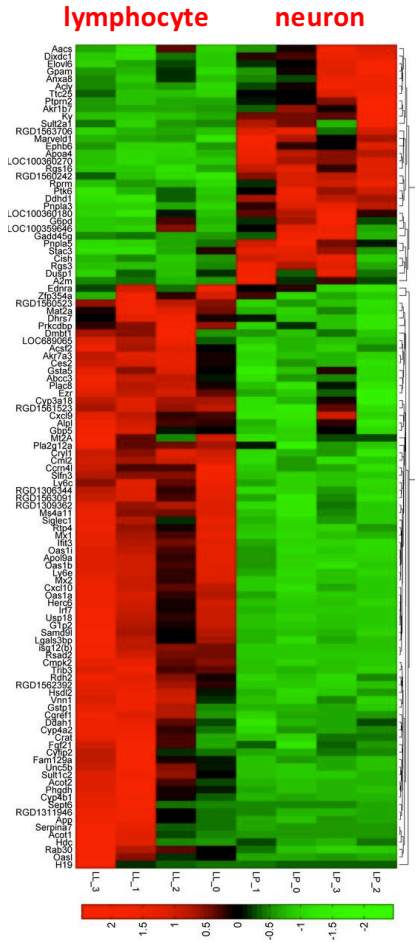
Crick, F (1970). "Central dogma of molecular biology." Nature 227 (5258):
The central dogma of de molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid.



Gene expression control for cell specification and development

GENOME coding and non-coding genes

Specific gene expression programs



Cell function

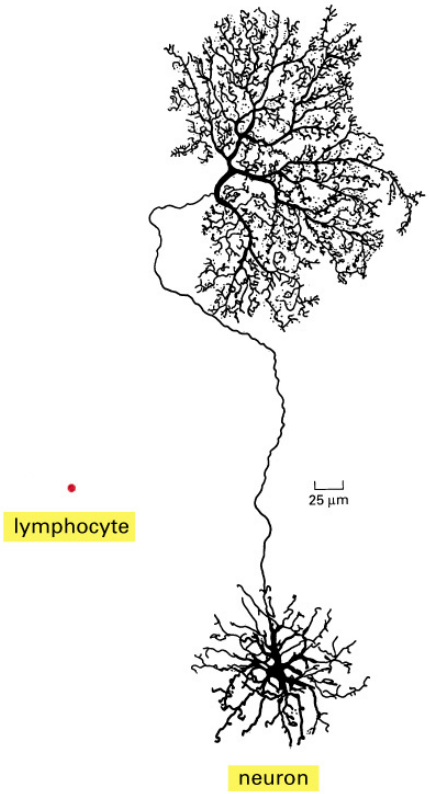


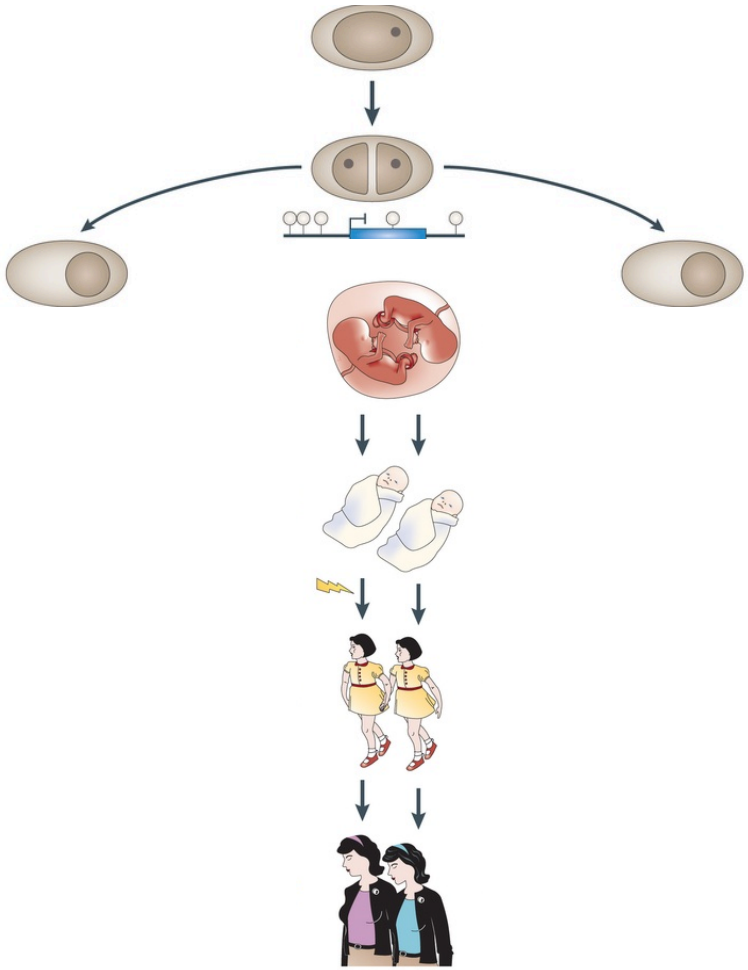
Figure 7–1. Molecular Biology of the Cell, 4th Edition.

1 Genome → >200 different cel types

Genetic information is used in a highly controlled manner

Observations that demonstrate that gene regulation also acts on top of genetics

→ Monozygotic twins and disease



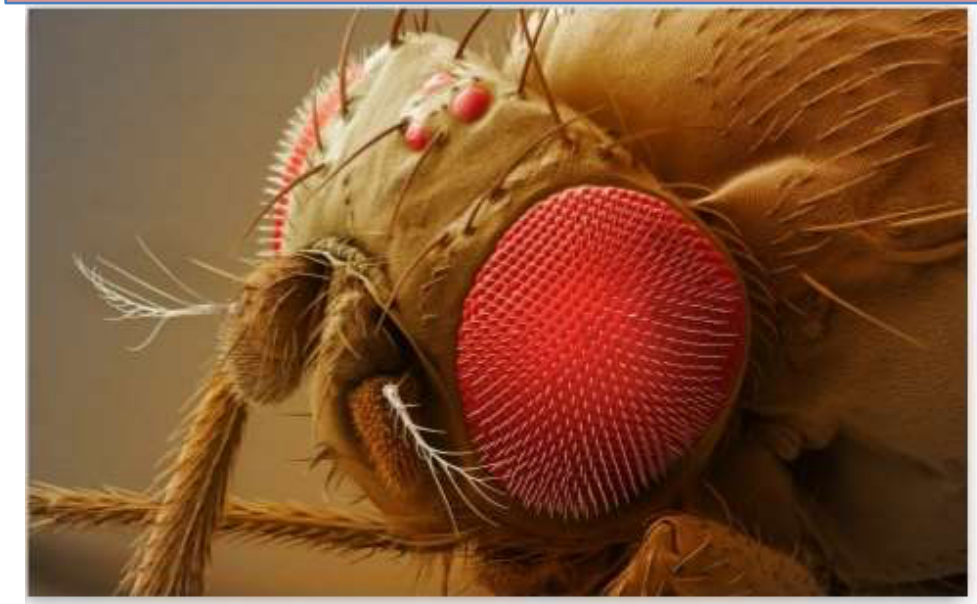
Monozygotic twins have 100% identical DNA
ONLY ONE TWIN DEVELOPS MULTIPLE SCLEROSIS

…but disease (multiple sclerosis) only in one of the two twins

…. There must be a special type of genetic information other than that of DNA

Observations that demonstrate that gene regulation also acts on top of genetics

→ The drosophila eye – a role model system for genetic/epigenetic research….Muller 1950

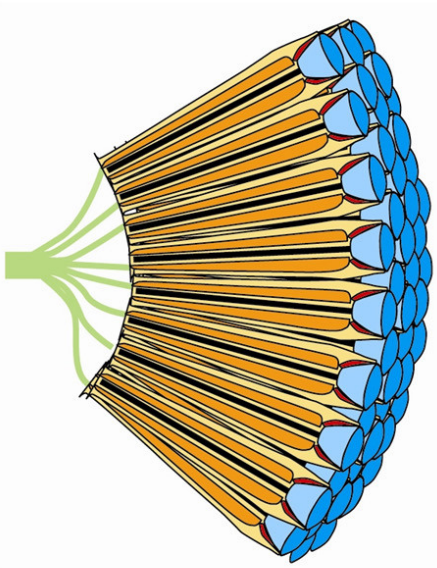


Drosophila compound **eye** is composed of 16.000 cells and contains a simple repetitive pattern of **700 to 750** of ommatidia, Each ommatidium consists of 14 neighboring cells: 8 photoreceptor neurons in the core, 4 non-neuronal cone cells and 2 primary pigment cells.

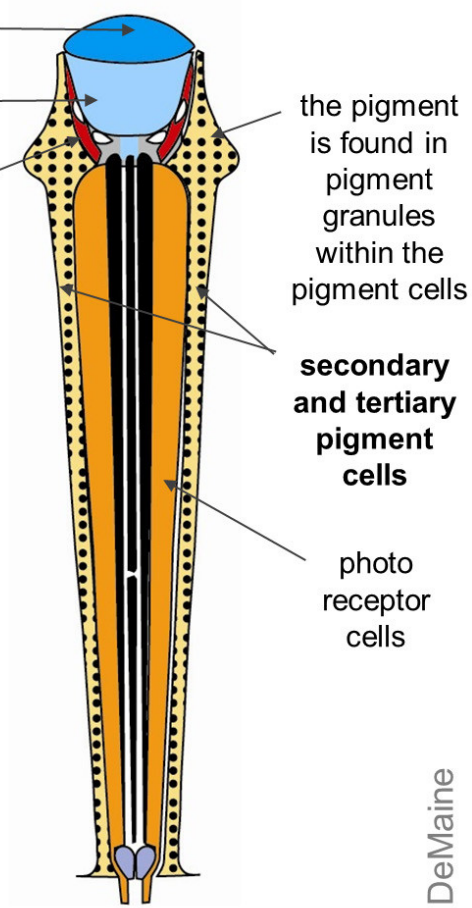
***Drosophila melanogaster* compound eye**

The photoreceptor organ

700 omatids with identical function, perfectly alligned and easy phenotypic readout in genetic experiments (i.e. introducing mutations in genetic screens



compound eye structure

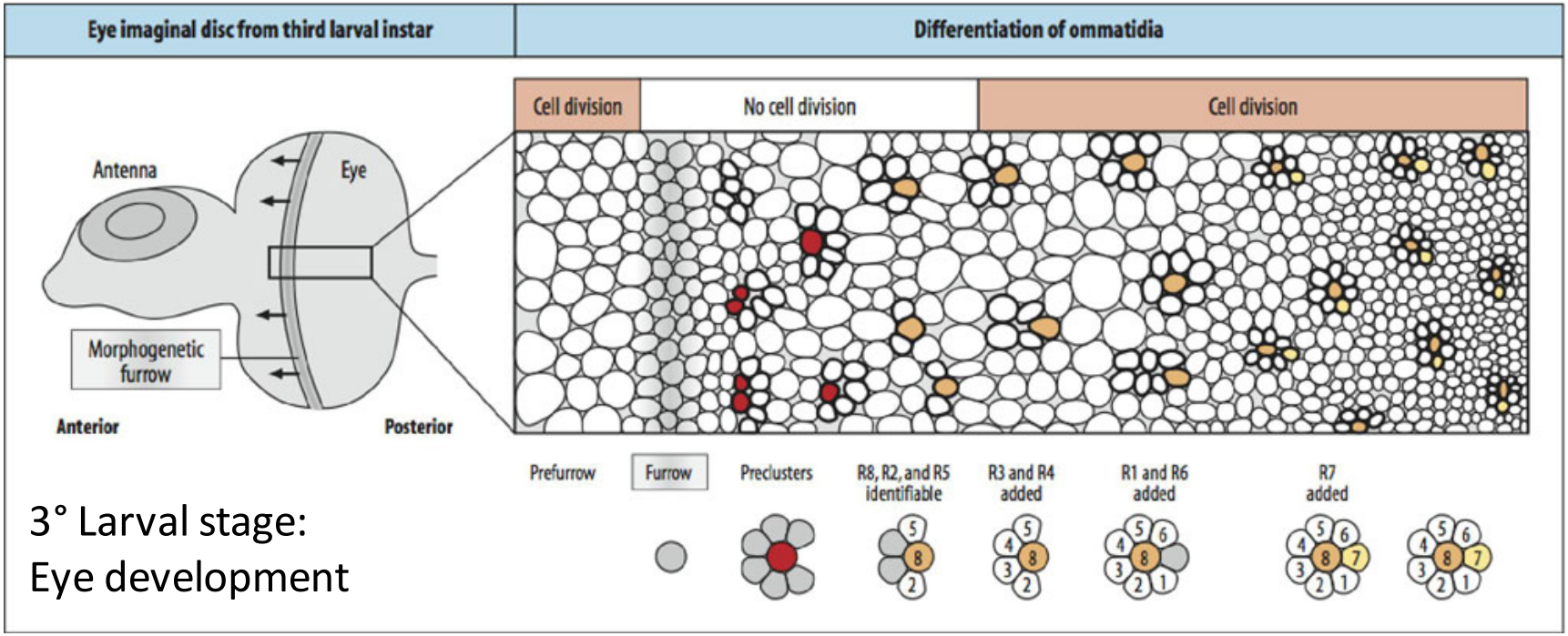


ommatidium

S. DeMaine

Observations that cannot be explained by genetics…..
Muller 1950

Position effect variegation: Drosophila melanogaster



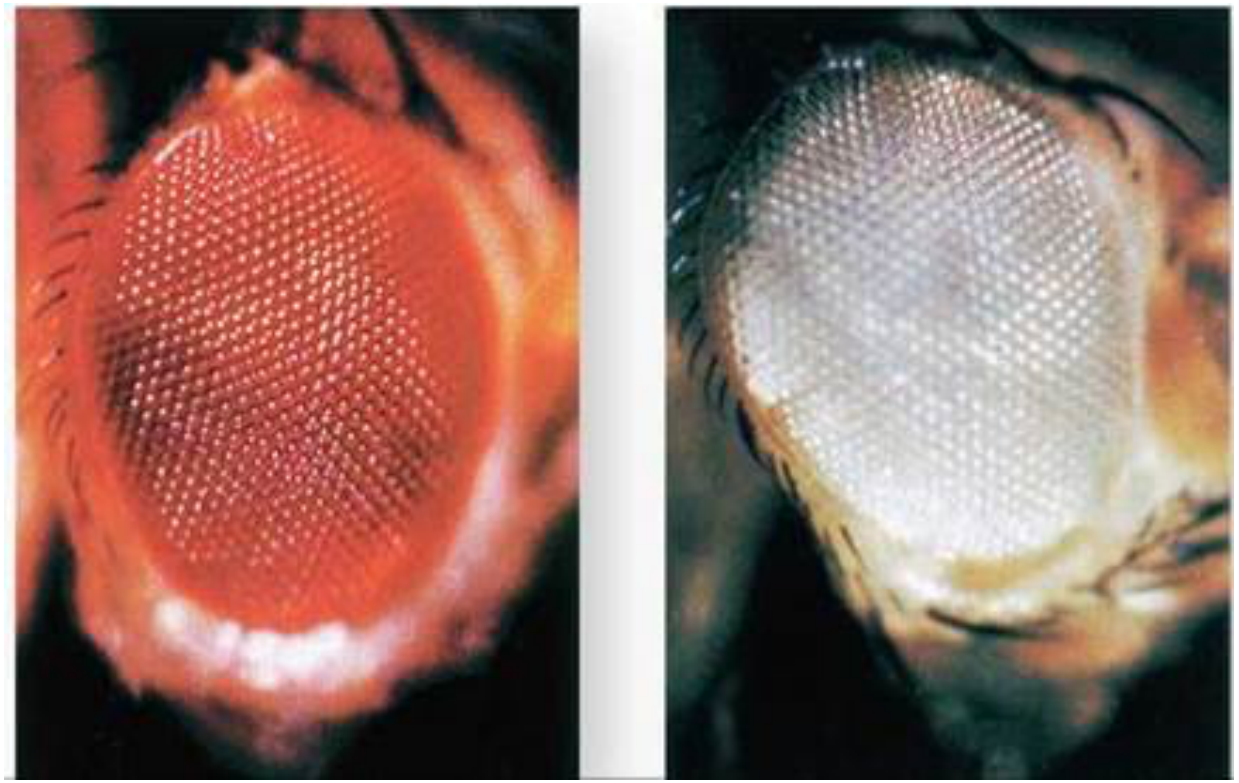
The Drosophila compound eye has 800 ommatidia (the photoreceptor organ), each which has 8 photreceptor neurons (R1-8), 4 cone cells (lens secretion) and **pigment cells**. Great model system to study a small group of cells.

- The eye develops as a single cell epithelial layer. During 3rd larval instar, posterior part of the eye begins to develop.
- Over two days the patterning moves towards the anterior while the disc grows 8 fold in size. The morphogenetic furrow forms early in the posterior eye disc and sweeps across the disc (P->A) to leave clusters of cells spaced in a hexagonal array in its wake.
- The morphogenetic furrow moves at a rate of 2 hours per row of ommatidial clusters (2 days for the eye). Behind the furrow, cells differentiate to become regularly spaced ommatidia, each row out 1/2 register from the next to give the hexagonal arrangement.
- The R8 photoreceptor neurons differentiate first separated by ~8 cells. Each R8 starts a series of signals that recruit a cluster of 20 cells. R2 &R5 form two identical neurons on either side of the R8, then R3 & R4 (different photreceptor types), then R1 & R6 abd finally R7 to surround the R8 cell.

The drosophila eye – a role model system for genetic/epigenetic research…..

Thomas and Lilian Morgan 1910

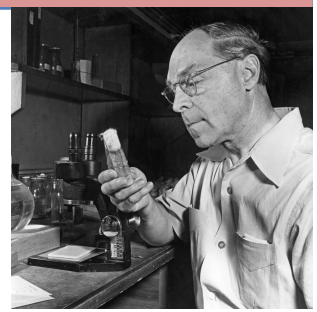
Wild-type



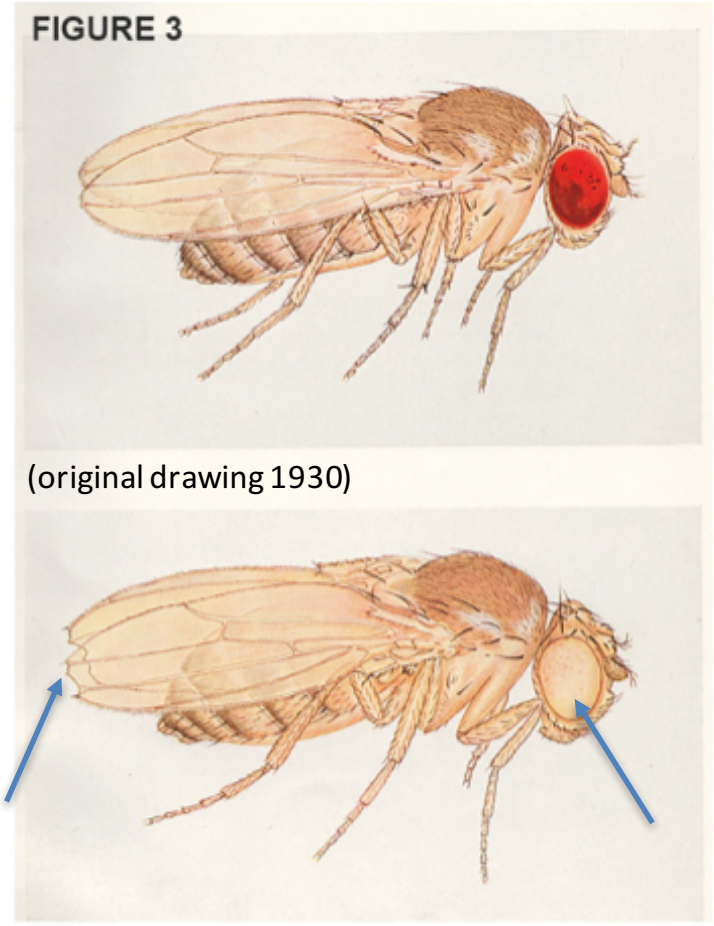
Genetic *white* mutant

The protein coded by the **white gene** functions as an ATP-binding cassette (ABC) transporter. It carries the precursors of the red and brown eye color pigments, guanine and tryptophan, into the developing eyes during pupation. The white gene is located on the X chromosome

Observations that cannot be explained by genetics…..
Herman Joseph Muller 1930



Muller 1930: X-ray irradiation was used as a mutation inducer to generate flies with translocations events



(original drawing 1930)

Wild-type

Mutant after X-Rays:
Notched wings (ali dentellate)
White eyes (“White allele”)

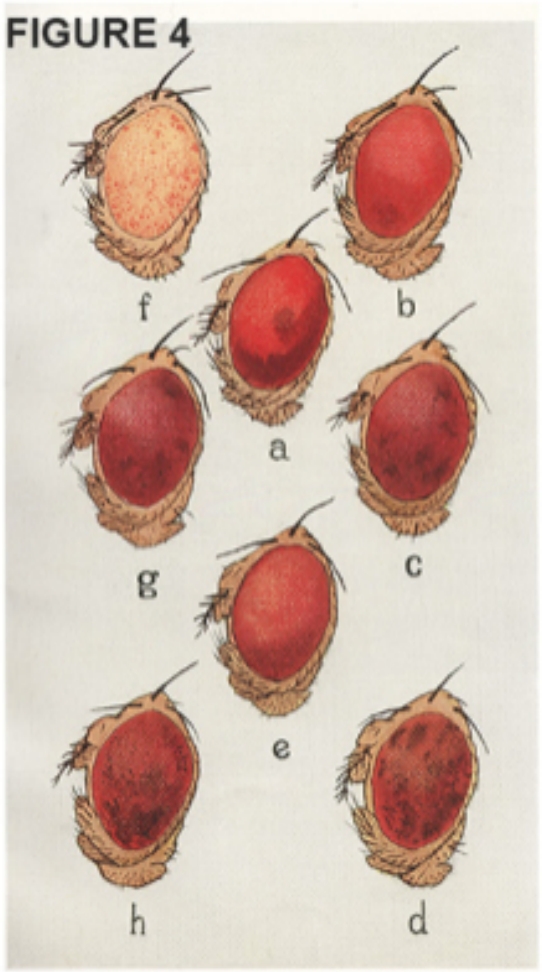
(Note: the white allele is located cloesely to the notch allele)

Observations that cannot be explained by genetics…..
Herman Joseph Muller 1930

Mutant after X-Rays

x

OTHER STRAIN WITH WHITE EYE PHENOTYPE (*white allele*)



"Mottled eye phenotype"

Mutated allele is inherited but shows phenotypic variation in offsprings from cross (reversible phenoptype of red pigmentation)

(Muller et al. J. Genet. July **1930**, Vol. 22, Issue 3)

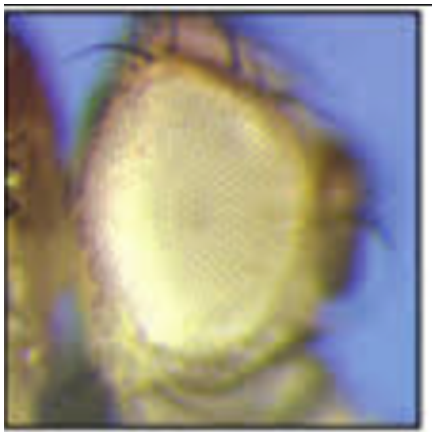
"To the great surprise of the writer, the Notch winged offspring of this cross had neither white nor normal red eyes nor even eyes of any uniform intermediate colour. They had mottled eyes, and exhibited various grades and sizes of lighter and darker areas…"

Observations that cannot be explained by genetics…..
Muller 1930

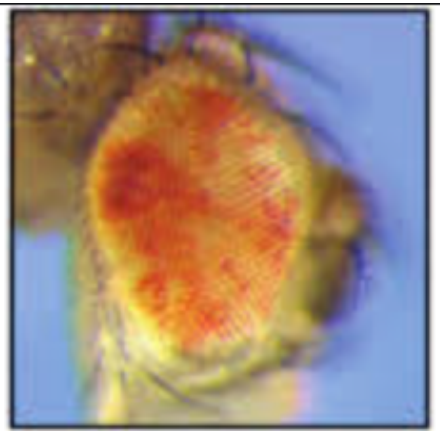
The ”mottled eye phenotype” : Kick off of epigenetics research

Omatides enable to observe genetic but also non-genetic events that define gene expression

X-Ray



White: mutant



White: functional/mutant

???? What has happenedd???

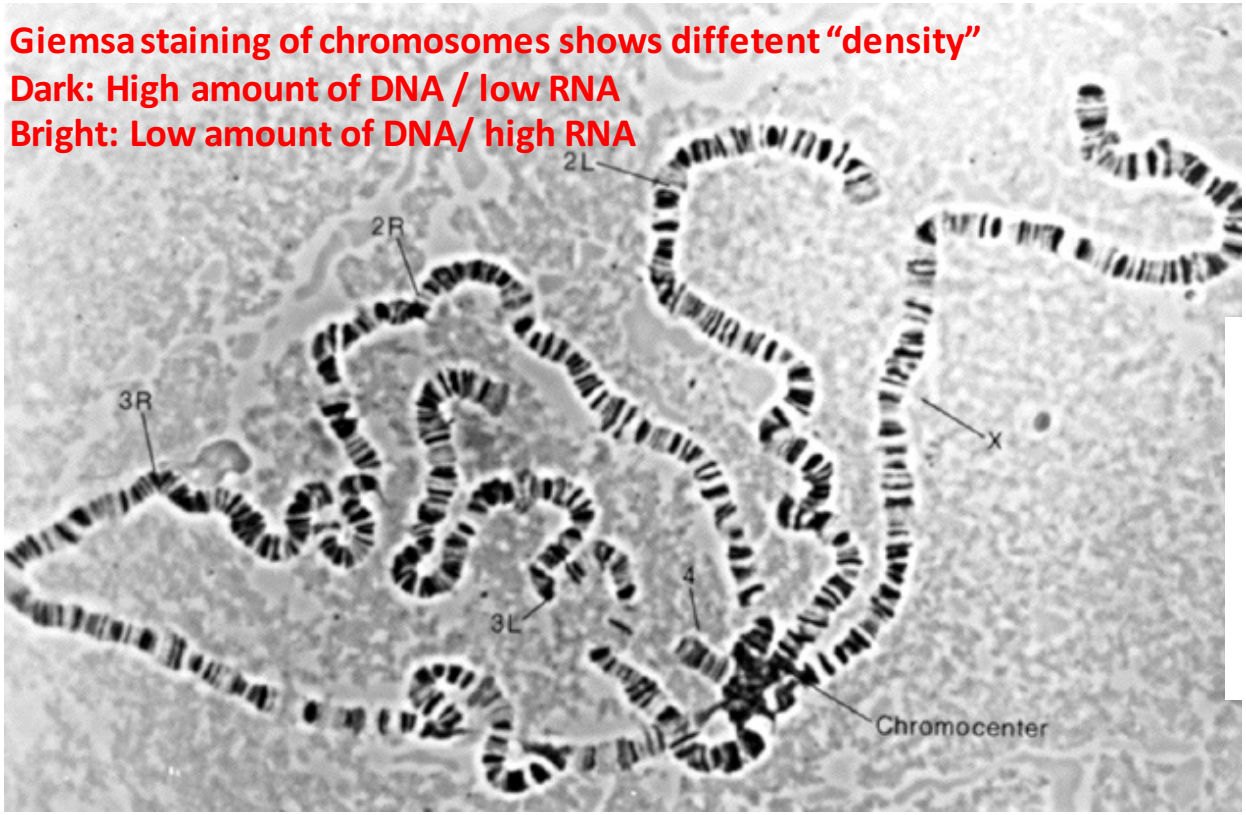
White: functional

Position-effect variegation (PEV) is a variegation caused by the silencing of a gene in **some cells** through **its abnormal juxtaposition with heterochromatin** via rearrangement or transposition.

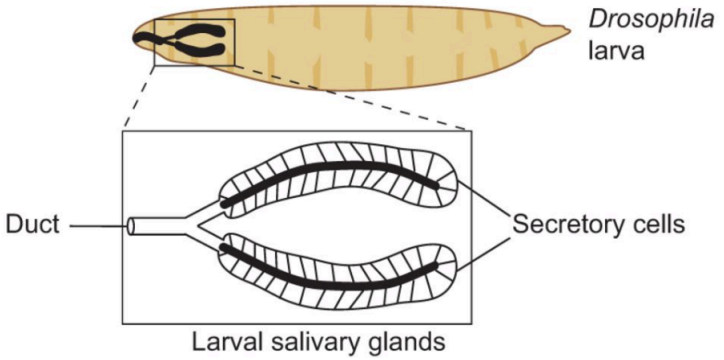
1. Introduction in epigentics and groudbreaking discoveries

Observations that cannot be explained by genetics…..
Muller 1950

Giemsa staining of chromosomes shows diffetent "density"
Dark: High amount of DNA / low RNA
Bright: Low amount of DNA/ high RNA



Drosophila melanogaster larvae



Polythen chromosomes are generated by endoduplication

Polytene chromosomes are large chromosomes which have thousands of DNA strands. They provide a high level of function in certain tissues such as salivary glands. High copy number pushes gene expression to produce large quantities of proteins - for example the adhesive mucoprotein ("glue") before pupation.

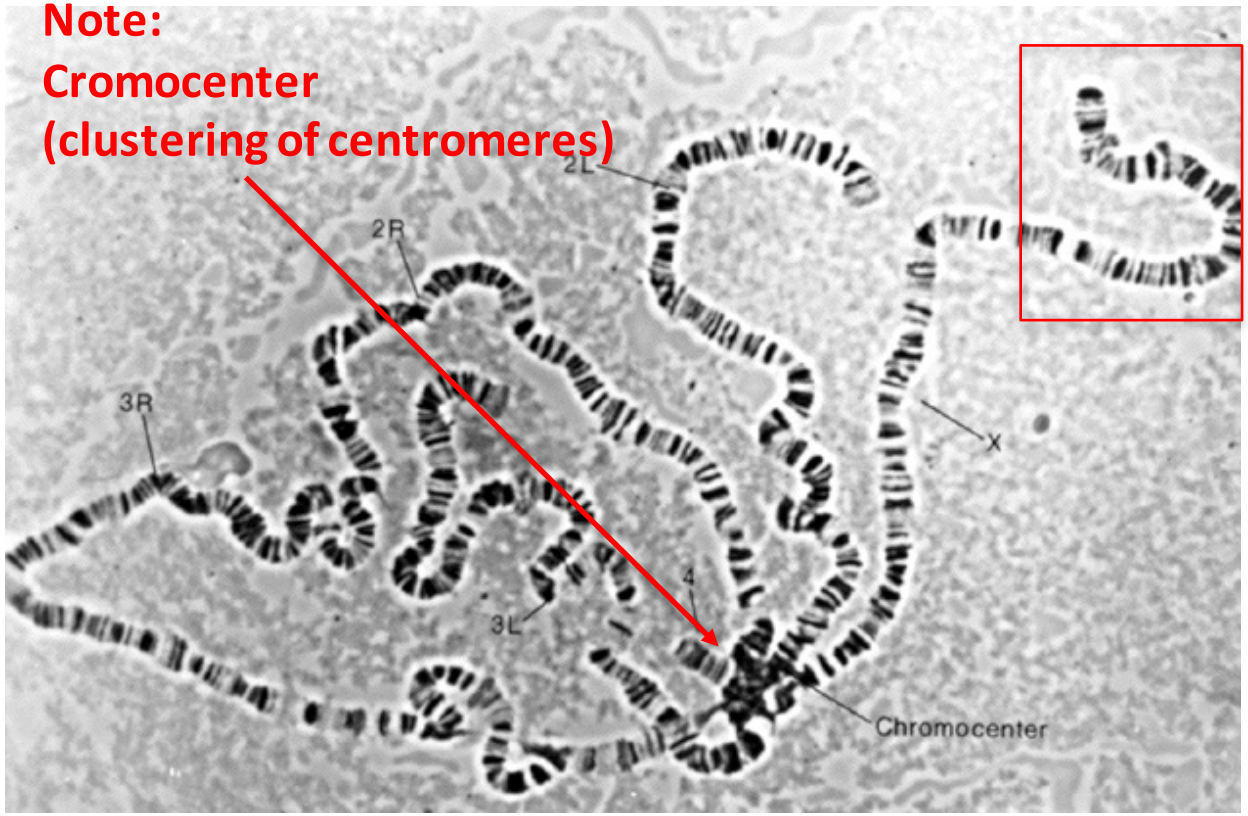
They are produced when **repeated rounds of DNA replication without cell division forms a giant chromosome (ENDOREPLICATION)**. Thus polytene chromosomes form when multiple rounds of replication produce **many sister chromatids which stay fused together**. Polytene chromosomes, at interphase, are seen to have distinct thick and thin banding patterns. These patterns were originally used to help map chromosomes, identify small chromosome mutations, and in taxonomic identification.

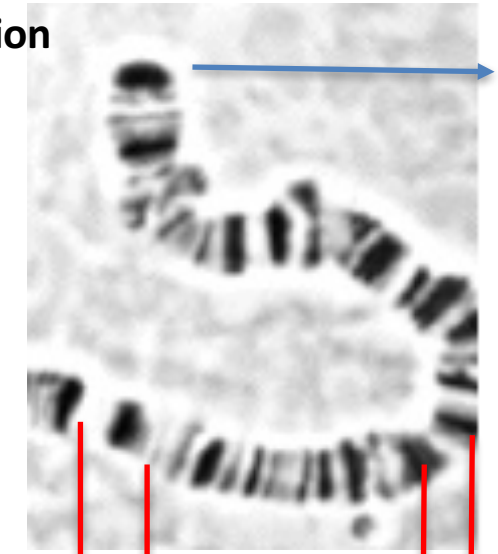
In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "**salivary gland chromosomes**".

Observations that cannot be explained by genetics…..
Muller 1950

Genomic DNA is organized into regions with high and low compaction

Note:
Cromocenter
(clustering of centromeres)





Note:
telomeres

Low Giemsa Intesity
Low Compaction
= Euchromatin

High Giemsa Intesity
High Compaction
= Heterochromatin

Polythen chromosomes are generated by endoduplication

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In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "salivary gland chromosomes".

Observations that cannot be explained by genetics…..
Muller 1950

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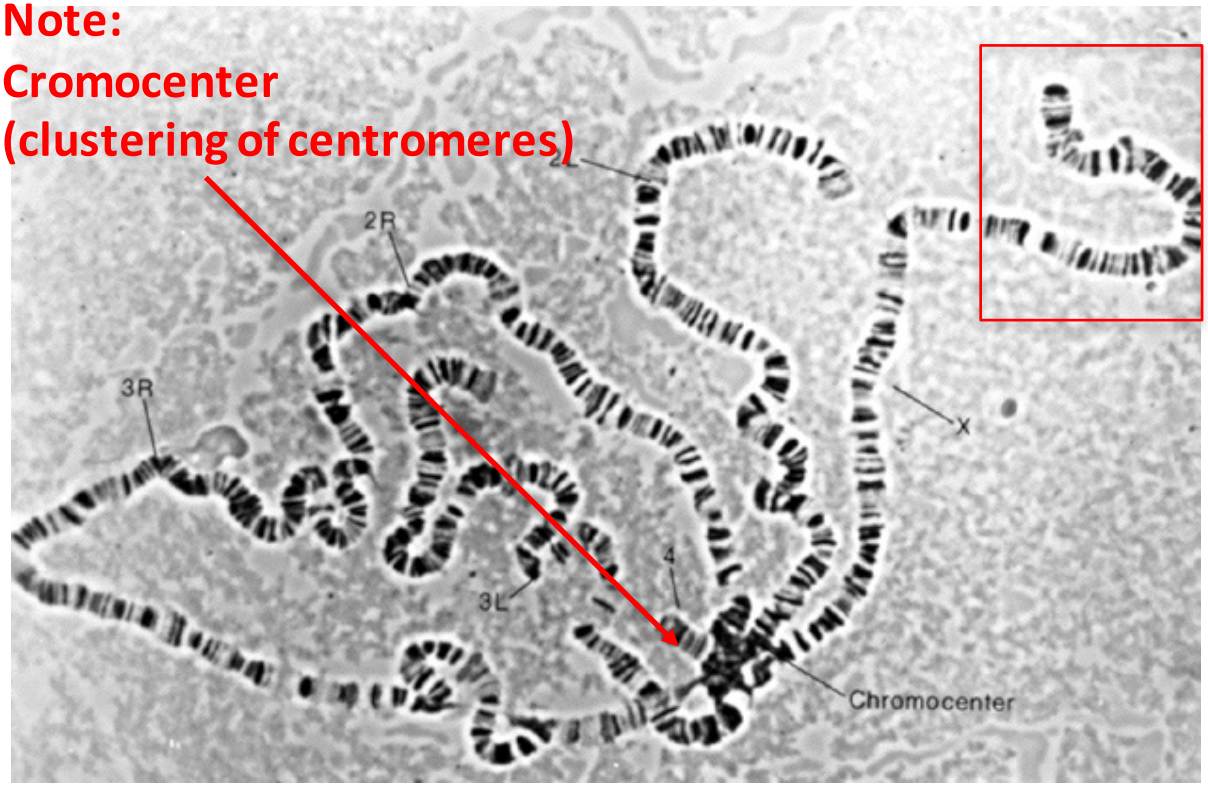
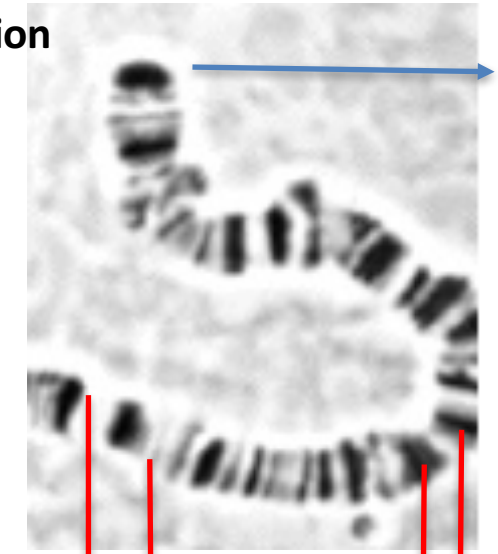




Note:
telomeres

Low Giemsa Intesity
Low Compaction
= Euchromatin

High Giemsa Intesity
High Compaction
= Heterochromatin

Giemsa staining (or other DNA stains) reveals different compaction levels of genomic DNA:

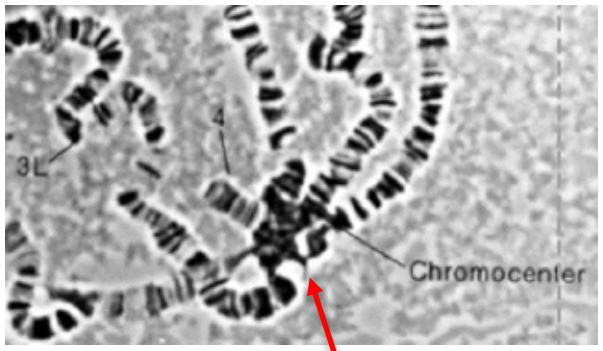
→ Factors must exist that compact DNA = **Proteins**

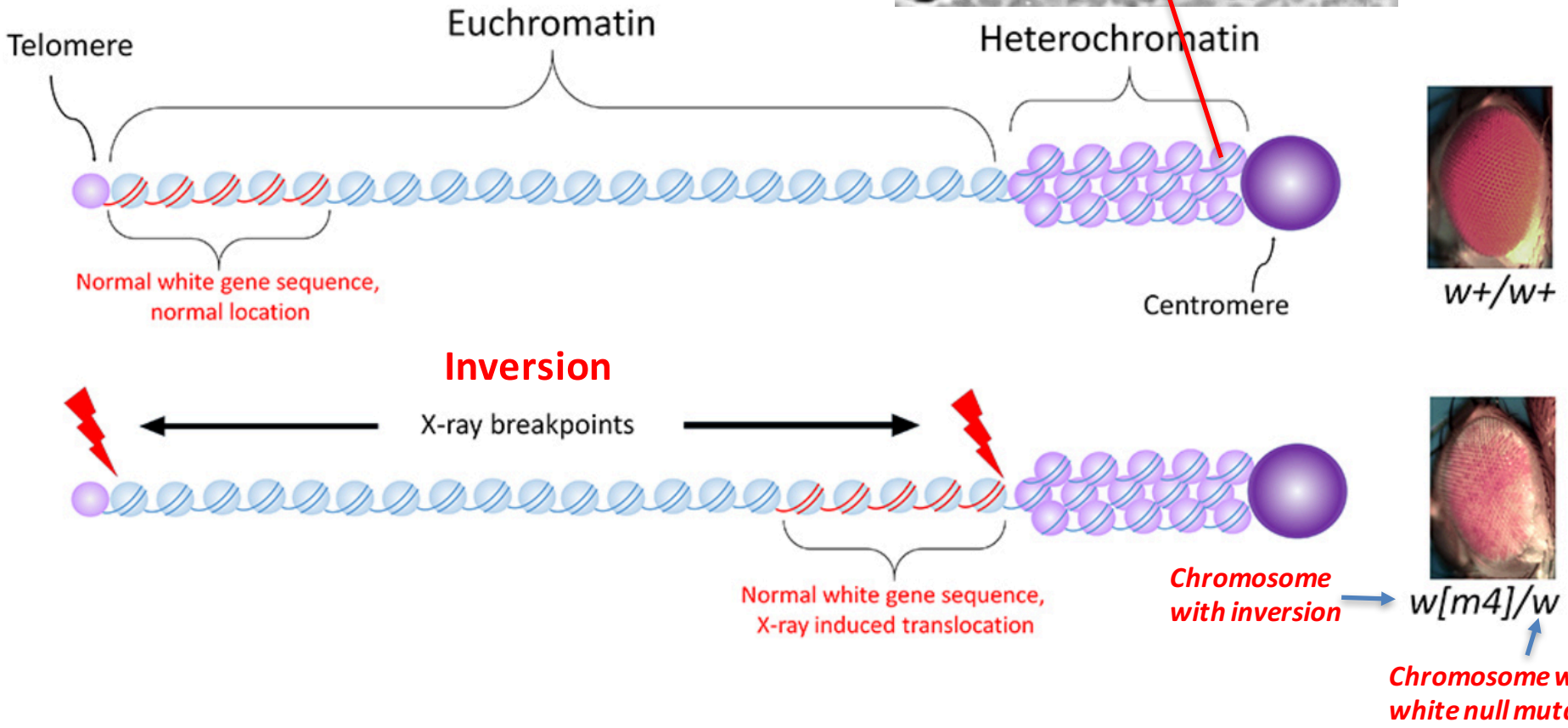
CHROMATIN = DNA + all proteins directly or indirectly associated with DNA

→ Heterochromatin: compacted DNA → "closed chromatin"
→ Euchromatin: : un-compacted DNA → "open chromatin

Observations that cannot be explained by genetics…..
Muller 1950

Muller 1930: X-ray irradiation produced a mutant fly that is characterized an inversion of a chromosome fragment, without affecting the coding potential of the white allele.

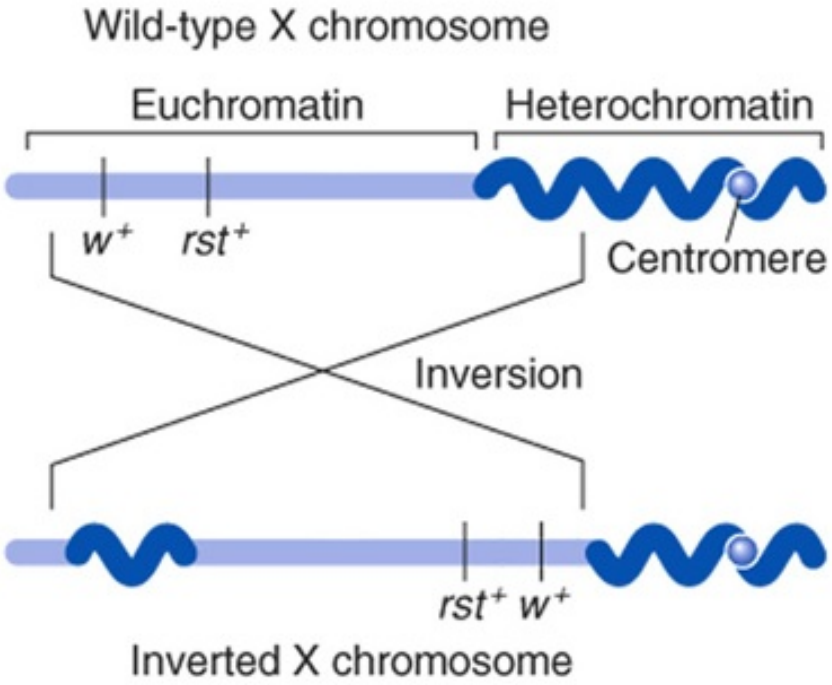




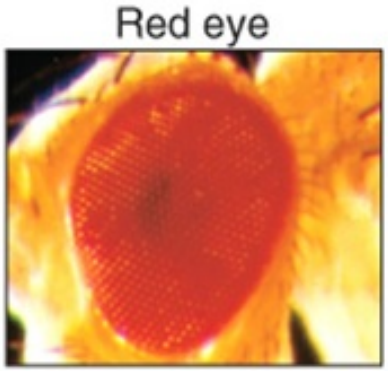
Note: Flies survived X ray treatment and show normal development → all genetic information is present

Observations that cannot be explained by genetics…..
Muller 1950

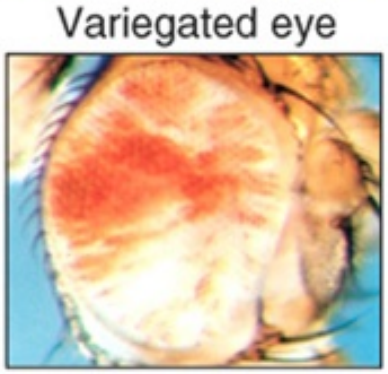
Position-effect variegation



Insertion of white gene into compacted DNA (heterochromatin)



Normal fly:
w^+ :red pigment expressed

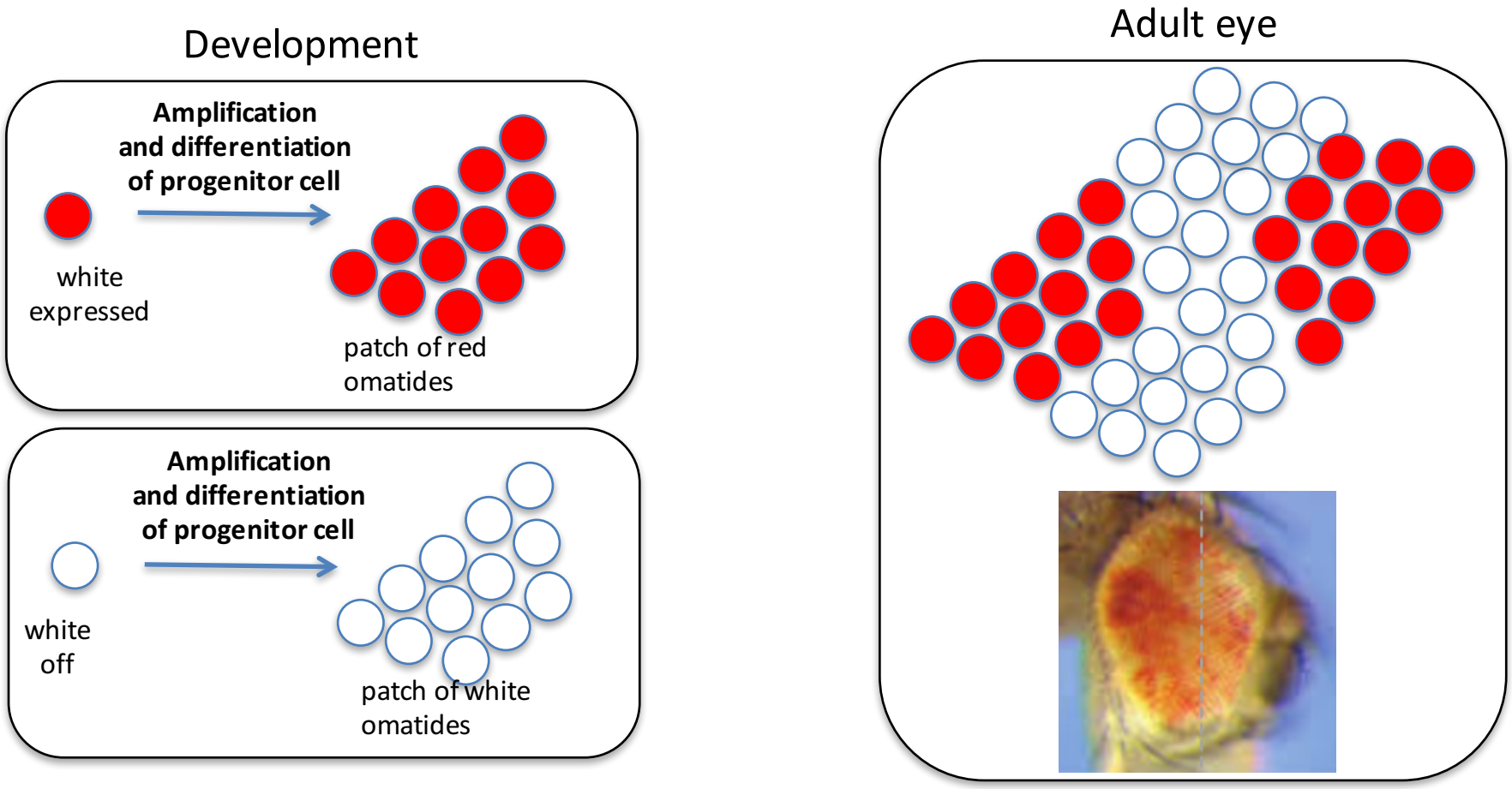


Genome rearrangement:
All genes are present but white gene
is close to the centromere
White gene is still wild type but no longer expressed in all ommatidia

W = white gene:wncodes a red pigment that is incorporated into ommatides of the Drosphila melanogaster
when mutated, eyes become white

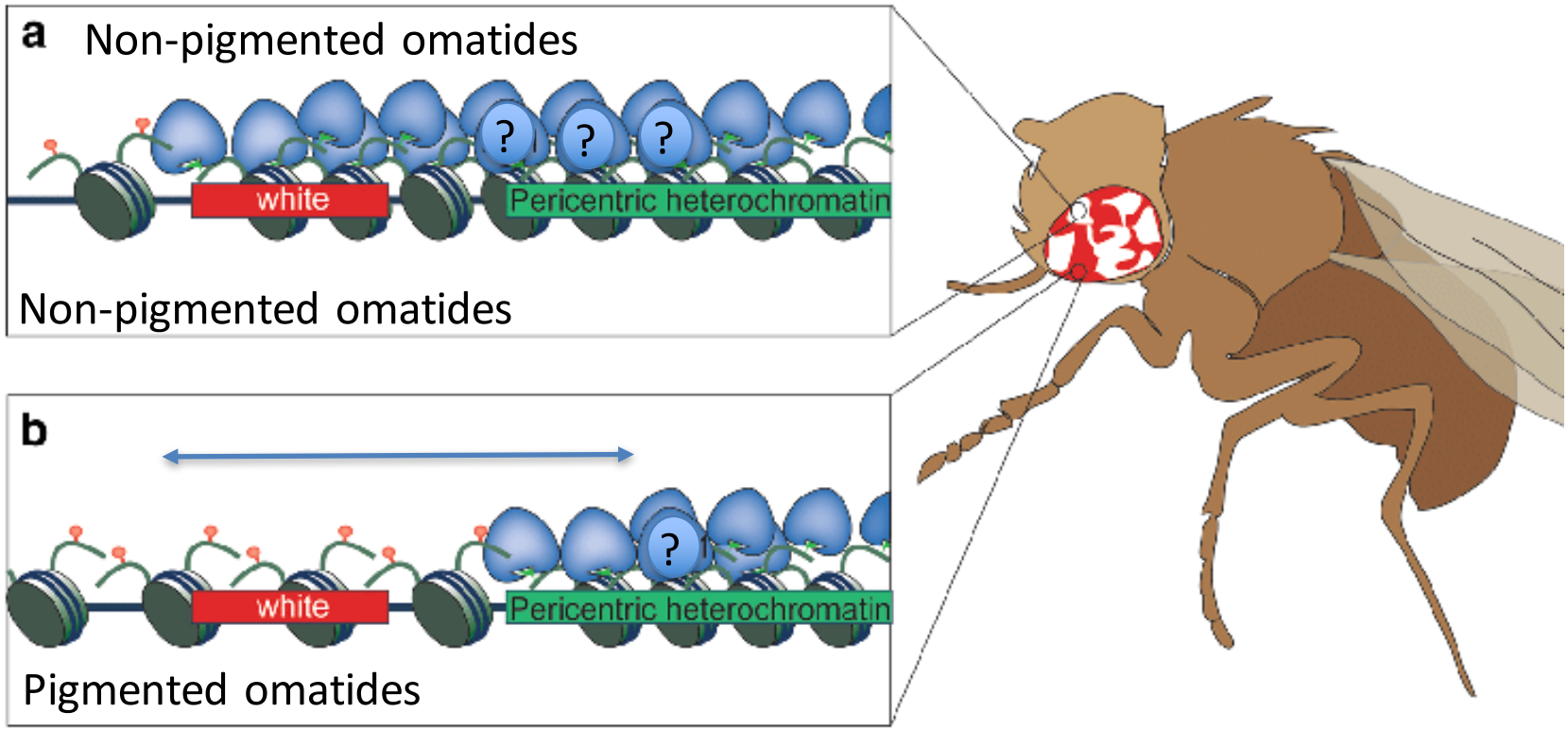
Position effect variegation in D. melanogaster

How is the "patchy style" of mottled phenotype generated?



Variegated Phenotype is stable during progressive cell division
→Is propagated to daughter cells
=staus of white gene expression is MAINTAINED

Position effect variegation in D. melanogaster



What are the factors that define the expression status of the white gene when juxtaposed to compact chromatin close to cenromeres (pericentric heterochromatin)

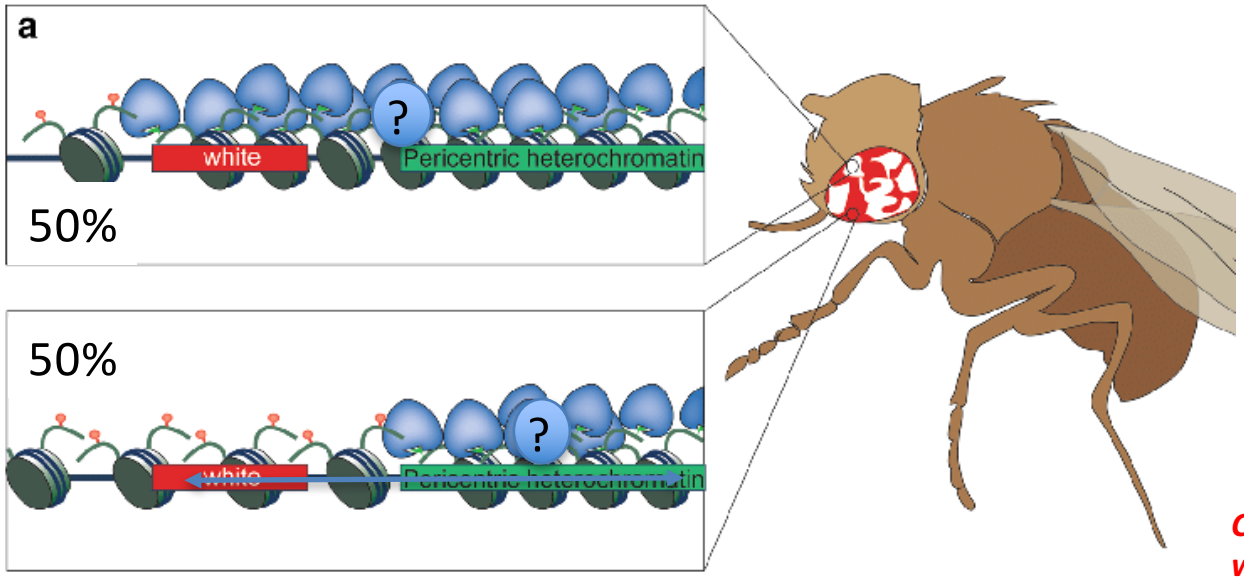
? .....factors that build chromatin

⟷ .....factors that allow to expand or retract the extension of compact chromatin

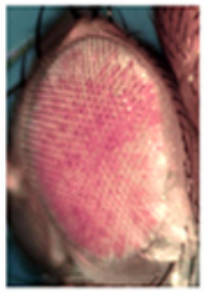
} Should act as **modifiers of PEV**

Genetic screens to identify modifiers of PEV

Non-pigmented omatides



Pigmented omatides



w[m4]/w

Chromosome with inversion

Chromosome with white null mutation

Perform forward genetics - Mutagenesis screens

= using mutagensis to create random mutations, then searching for the genotypes that underlie the resulting phentoypes = modifiers of PEV

Pigmented/non pigmented = 1/1

Parental strain i.e. w[m4]/w

→ *Mutagenesis*

Strain X: Pigmented/non pigmented = 3/1

Strain Y: Pigmented/non pigmented = 1/5

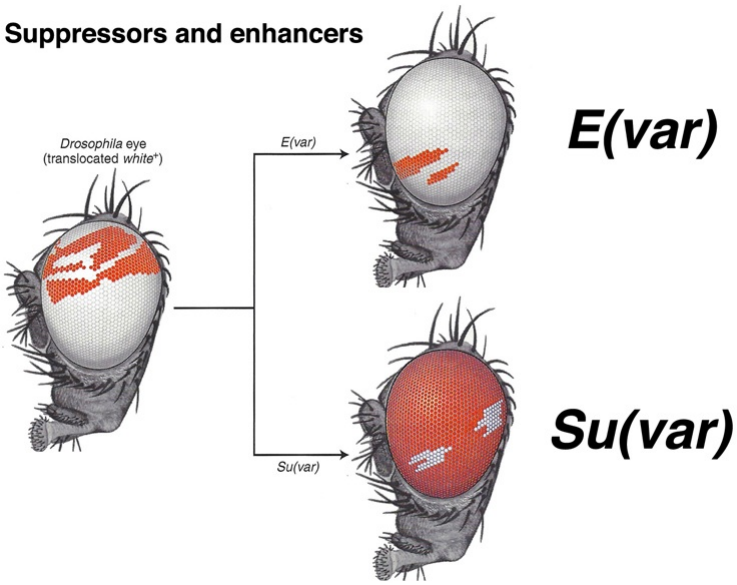
Strain Z: Pigmented/non pigmented = 1/2

Visual inspection of Mutant offspring strain: Check for flies that do not have partental patterning

Strain A: Pigmented/non pigmented = 1/1

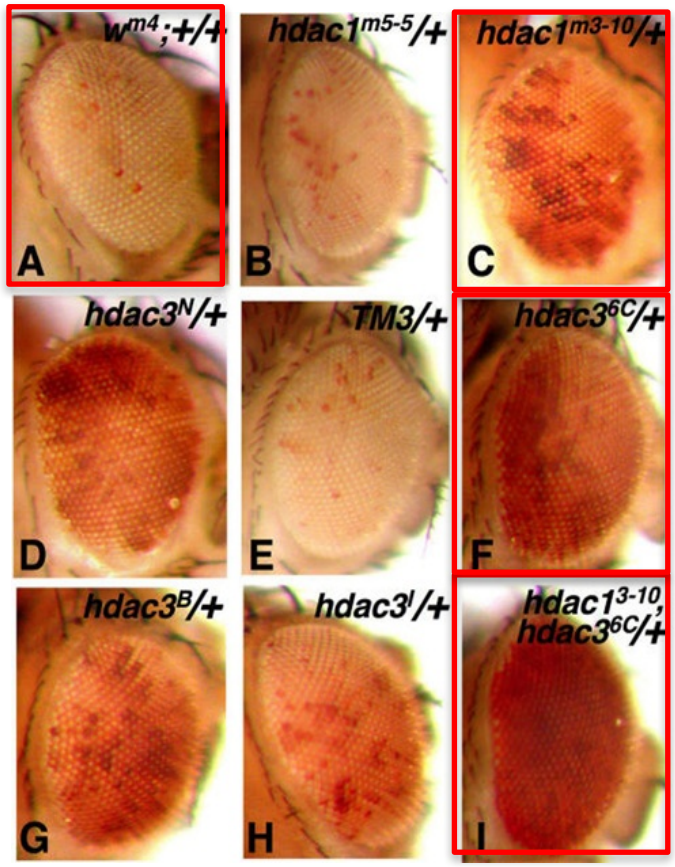
Observations that cannot be explained by genetics…..

Mutations in certain genes can suppress or enhance PEV



E(var)

Su(var)



Additive effects of two *Su(var)* mutations

Su(var) = hdac

***hdac* = histone deacetylase**

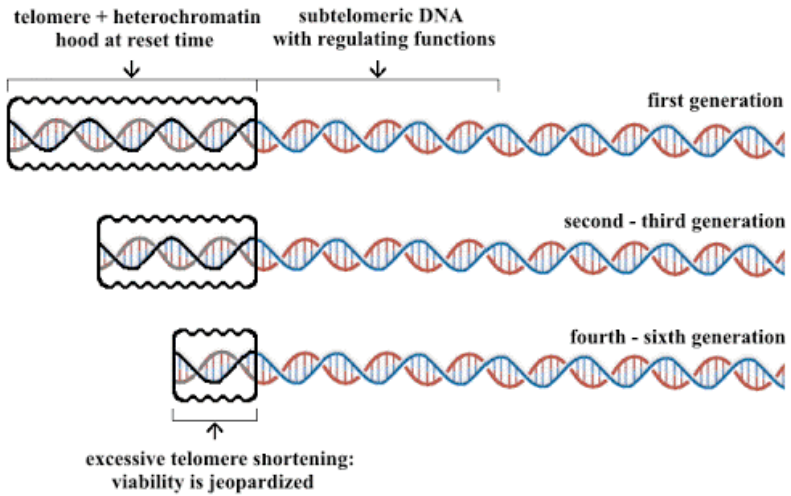
Su(var): Suppressor of Variegation: when mutant, white gene is **expressed** in higher number of omatides = more pigmentation : gene encoded a factor that **promotes chromatin compaction** around centromeres

E(var):Enhancers of Variegation: when mutant, white gene is **repressed** in higher number of omatides = less pigmentation: gene encoded a factor that **antagonizes chromatin compaction** around centromeres

LOCALIZE MUTATION IN MUTANT STRAIN AND IDENIFY GENE – DISCOVER GENE FUNCTION: Example HDACs

Observations that cannot be explained by genetics…..

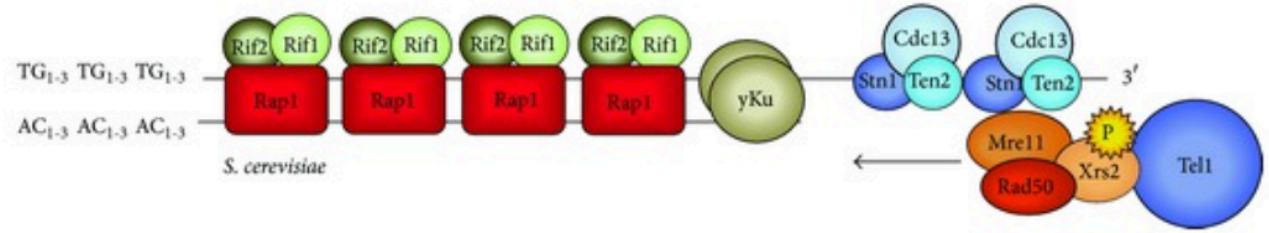
Telomere position effect: Saccharomyces cerevisiae



End-replication problem
Telomere shortenting

Telomere dysfunction - senescence

3’ telomere overhang

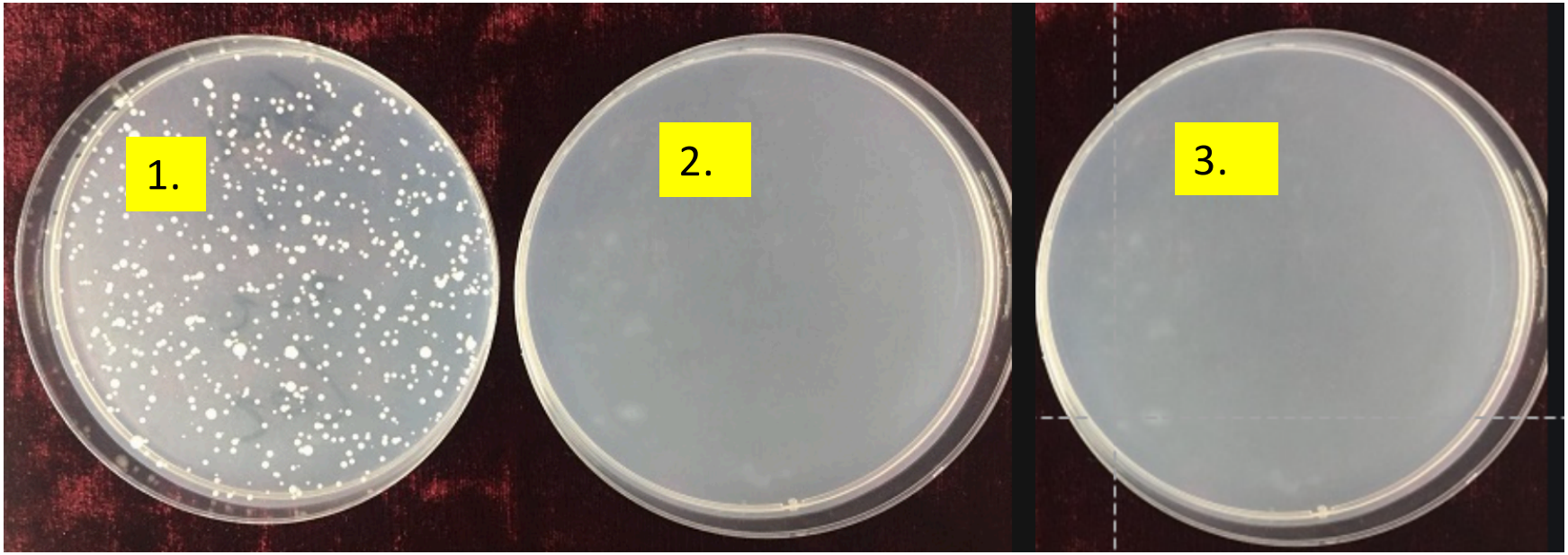


Telomere length homeostasis and function is regulated by telomere repeat binding proteins and the telomerase complex =telomeric chromatin

telomerase

Observations that cannot be explained by genetics…..

Telomere position effect: Saccharomyces cerevisiae



1.	2.	3.
URA^+	URA^-	URA^+
- Lack of Uracil	- Lack of Uracil	- Lack of Uracil
- Lack of Uridne	- Lack of Uridne	- Lack of Uridne
		+ 5-FOA (5-Fluoroorotic acid)

URA3 is a gene on chromosome V in Saccharomyces cerevisiae (yeast). URA3 is often used in yeast research as a "marker gene", that is, a gene to "label" chromosomes or plasmids. **URA3 encodes Orotidine 5'-phosphate decarboxylase (ODCase), which is an enzyme that catalyzes one reaction in the synthesis of pyrimidine ribonucleotides** (a component of RNA). **Loss of ODCase activity leads to a lack of cell growth unless uracl or uridine is added to the media.**

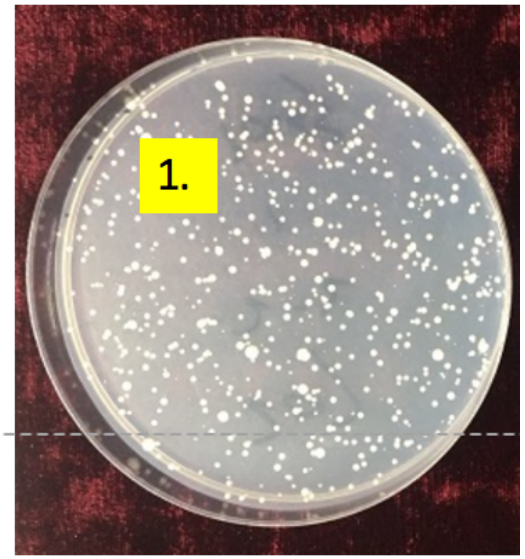
The presence of the URA3 facilitating growth on media not supplemented with uracil or uridine, thereby allowing selection for yeast carrying the gene. In contrast, **if 5-FOA (5-Fluoroorotic acid) is added to the media, the active ODCase will convert 5-FOA into the toxic compound** 5-fluorouracil causing cell death. This allows to select against yeast carrying the URA3 gene.

Observations that cannot be explained by genetics…..

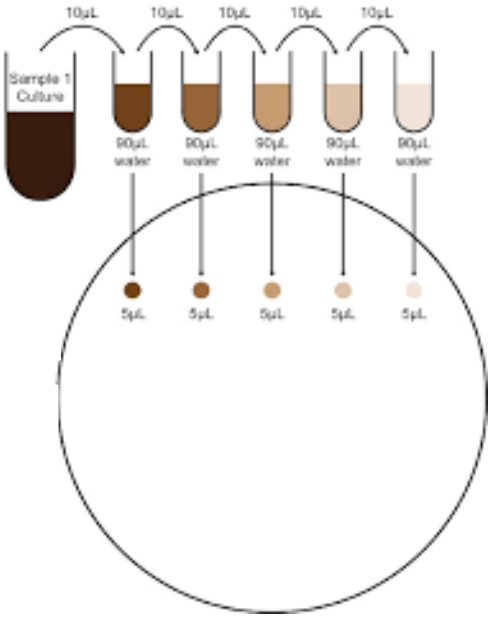
Telomere position effect: Saccharomyces cerevisiae

How to plate yeast:

Spreading a suspension of yeast cells equally on plate



Making serial dillution of suspension of yeast cells



Add drop (ca 10ul) of serial dillution of plate → enables to evlaute the effect of a drug/reporter gene

Concentration of plated cell suspension

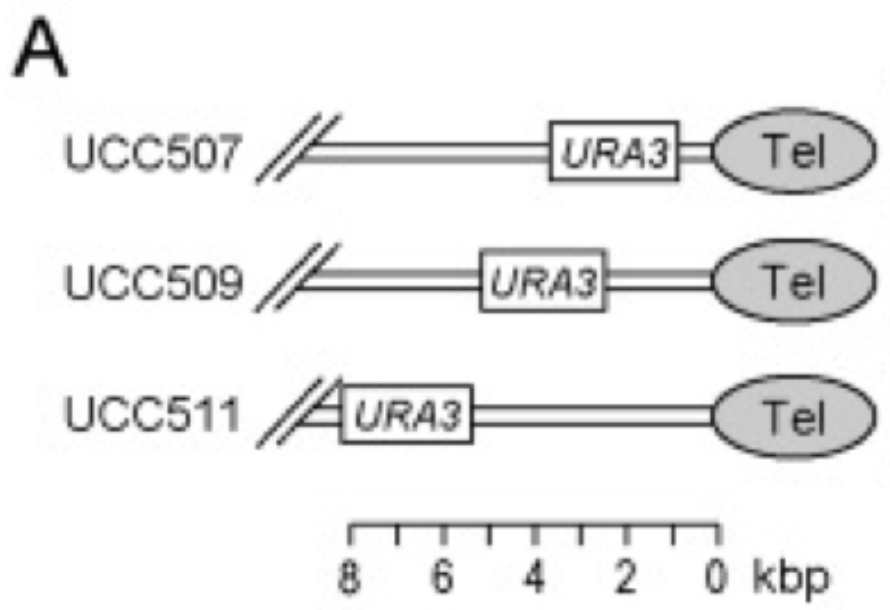
Generation of S.cerevisae stains with subtelomeric insertion of URA3 gene

(endogenouse URA3 gene has been previously deleted)

Observations that cannot be explained by genetics…..

Telomere position effect: yeast

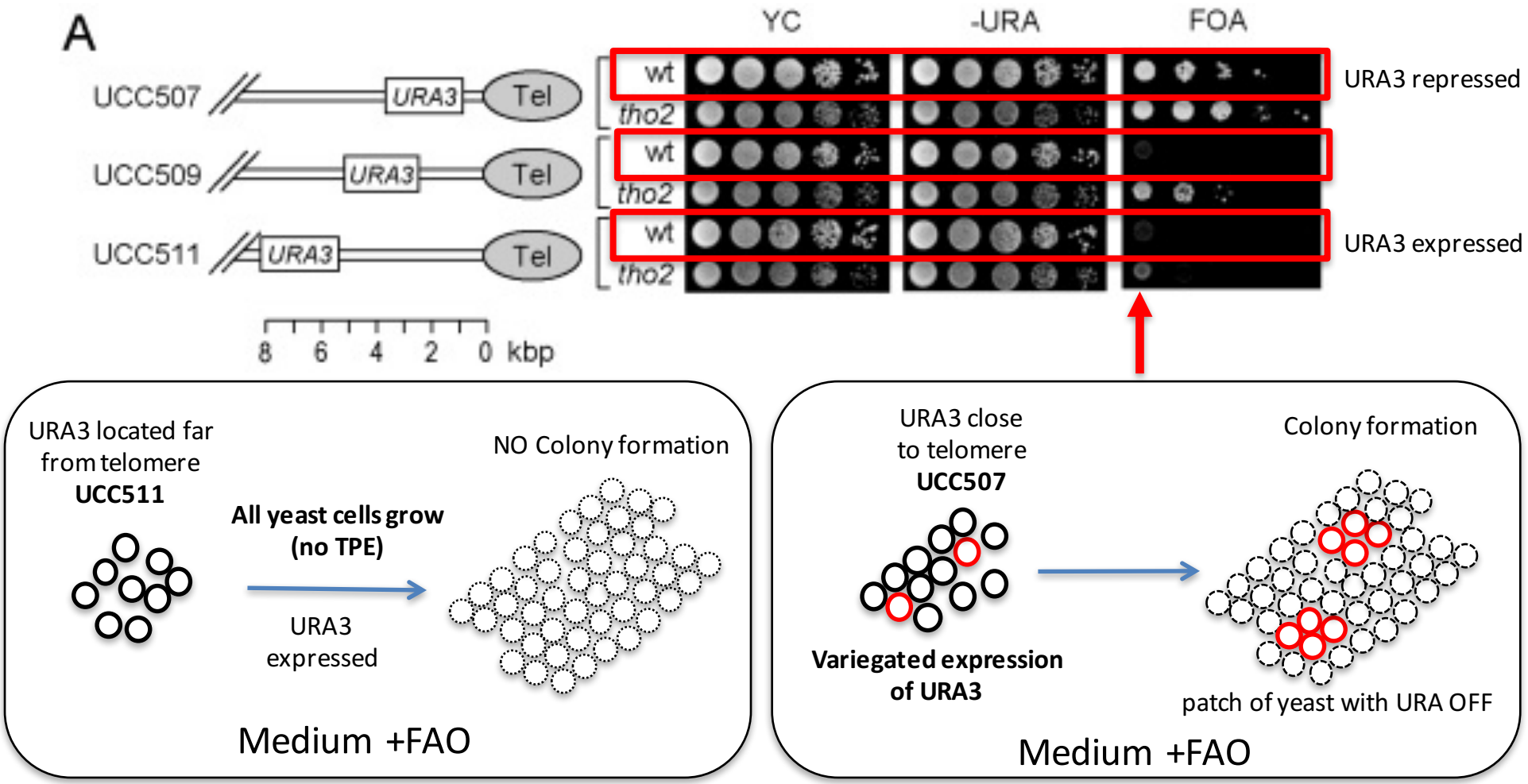
Generate yeast strains that have an insertion of the URA3 gene at different **subtelomeric** positions



	Growth in medium without Uracil and Uridine	Sensitivity to FAO
UCC507	++	+
UCC509	++	++
UCC511	++	+++

Observations that cannot be explained by genetics…..

Telomere position effect: yeast



Variegated expression of URA3 marker → few yeast cells silence URA3 → are FAO resistant

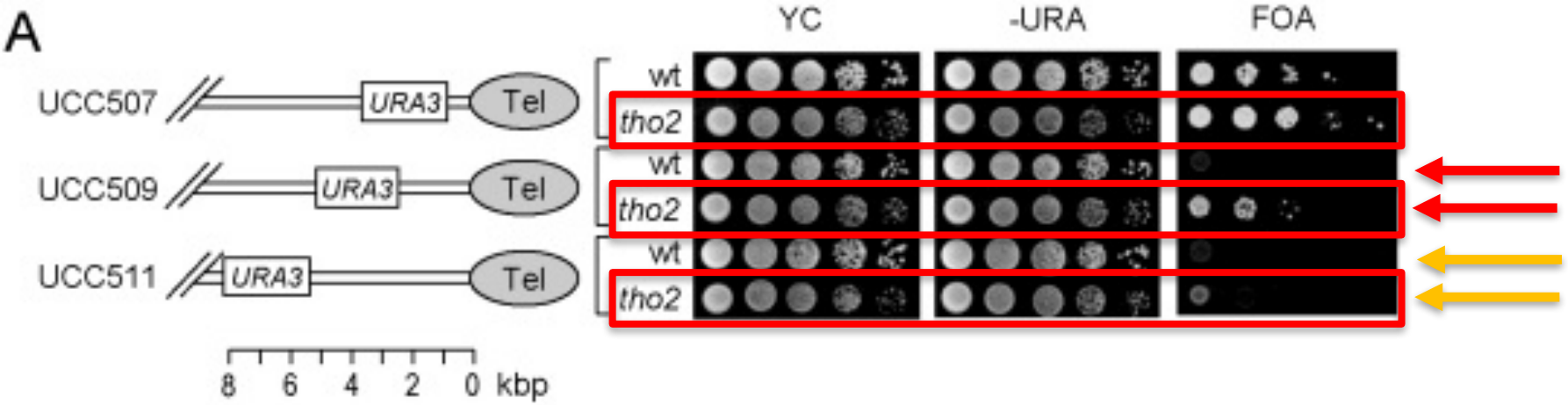
Important: Phenotype is stably during cell division

→Status is propagated to daughter cells =a sort of inheritance

Observations that cannot be explained by genetics…..

Telomere position effect: Saccharomyces cerevisiae

Mutagenesis screen + gene identification



Tho2 mutations cause a modifiction of the telomere position effect

→ Repressive effect on URA3 is enhanced

→ URA3 repression is enhanced in UCC509 and UCC511 strains and result improved suppression of URA3

→ improved resistance to FOA

→ Tho is an enhancer of variegation/modifier of TPE

URA3 is a gene on chromosome V in Saccharomyces cerevisiae (yeast). URA3 is often used in yeast research as a "marker gene", that is, a gene to “label” chromosomes or plasmids. **URA3 encodes Orotidine 5'-phosphate decarboxylase (ODCase), which is an enzyme that catalyzes one reaction in the synthesis of pyrimidine ribonucleotides** (a component of RNA). **Loss of ODCase activity leads to a lack of cell growth unless uracl or uridine is added to the media.**

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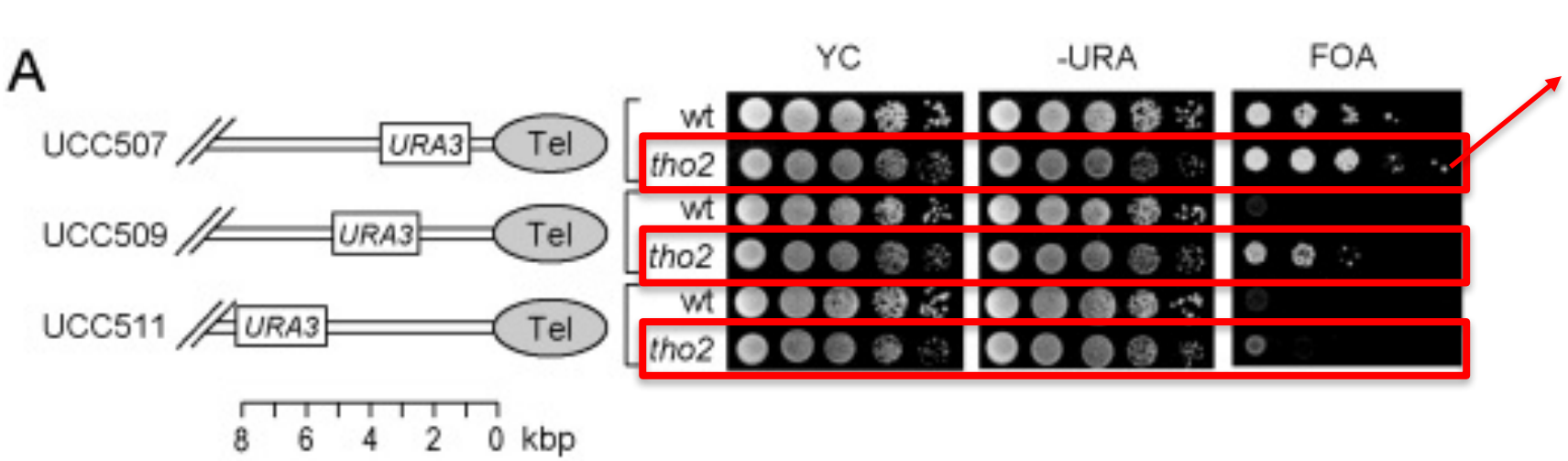
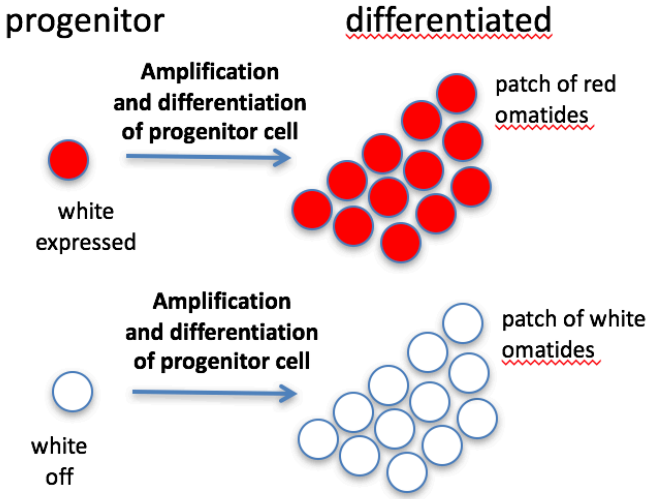
MODULATION OF GENE EXPRESSION IS PASSED ON TO DAUGHTER CELL

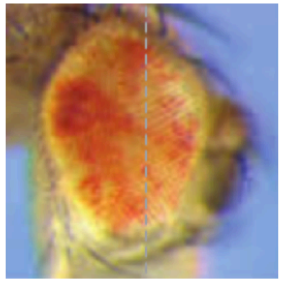
Telomere position effect: Saccharomyces cerevisiae:



Single colony consist of millions of single cells with same feature

Position effect varriegation: Drosophila melanogaster:





Pachy appearance

.....Marker has been artificially located into a heterochromatic region with already established epigenetic regulation
....epigentic context is subjected to models cell – to – cell variation
....however, "epigentic information" in individual cell will be passed on to next generation of cells

= Epigenetic inheritance or Maintenance of epigentic information

WHAT IS EPIGENTICS - 1996 ??

Waddington 1950
…Epigenetics is the study of processes that categorize all of the developmental events leading from the fertilized oozyte to the mature organism – that is, all of the regulated processes that, beginning with the genetic material, shape the final product

Riggs,1996; Riggs and Porter 1996:
…the study of meiotically/mitotically heritable changes changes in gene function that cannot be explained by changes in in DNA seqeunce"

….so far we have not considered an initiating event in TPE and PEV models

Berger 2009
…the initiation of a new epigenetic state involve a transient mechanism
Separate from the one required to maintain it
→ Particular importance for development and disease

WHAT IS EPIGENTICS - 2009 ??

2009: Shelley Berger

…the initiation of a new epigenetic state involve a transient mechanism, separate from the one required to maintain it

STEP 1: Molecule/Processes that initiate regulation (trigger)
STEP 2: Molecules/Processes that maintain regulation

PERSPECTIVE

An operational definition of epigenetics

Shelley L. Berger,[1,5] Tony Kouzarides,[2,5] Ramin Shiekhattar,[3,5] and Ali Shilatifard[4,5]

[1]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; [2]Gurdon Institute and Department of Pathology, Cambridge CB2 1QN, United Kingdom; [3]Wistar Institute, Philadelphia, Pennsylvania 19104, USA; [4]Stowers Institute for Medical Research, Kansas City, Missouri 64110, USA

A recent meeting (December 2008) regarding chromatin-based epigenetics was hosted by the Banbury Conference Center and Cold Spring Harbor Laboratory. The intent was to discuss aspects of epigenetic control of genomic function, and to arrive at a consensus definition of "epigenetics" to be considered by the broader community. It was evident that multiple mechanistic steps lead to the stable heritance of the epigenetic phenotype. Below we provide our view and interpretation of the proceedings at the meeting.

and subsequent generations. These classes are depicted in Figure 1 and are explained below.

Epigenator

The epigenetic phenotype is likely triggered by changes in the environment of the cell. Everything occurring upstream of the first event on the chromosome would be part of the Epigenator signal, including an environmental cue or niche and the subsequent signaling pathways leading to the

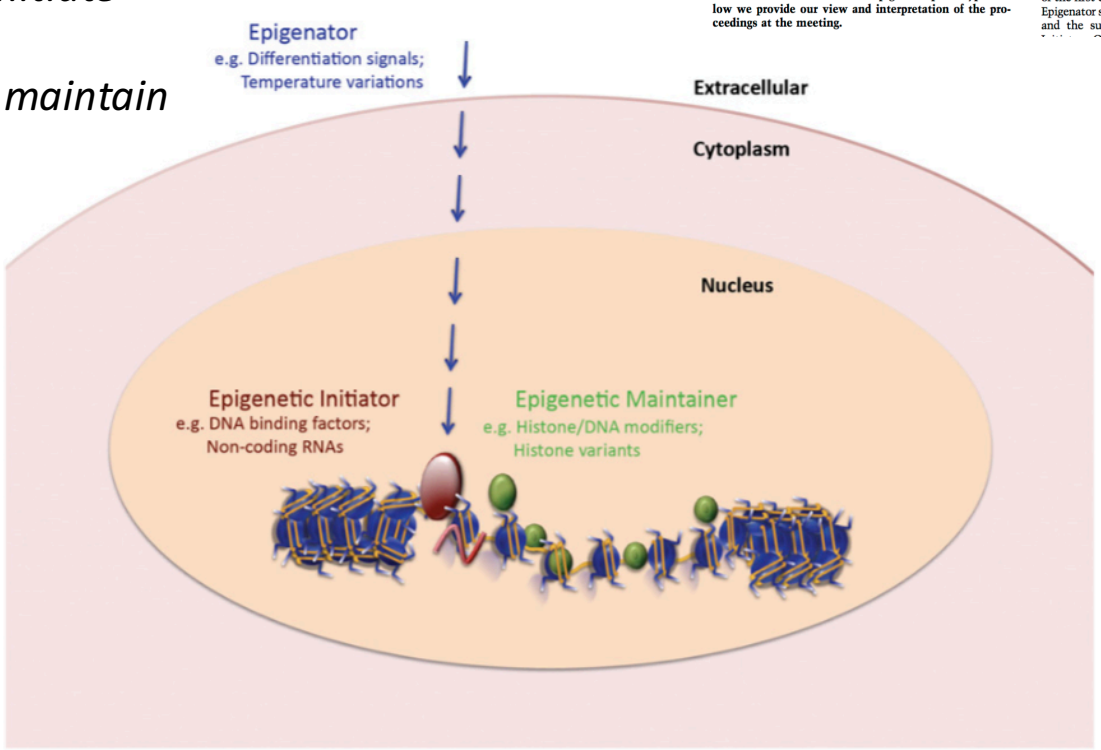


Figure 1. The epigenetic pathway. Three categories of signals are proposed to operate in the establishment of a stably heritable epigenetic state. An extracellular signal referred to as the "Epigenator" (shown in blue) originates from the environment and can trigger the start of the epigenetic pathway. The "Epigenetic Initiator" (shown in red) receives the signal from the "Epigenator" and is capable of determining the precise chromatin location and/or DNA environment for the establishment of the epigenetic pathway. The "Epigenetic Maintainer" (shown in green) functions to sustain the chromatin environment in the initial and succeeding generations. Persistence of the chromatin milieu may require cooperation between the Initiator and the Maintainer. Examples for each category are shown *below* each heading. Chromatin is depicted in blue.

The "packaging" of genetic information is essential for gene expression

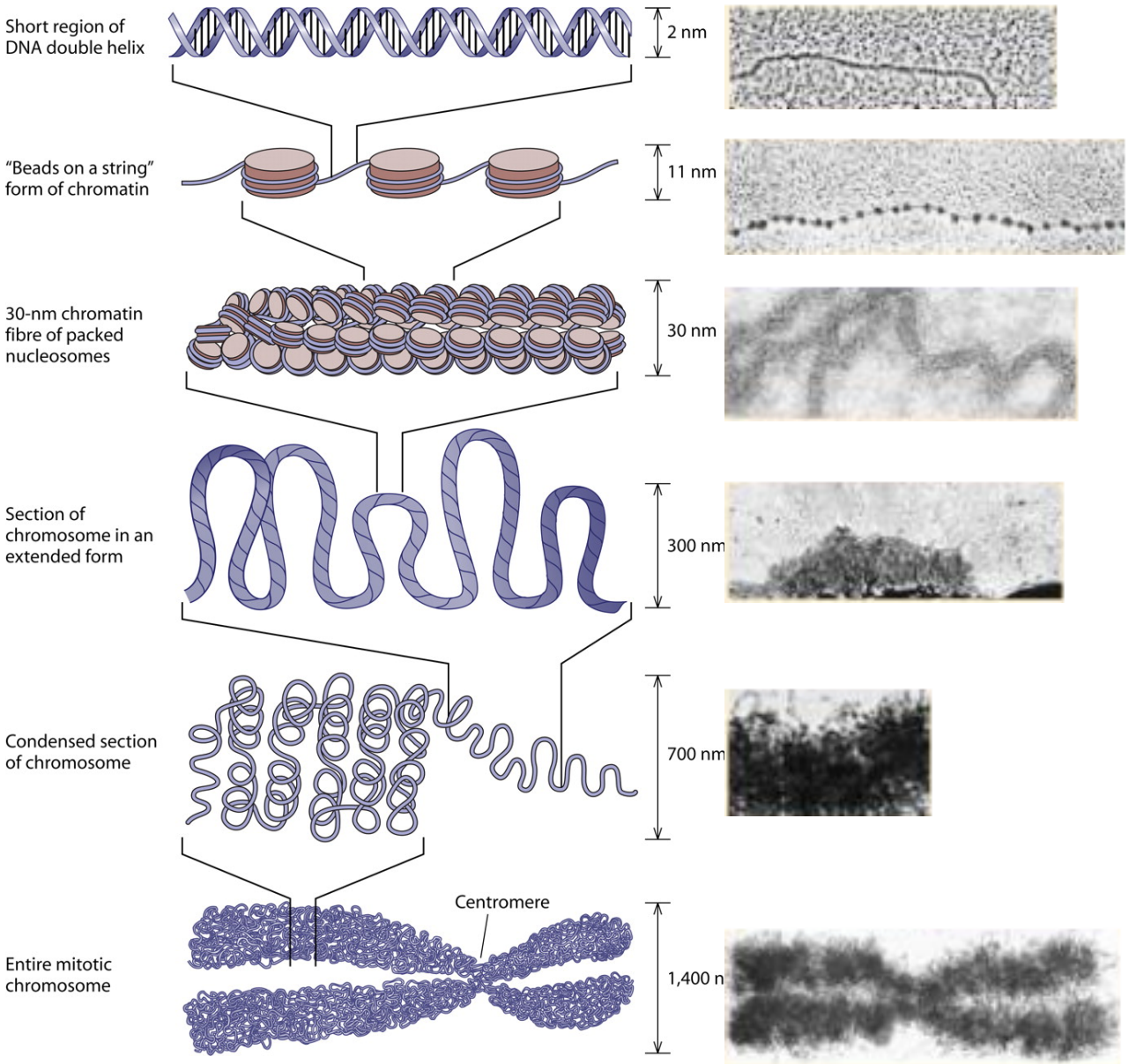
1950: Stedman and Stedman: all cells contain the same DNA information. It must have different histones that bind to DNA that allow the differentiation in to all different cell types of an organism

The DNA is not a naked Molecule In the nucleus.

DNA is bound to proteins

DNA+DNA-bound proteins = CHROMATIN

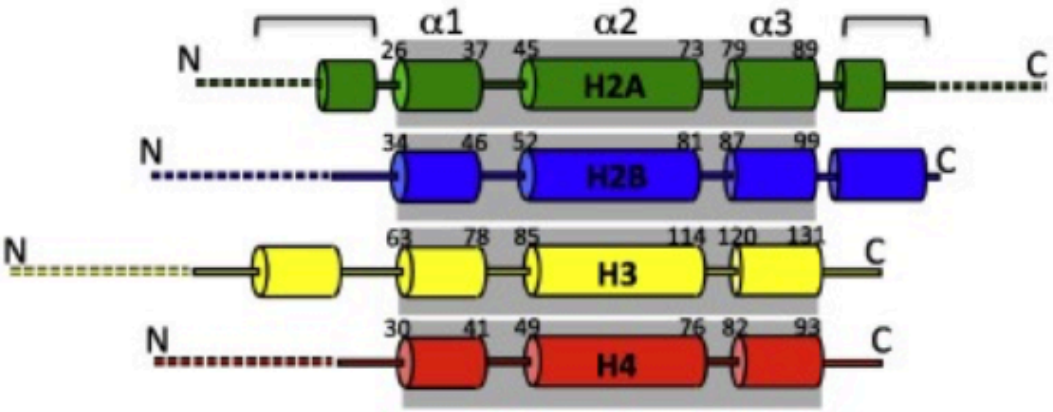
Chromatin is organized at different levels



The "packaging" of genetic information is essential for gene expression

Histones have common protein domain organizaion: Histone fold domain

eukaryotes



11-15kDA

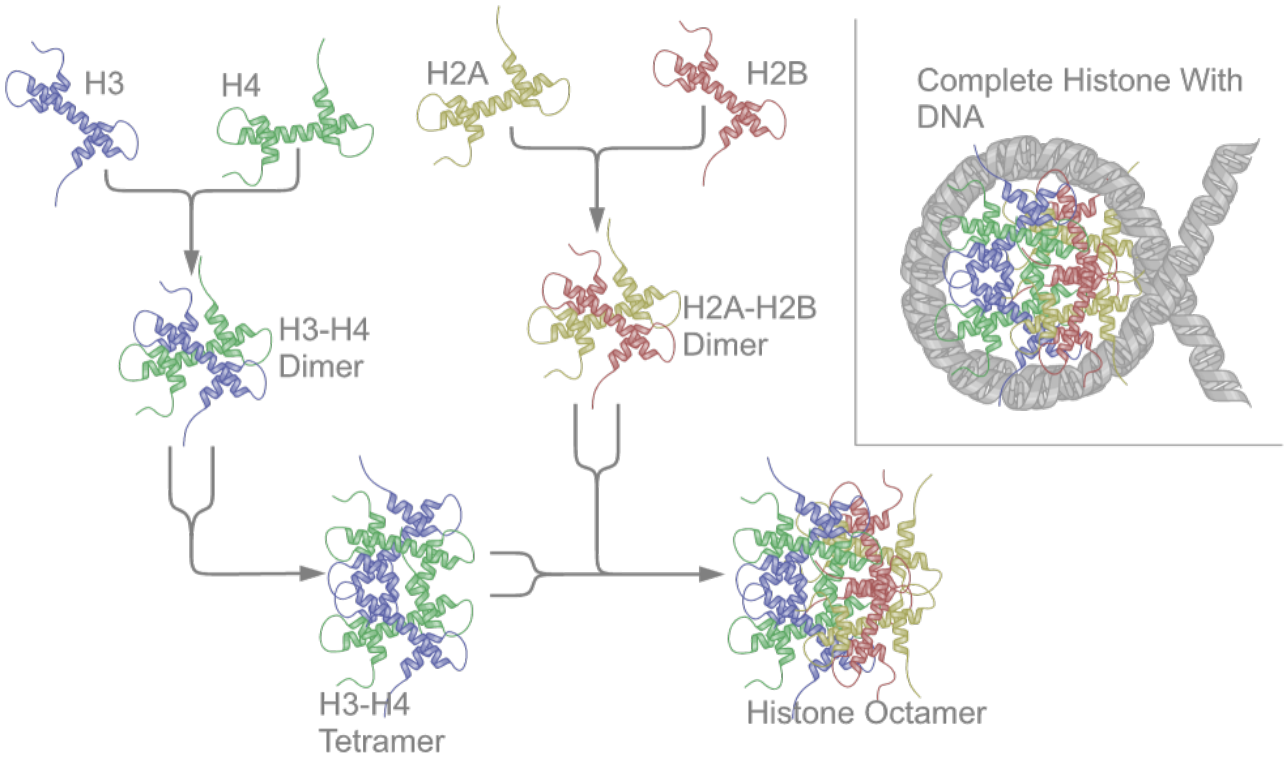
Top: Schematic showing secondary structure of the core histone proteins:

- α-helices represented by columns.
- Dashed lines indicate approximate residues within 'tail' domains;
- shaded boxes indicate the 3-helix histone fold domains within each protein, with first and last residues within α1, α2 and α3 helices indicated.
- Additional helices outside the histone fold domain are indicated by brackets

Bottom: Primary contacts between the core histone proteins in the nucleosome core.
Core histone dimerization partners are separated by dashes; dimer–dimer interactions via 4-helix bundles are indicated by colons.

The "packaging" of genetic information is essential for gene expression

histones



DNA wrapped around: 147/146nt

HISTONES CAN CONSERVE/MAINT AIN EPIGENETIC INFORAMTION

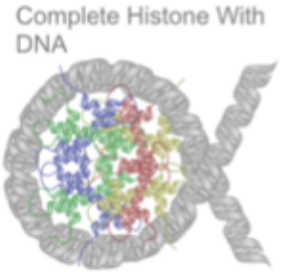
TPE, PEV: higher order gene regulation – but not the presence of regulatory DNA elements – impact on expression of reporter

How can it be tested if histones have an important role in regulating gene expression???

Histone tails are essential for epigenetic information

Cell, Vol. 65, 1023–1031, June 14, 1991, Copyright © 1991 by Cell Press

Yeast Histone H4 N-Terminal Sequence Is Required for Promoter Activation In Vivo



Model system:

CONCEPT: Yeast strain with a deletion of both histone H4 genes; contemporarily the yeast strain contains an extra copy of a histone H4 gene (was introduced) that is controlled by the **Galactose-inducible GAL promoter**. Upon addition of Galactose, yeast grown in media containig galactose (and lack Glucose), galactose activated the GAL promoter amd histone H4 is expressed → yeast survives

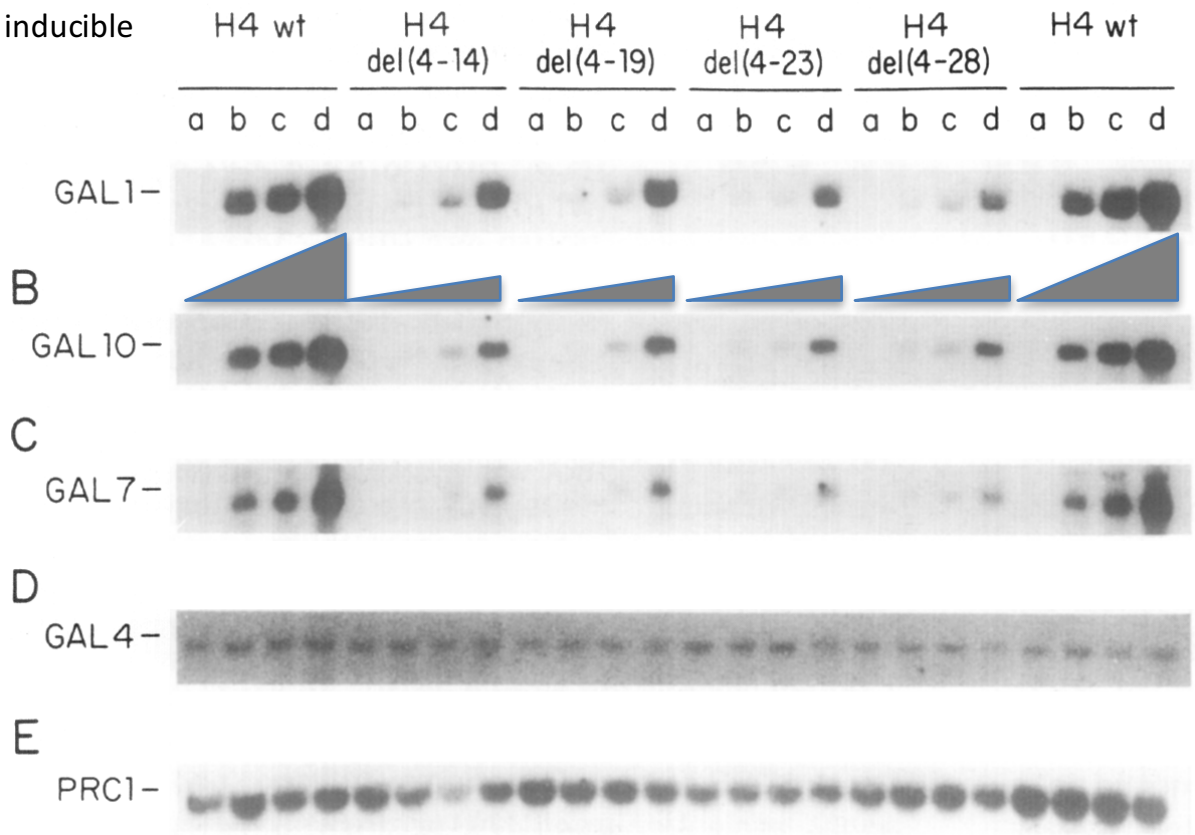
NOTE: Galactose also activates endogenous genes such as GAL1, GAL10, GAL7 and GAL4 that are necessary to process galactose

Deletion analysis: Model system for the study:

CONCEPT: Yeast lacking both endogenous H4 alleles and carry inducible wild-type or mutant versions of histone H4.

Histone H4

Histone tails are essential to control gene expression



Yeast cells were treated with Galactose to induce H4 (wt or mutant) expression.

Galactose indices activation of GAL1, GAL10, GAL7 and GAL4 gene.

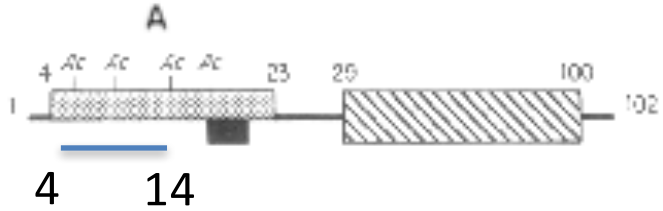
QUESTION: which H4 version is able to support gene activation?

METHOD: Northern blot; a, b, c, d: increased amount of RNA loaded on gel.

NOTE PRC1 cannot be activate by galactose treatment

a, b, c, d: increased amount of RNA loaded on gel

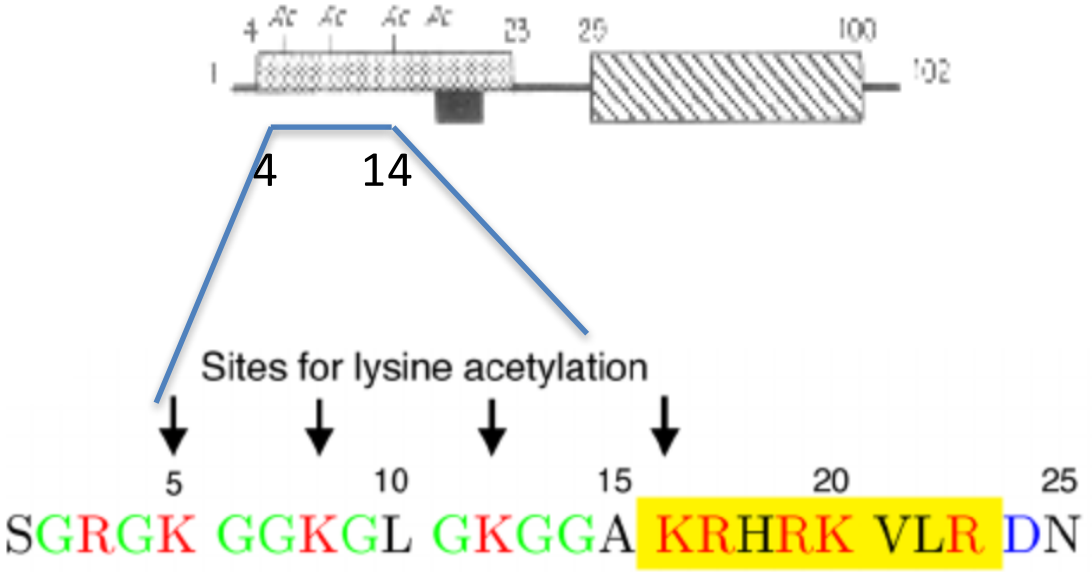
Cells with mutant H4 tails only inefficiently activate Gal1, 10, 4, 4 expression

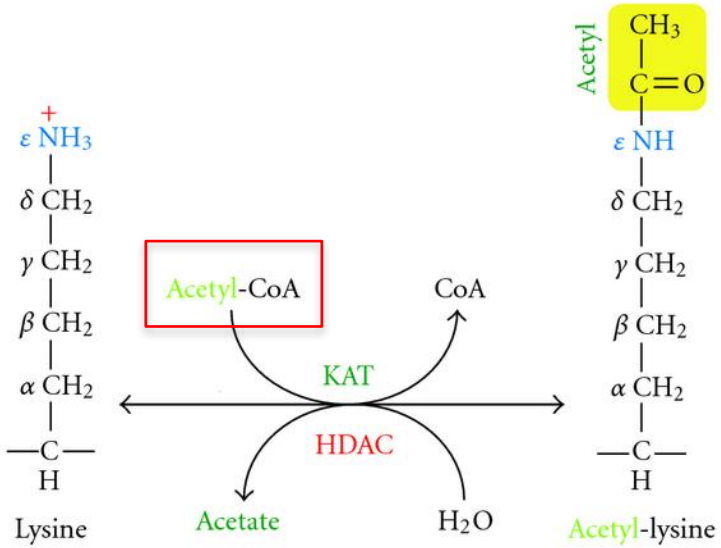


H4 TAIL CONTAINS INFORMATION TO ACTIVATE GENE EXPRESSION

Histones can be chemically modified

Lysines can be chemically modified in vitro
First modification tested: Acetylation





Can cells chemically modify lysine residues in histones to change gene epression levels??

Histones can be chemically modified

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6364–6368, July 1995
Cell Biology

An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei

(acetylation/chromatin)

JAMES E. BROWNELL AND C. DAVID ALLIS*
Department of Biology, Syracuse University, Syracuse, NY 13244

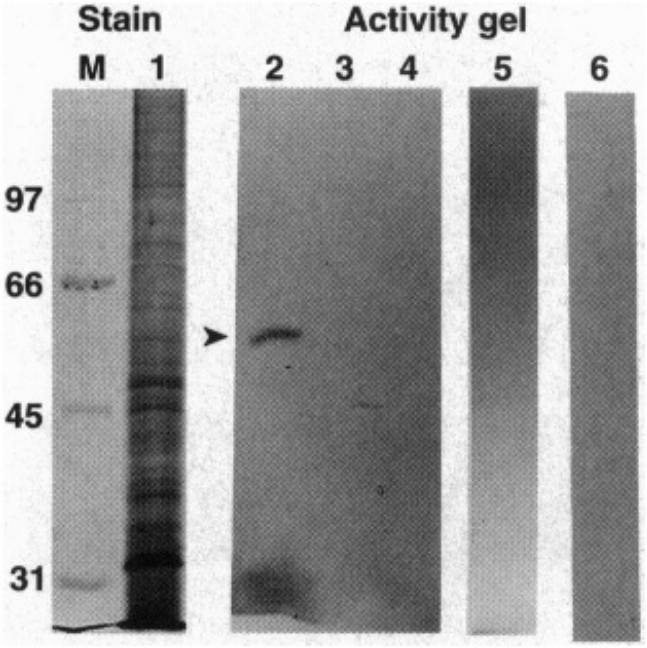
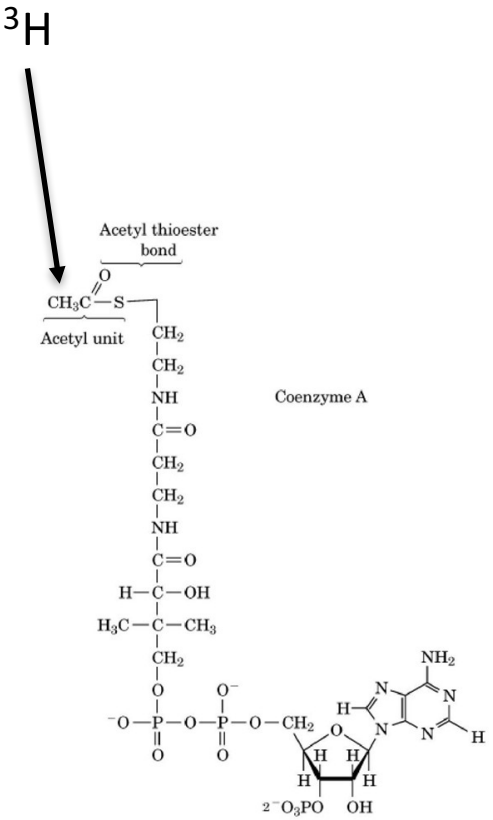


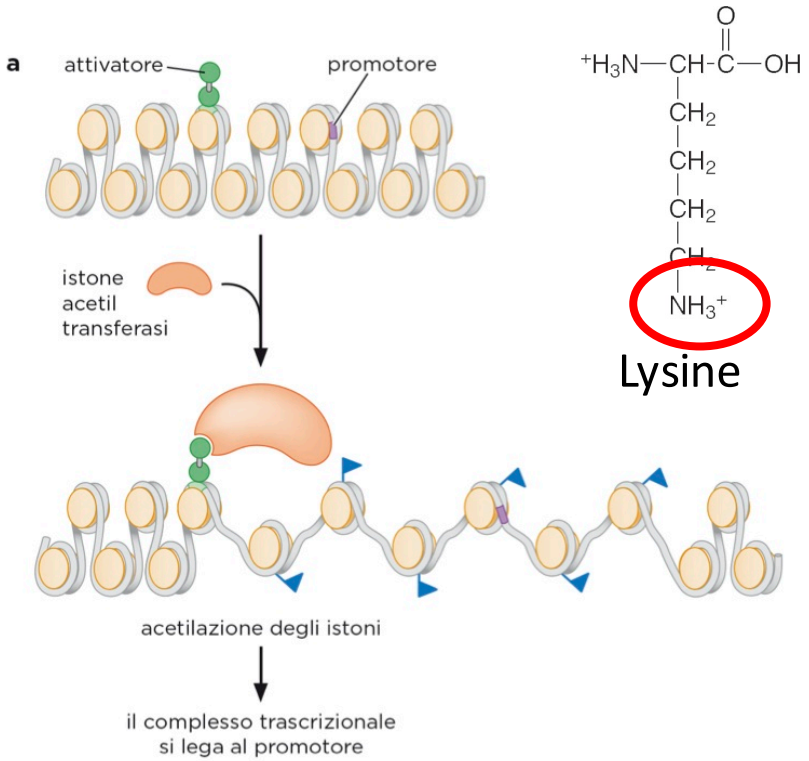
FIG. 1. A 55-kDa polypeptide specifically labels histones in an acetyltransferase activity gel assay. Crude macronuclear histone acetyltransferase activity was subjected to electrophoresis in SDS/8% polyacrylamide gels in which histones (lanes 2, 3, and 4), bovine serum albumin (lane 5), or no protein substrates (lanes 1 and 6) were incorporated prior to polymerization. Following electrophoresis, the gels were prepared for the activity gel assay and processed for fluorography (lanes 2–6) or silver stained (lane 1); M, molecular weight markers. In some cases, the enzyme was inactivated prior to loading the gel either by boiling for 5 min in sample buffer (lane 3) or by incubation with 10 mM *N*-ethylmaleimide (lane 4). [^{3}H]Acetate was incorporated into histones in a single region of the gel corresponding to a molecular mass of 55 kDa (arrowhead, lane 2). The gel was exposed for 1 week.

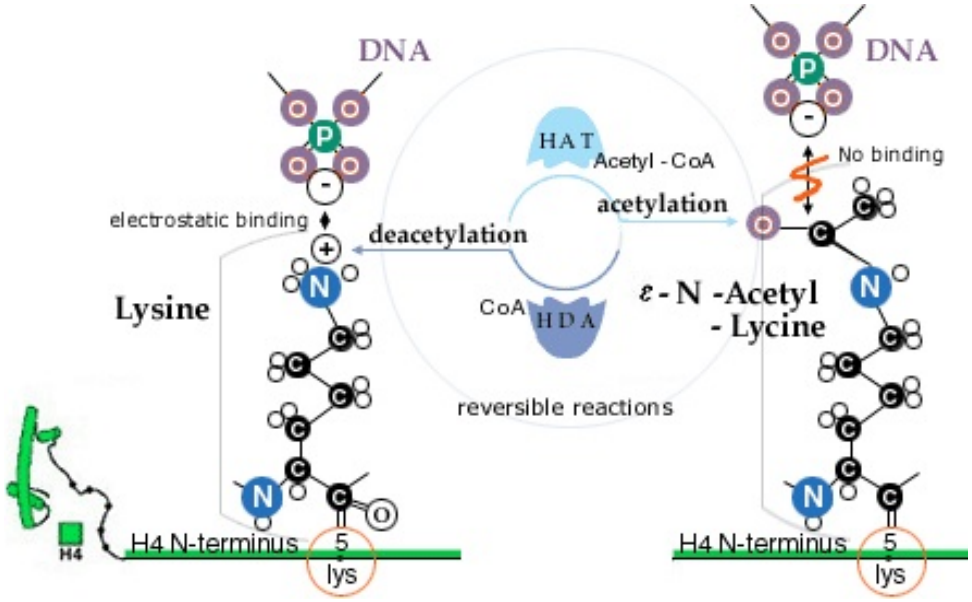
1. Make Polyaclylamide gel with incorporated, purified histones (lanes 2, 3, 4) or BSA (lane 5)
2. Lane 3: extract boiled; Lane 4: N-ethylmaleimide – binds Cys

2. Take protein extract from Tetrahymena Macronuclei and load run on gel.
3. After stop of the gel the enzymatic assay will be performed: radioactive [^{3}H]-Acetyl-CoA (contains acetyl group – is a major enzymatic cofactor in cells) on top of gel.
4. After autoradiography, a band appears that marks acetylated histones that co-lolocalize with a “histone acetyltransferase” present in the Tetrahymena extract



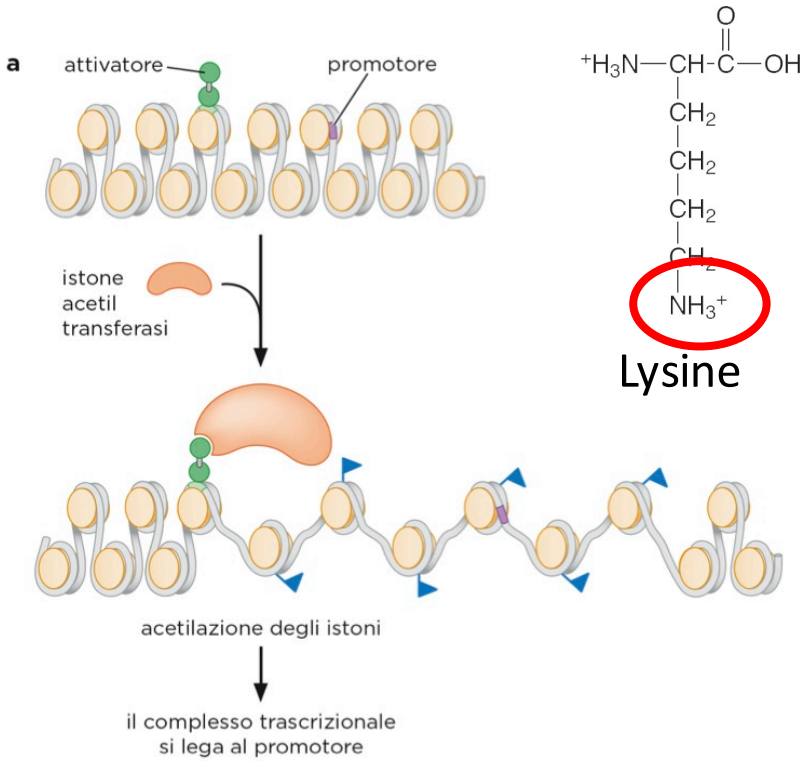
Post-translational modifications can change the topology of chromatin

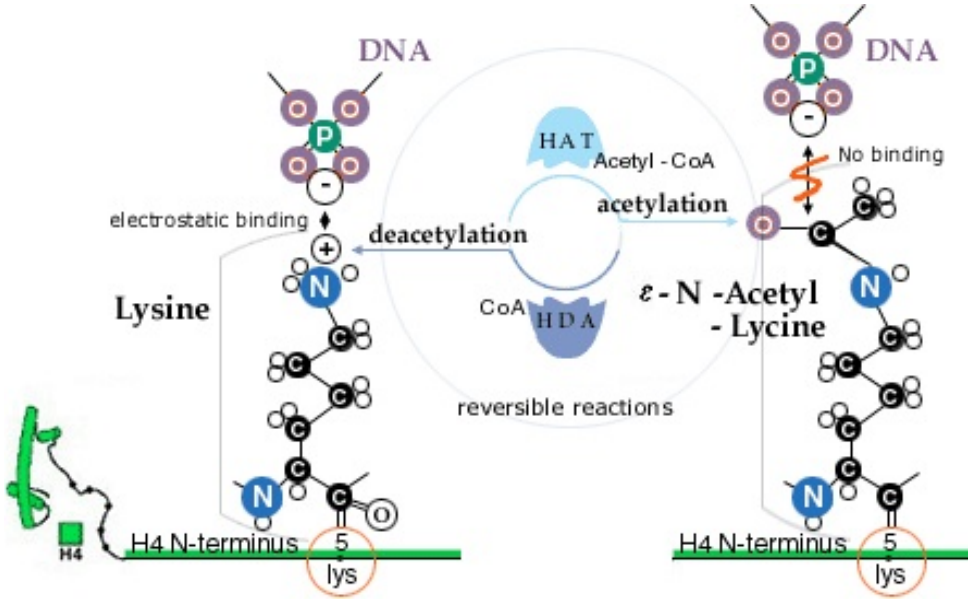




"EPIGENETIC GENE REGULATION" IS MAINLY BASED ON CHEMICAL MODIFICATIONS OF HISTONES AND DNA

Post-translational modifications can change the topology of chromatin





"EPIGENETIC GENE REGULATION" IS MAINLY BASED ON CHEMICAL MODIFICATIONS OF HISTONES AND DNA

Post-translational modifications decide on gene expression
TELOMERE POSITION EFFECT AND ACETYLATION

Observation:

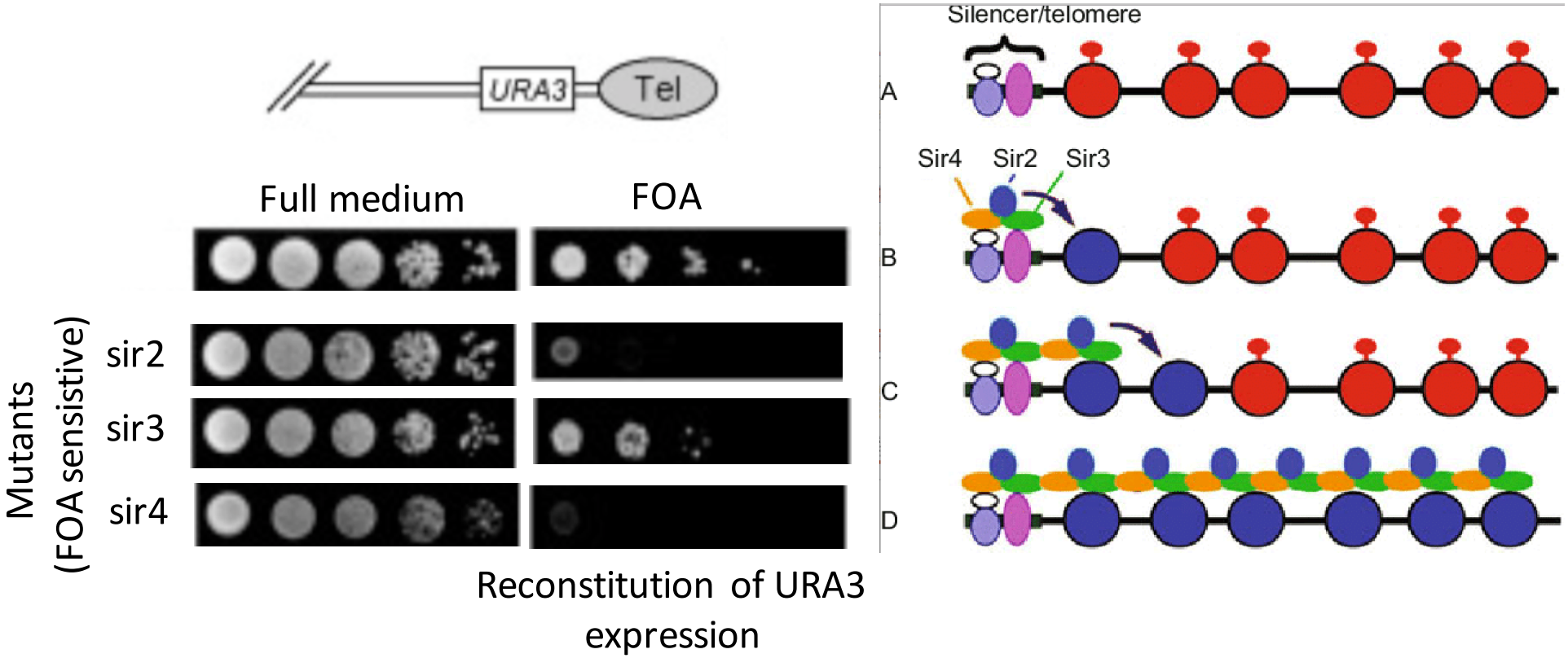
→URA3 Reporter gene inserted into central position in chromosome: EXPRESSED

→ URA 3 Reporter gene inserted in proximity to chromosome ends: SILENT

→ make mutatant S. cerevisiae that release reporter from silencing

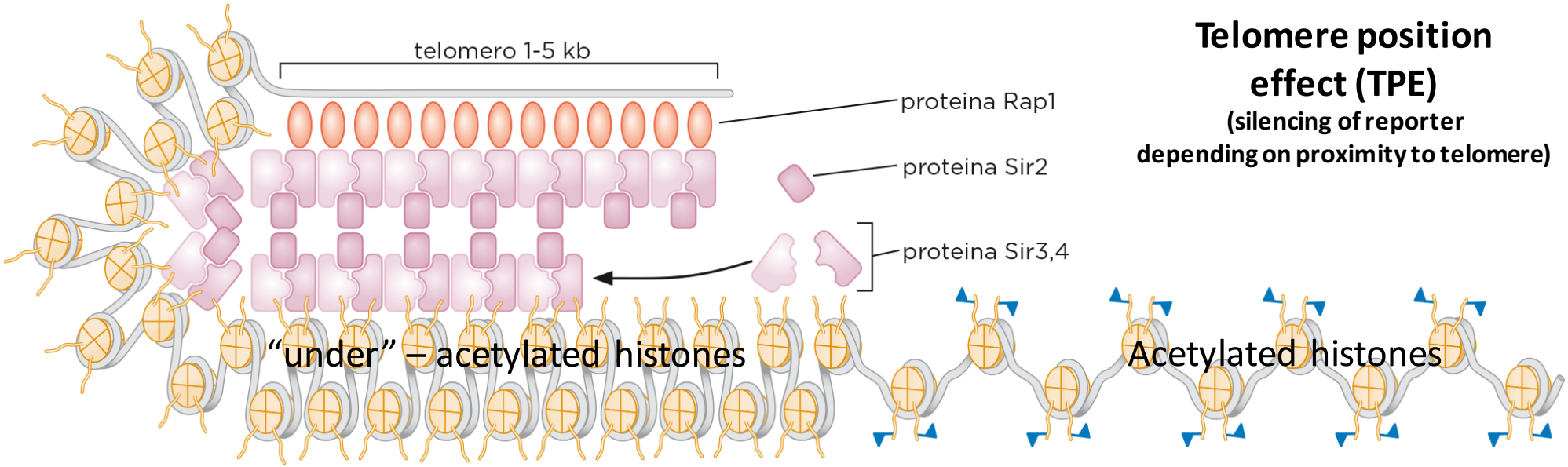
→ identify genes that are mutated

= SILENT INFORMATION REGUALTORS (SIR) GENES



(Not orignal data)

Post-translational modifications decide on gene expression
TELOMERE POSITION EFFECT AND ACETYLATION

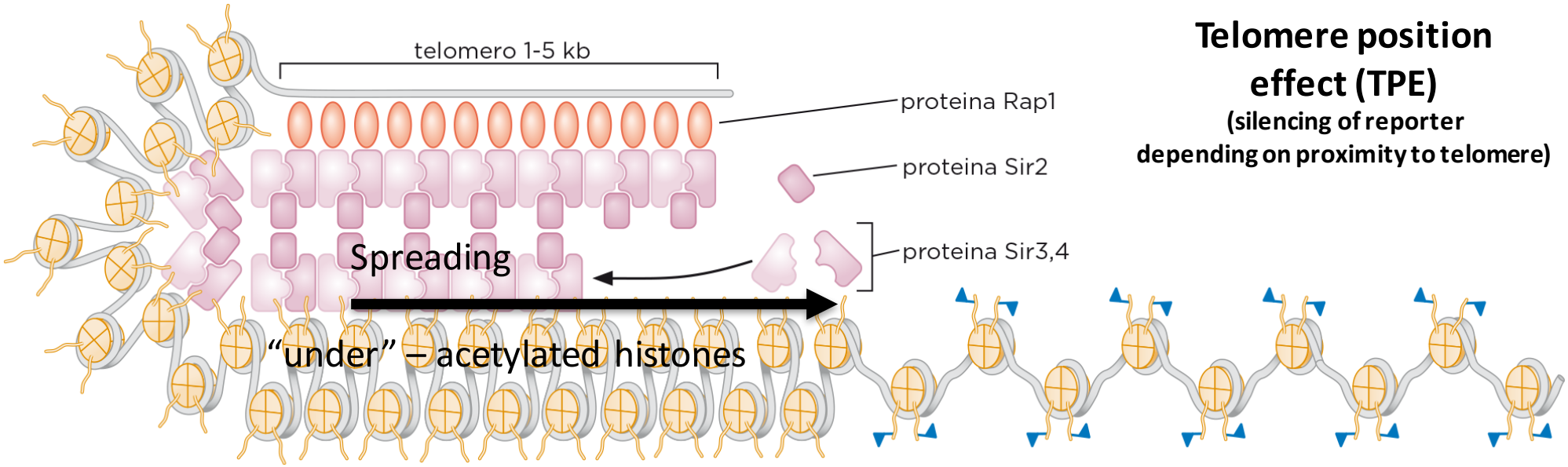


Telomere position effect (TPE)
(silencing of reporter depending on proximity to telomere)

Mechansim

1. Rap1 specifically binds to telomeric repeat sequences $(G_{(2-3)}(TG)_{(1-6)}T$ consensus)
2. **Rap1 recruits the SIR complex (SIR2,3,4)** SIR binding is co-opertative and Sir2,3,4 multimerization expands onto subtelomeric chromatin that contained histones
3. SIR proteins stabilize a folded chromosome end structure (protection, suppression of gene expression)
4. **SIR2 is a HDAC → silencing of chromatin by histone deacetylation**
5. SIR complex spreads towards the centromere until meets antagonizing chromatin signature

Post-translational modifications decide on gene expression
TELOMERE POSITION EFFECT AND ACETYLATION



Telomere position effect (TPE)
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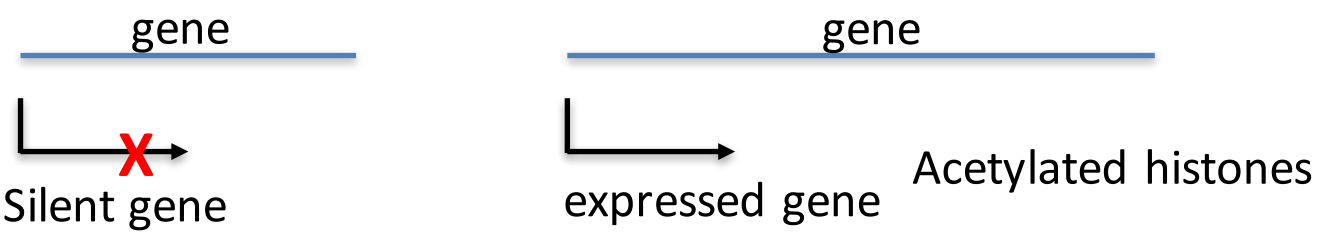


How is spreading of telomric heterochromatin controlled?

Post-translational modifications decide on gene expression
TELOMERE POSITION EFFECT AND ACETYLATION

HOW IS SPREADING REGUALTED??

Silencing complex spreads until meets gene-rich regions that contain H2A variant H2AZ = **barrier** (S. cerevisiae name: Htz1 and H4K16-acetylation (active regions) = strong information for active gene expression → competition between repressive and active chromatin

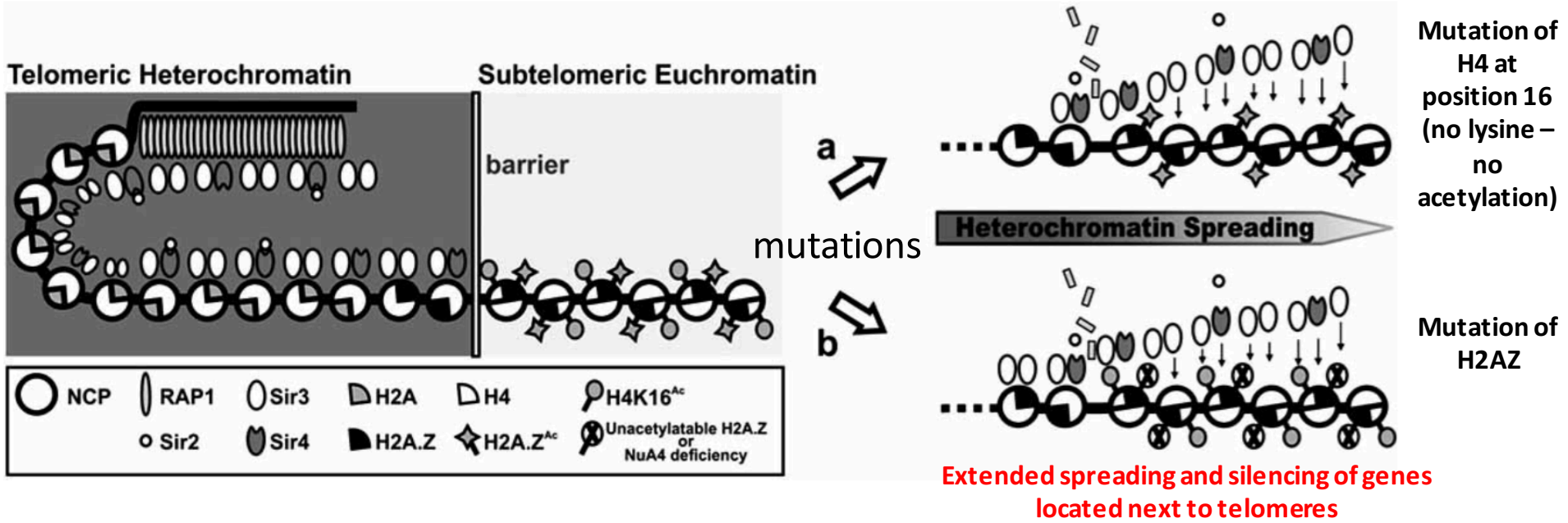
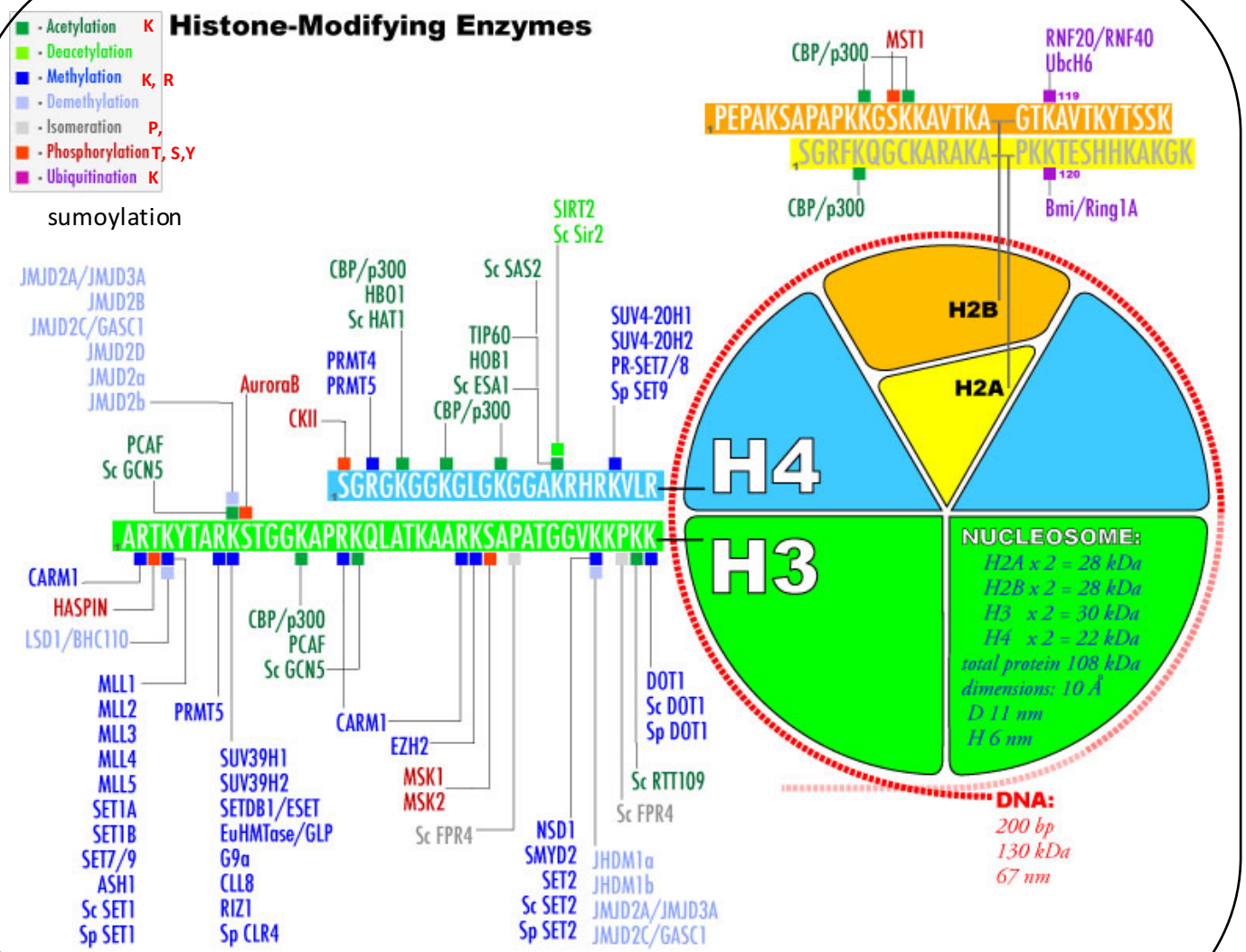
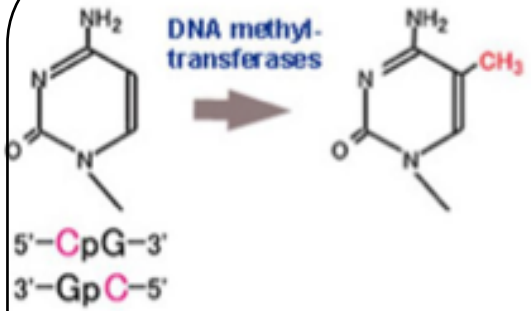


Fig. (2). A model for yeast telomeric heterochromatin adapted from [39]. Heterochromatin formation and maintenance involve the RAP-1 containing telosome, different Sir proteins and the interaction of these elements with histone H4. H2A.Z participates in preventing the spread of telomeric heterochromatin to subtelomeric euchromatin regions by creating a barrier effect, and this boundary function is highly dependent on acetylation. For instance, the absence of H4-K16^{Ac} results in the disruption of this barrier even in the presence of acetylated H2A.Z (pathway a [7, 8, 17**]). Furthermore, absence of H2A.Z acetylation or NuA4 deficiency (pathway b) also break this barrier even in the presence of H4-K16^{Ac} [11**, 27**]. Thus, the boundary function played by H2A.Z and H4 histones is mainly regulated by their acetylation rather than by their mislocalization at subtelomeric regions.

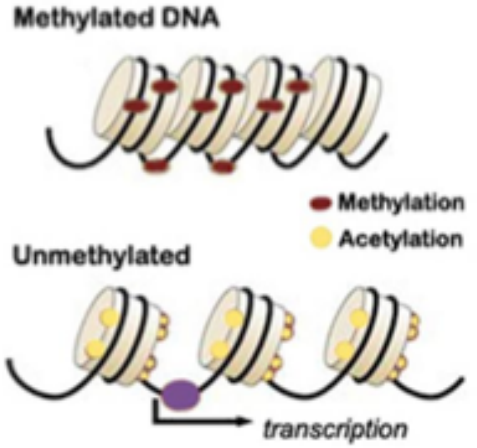
Histones carry multiple chemical modifications
=POST TRANSLATIONAL HISTONE MODIFICATIONS



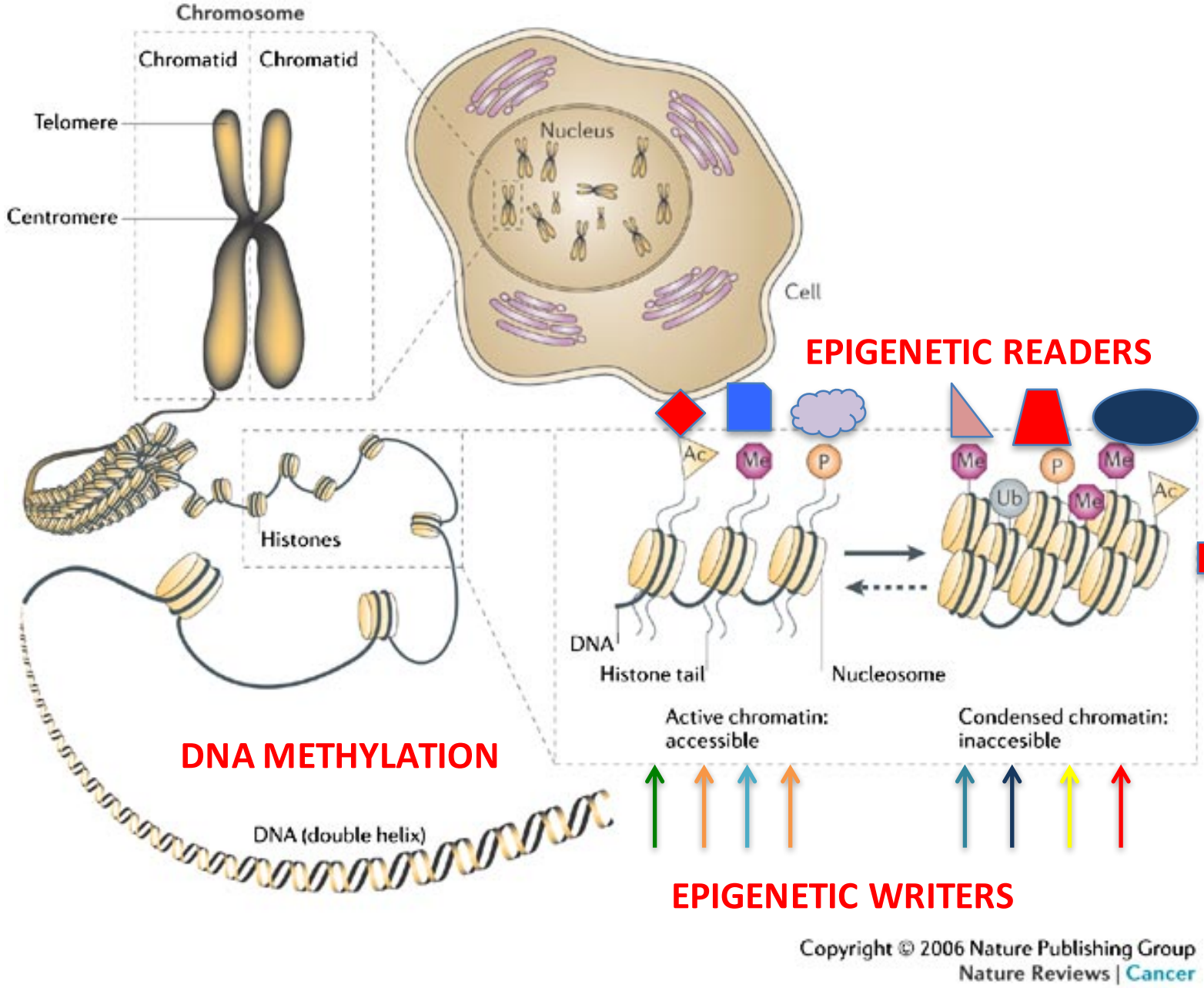
THE "HISTONE CODE"



DNA methyltransfererases DNMT1, DNMT3a, DNMT3b act only on CpG di-nuceotides



Histones can carry multiple chemical modifications =POST TRANSLATIONAL HISTONE MODIFICATIONS



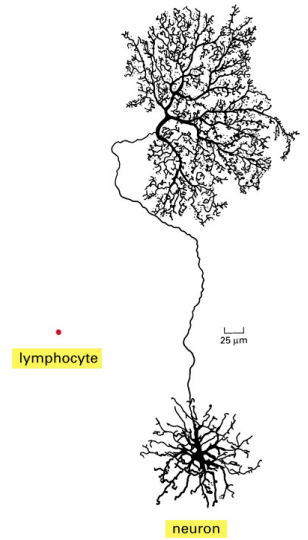


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

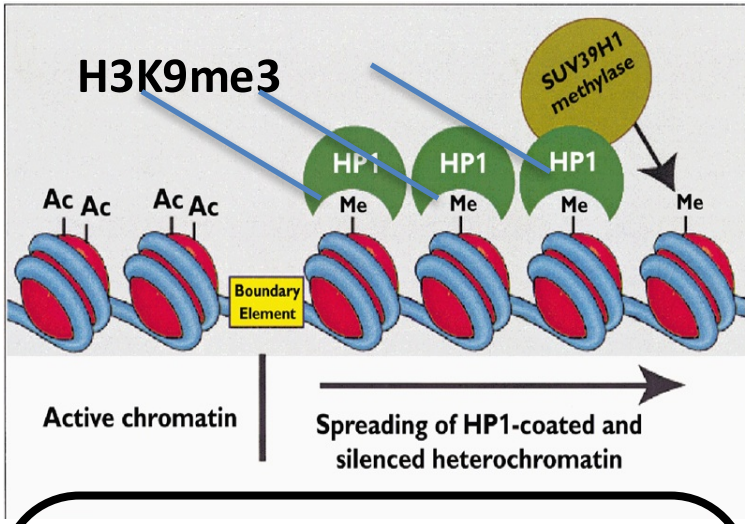
GENE EXPRESSION PROGRAMS:
→CELL TYPE SPECIFIC

→NORMAL/ CANCER

EPIGENTIC WRITERS AND READERS

HMTase generates post-translational histone modifications can recruit specialized proteins (WRITERS and READERS)

Example: SUV39H1 (Su(var)3-9) writes H3K9me3 that recruits HP1 to form heterochromatin at centromeres in flies and vertebrates



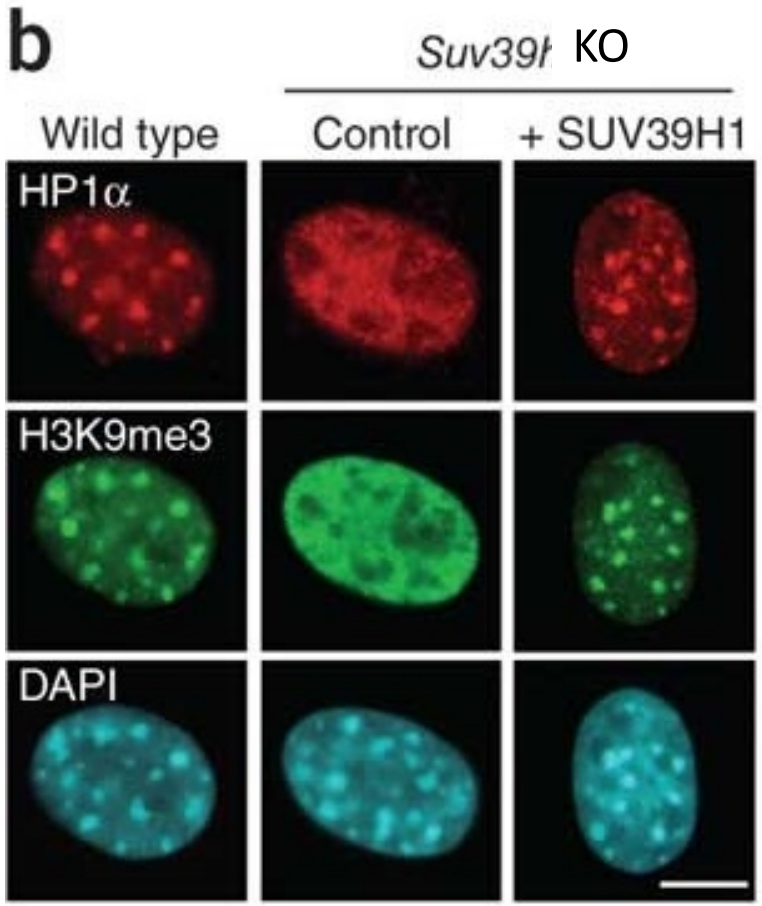
Suv39h1/2 tri-methylate Lysine 9 on histone H3

↓

tri-methylated Lysine 9 on histone H3 recruits HP1

↓

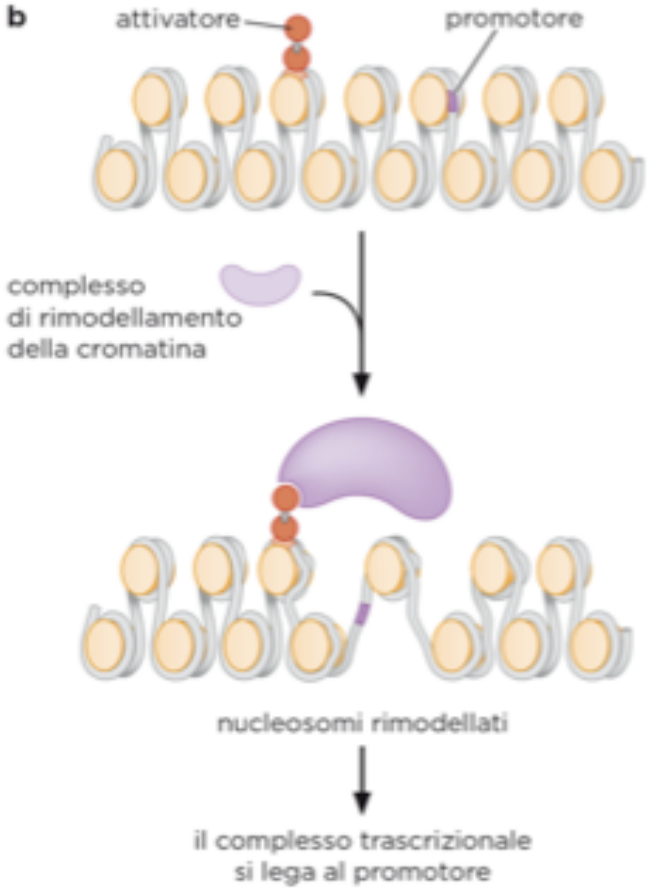
heterochromatin

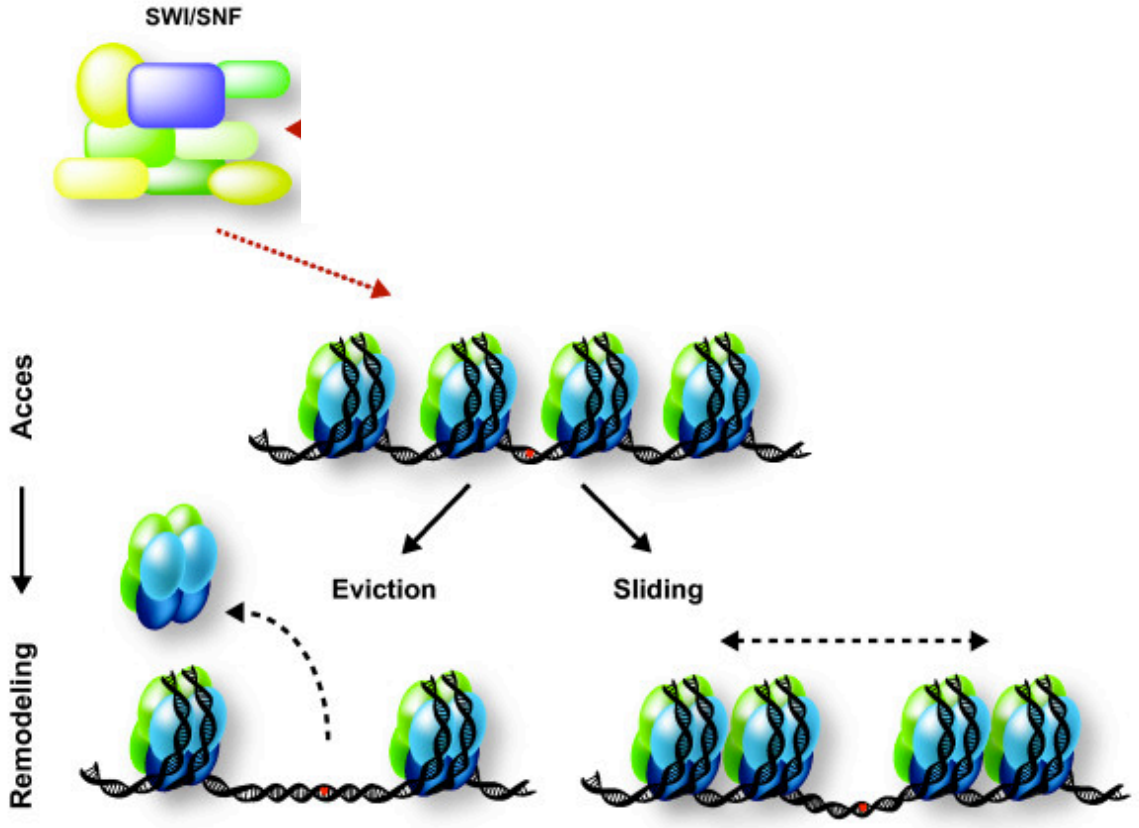


histone modifications can reach high levels in cells and can be visualized by immunofluorescence

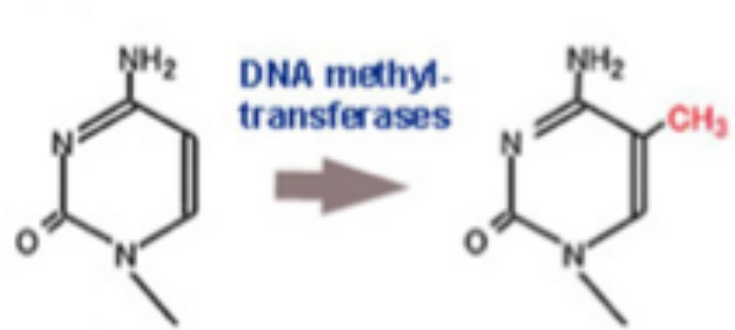
CORE HISTONES CAN BE MOVED OR ELIMINATED = CHROMATIN REMODELLING COMPLEXES

-Activator can recruit a **chromatin remodeling complex** → SWI/SNF complex, moves nucleosomes to make promoter/response elements accessible (ATP dependent!)
-Activator (i.e TF or Co-TF) can recruit a **histone acetyl transferases** → add acetyl groups to lysine histone tails (p300,, GCN5, MOF, etc)
→arrangement of nucleosomes change at response elements
→acetylated tails serve as a binding site for bromo-domain proteins (TFIIH contains such protein)

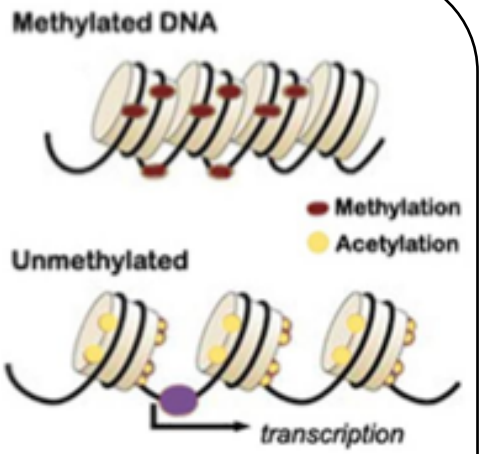


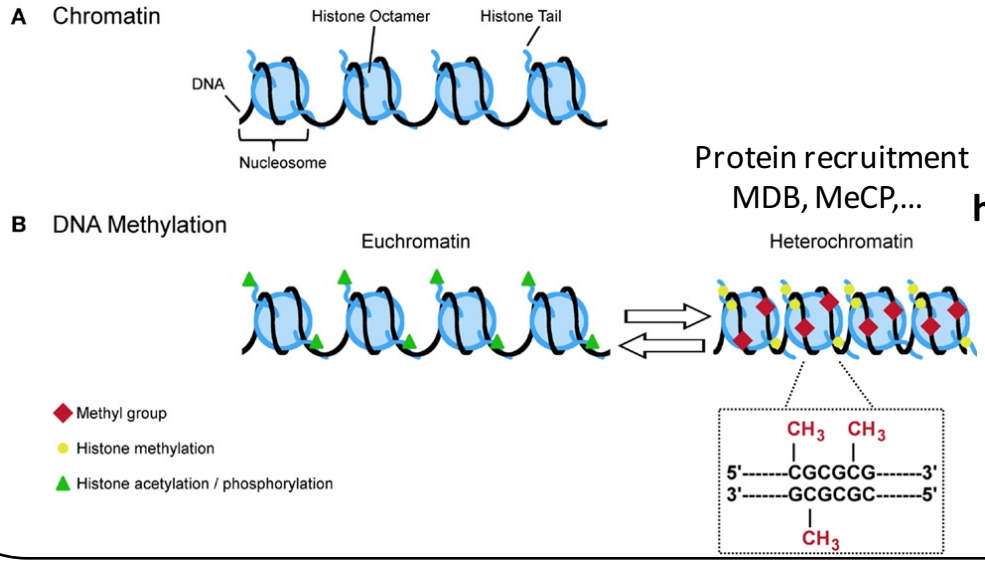


DNA CAN CARRY INFORMATION THAT PREVENT THE ACTIVATION OF GENES



DNA methyltransfererases DNMT1, DNMT3a, DNMT3b act only on CpG di-nuceotides





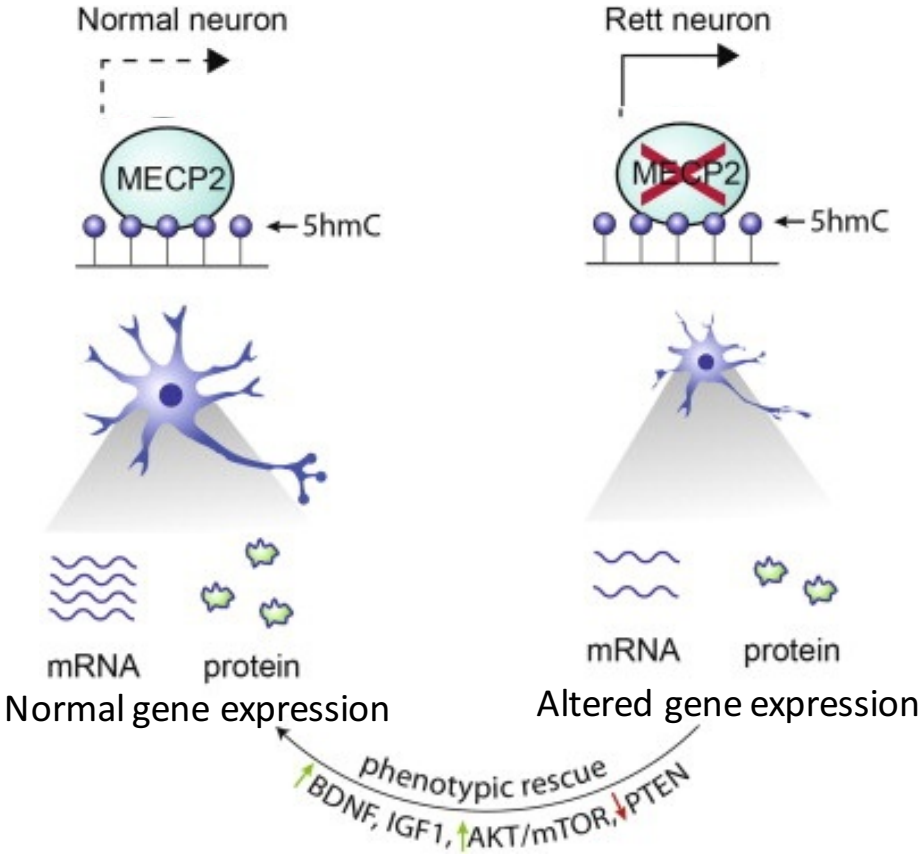
DNA methylation is paired with repressive histone modifications and lack of activating modifications

= FUNCTIONAL REDUNDANCY TO ENSURE REPRESSION

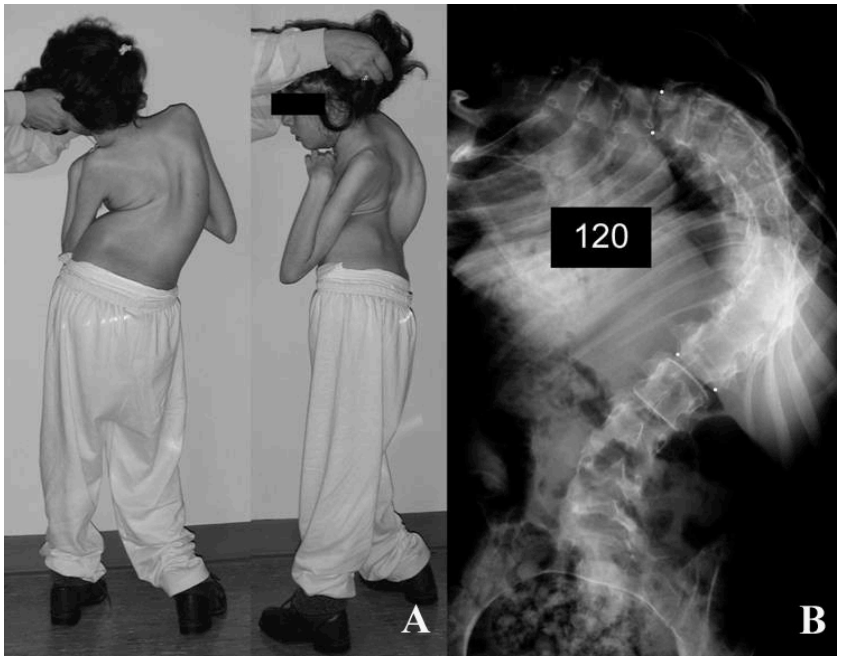
Yeast:
NO
C. elegans:
NO
D. melanogaster:
YES, but very low levels
Vertebrates: HIGH
Very important

DNA can carry information that prevent the activation of genes
DNA methylation

Methylated DNA is bound
By MeCP2 (and other methyl-DNA
Specific proteins such as MBD1, MBD2,
MBD4 and BAZ2)
= MeCP2 is a reader of DNA methyaltion



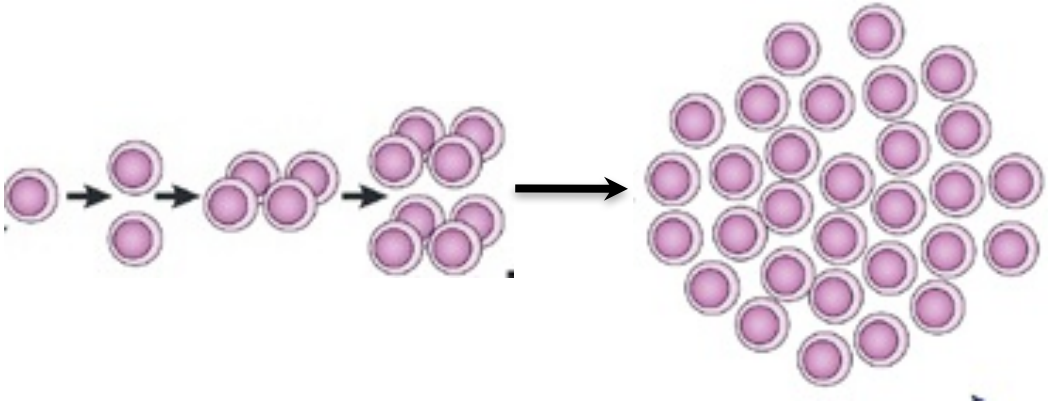
Rett syndrome is caused by mutations in MeCP2



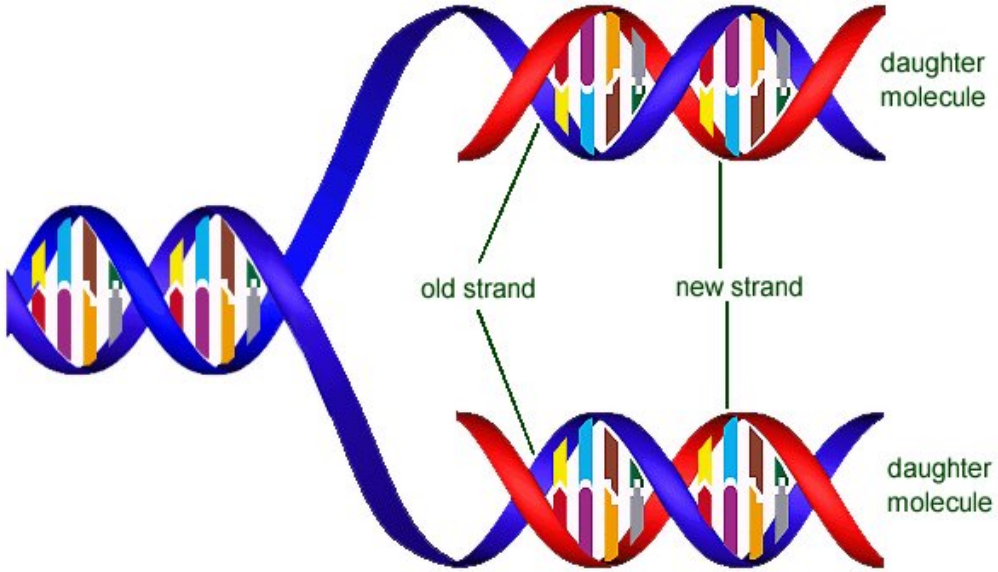
Rett syndrome (RTT), originally termed cerebroatrophic hyperammonemia is a rare genetic postnatal neurological disorder of the grey matter of the brain that almost exclusively affects females but has also been found in male patients. The clinical features include small hands and feet and a deceleration of the rate of head growth (including microcephaly in some). Repetitive stereotyped hand movements, such as wringing and/or repeatedly putting hands into the mouth, are also noted. People with Rett syndrome are prone to gastrointestinal disorders and up to 80% have seizures. They typically have no verbal skills, and about 50% of affected individuals do not walk. Scoliosis, growth failure, and constipation are very common and can be problematic.

CAN EPIGENTIC INFORMATION BE CONSERVED AFTER DNA REPLICATION??

Cell proliferation



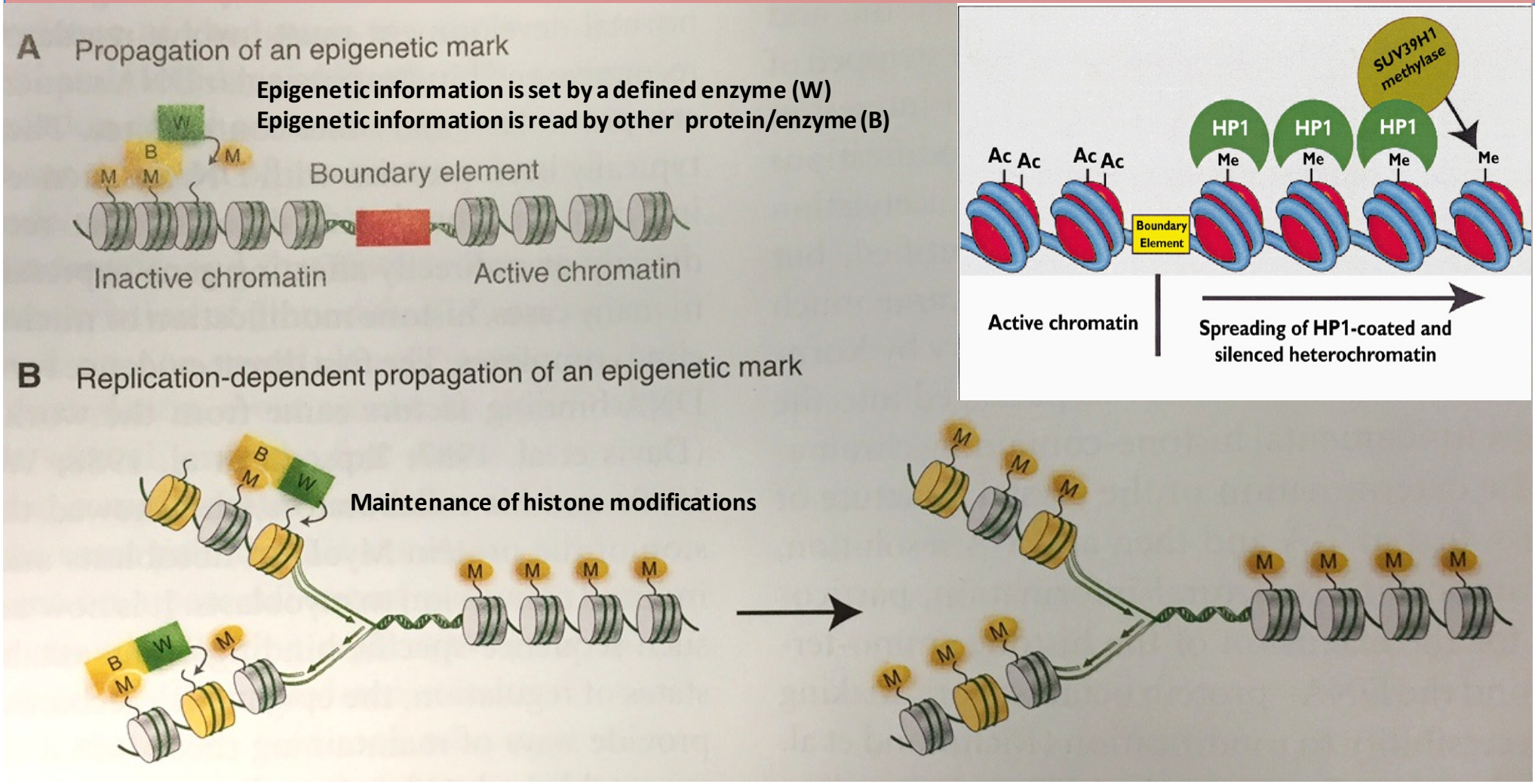
SEMICONSERVATIVE DNA REPLICATION



WHAT IS HAPPENING WITH EPIGENTIC INFORMATION DURING DNA REPLICATION

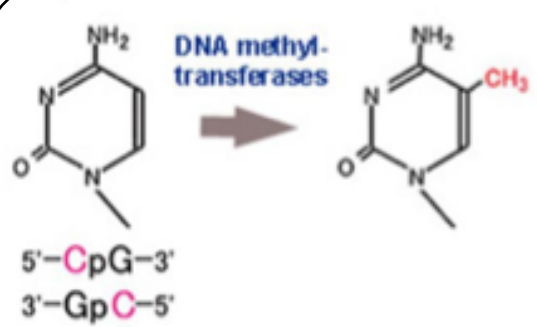
HOW CAN IT BE MAINTANED DURING SEMICONSERVATIVE REPLICATION??

The propagation of epigenetic marks

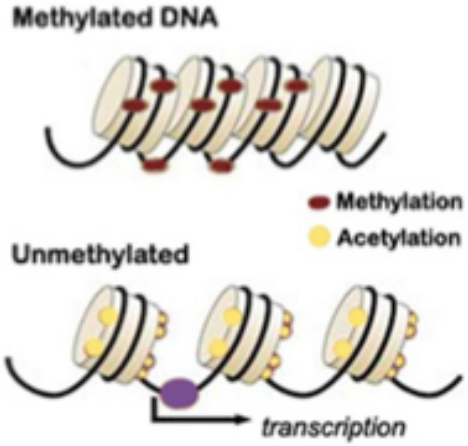


S-Phase: New, randomly deposited histones octameres without histone modifications are inserted during DNA replication. Epigenetic writers associated with the parental DNA now Impose the parental histone code to newly incorporated histones. Epigenetic code is maintained in both daughter cells

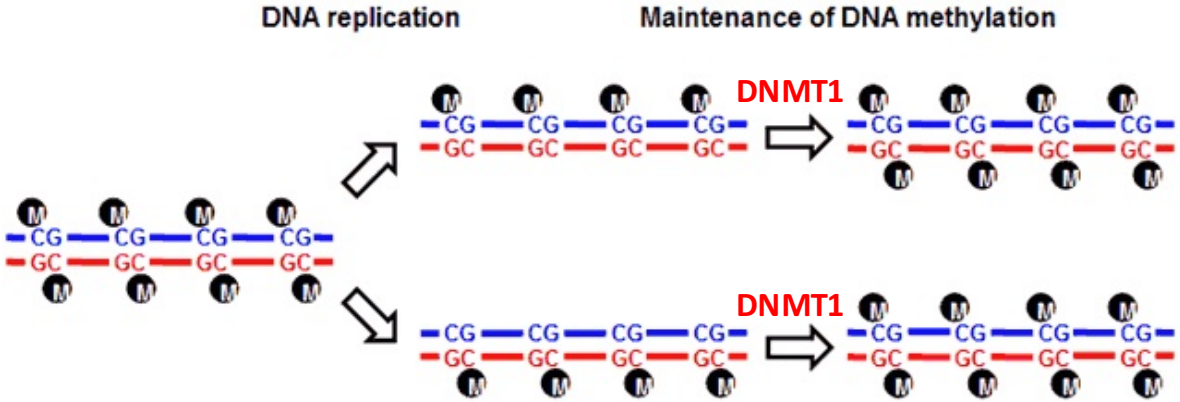
The propagation of epigenetic marks



DNA methyl transfererases DNMT1, DNMT3a, DNMT3b act only on CpG di-nuceotides



CAN EPIGENTIC INFORMATION BE CONSERVED AFTER DNA REPLICATION??



Newly synthesized DNA is without DNA methylation. DNMT1 specifically, reads hemi-methylated DNA and methylates the opposite C on the the newly synthesized, unmethylated DNA filament. Both Daughter cells contain the same DNA methylation pattern like the patental cell

KEY FEATURS OF EPIGENETIC REGULATION

→ INITIATION OF CHROMATIN REGULATION BY SPECIFIC RECRUITMETN AND ENZYMATIC ACTIVITY (writers)

→ RERUITMENT OF MAINTENANCE FACTORS (readers)

→ REDUNDACY – chromatin status is defined by more than 1 writer/reader (see PEV HDACs)

→ SPREADING OF CHROMATIN STATUS (writers + readers, see TPE PEV)

→ NUCLEOSOME PHASING

→ BARRIERS BLOCK SPREADING OF CHROMATIN STATUS (see TPE)

→ MAINTAINACE OF CHROMATIN STATUS DURING CELL DIVISION (see TPE, PEV)

→ REVESIBILITY OF CHROMATIN STATUS BY ENZYMATIC ACTIVITES (HATs – HDACs)

DEFINING EPIGENTICS

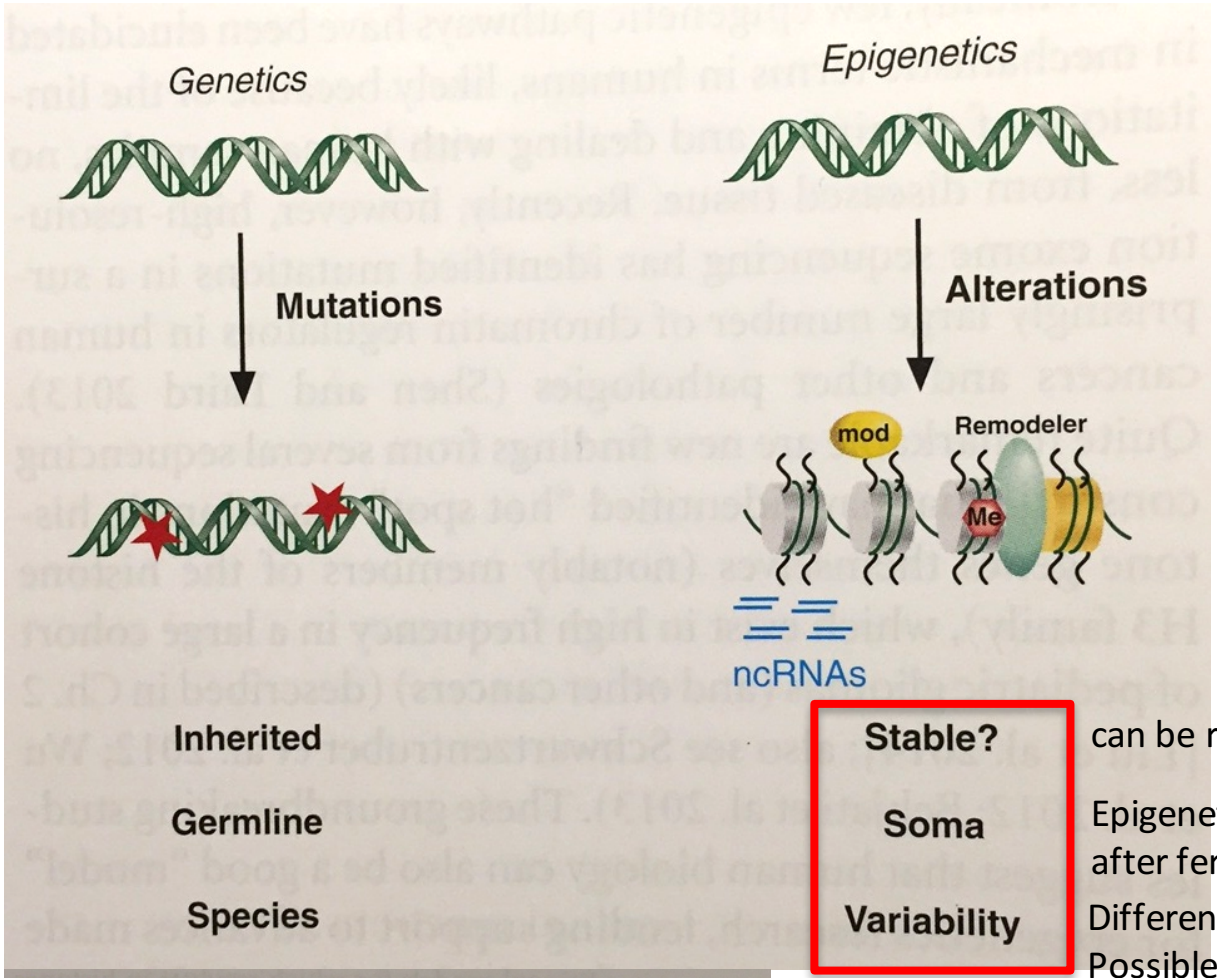


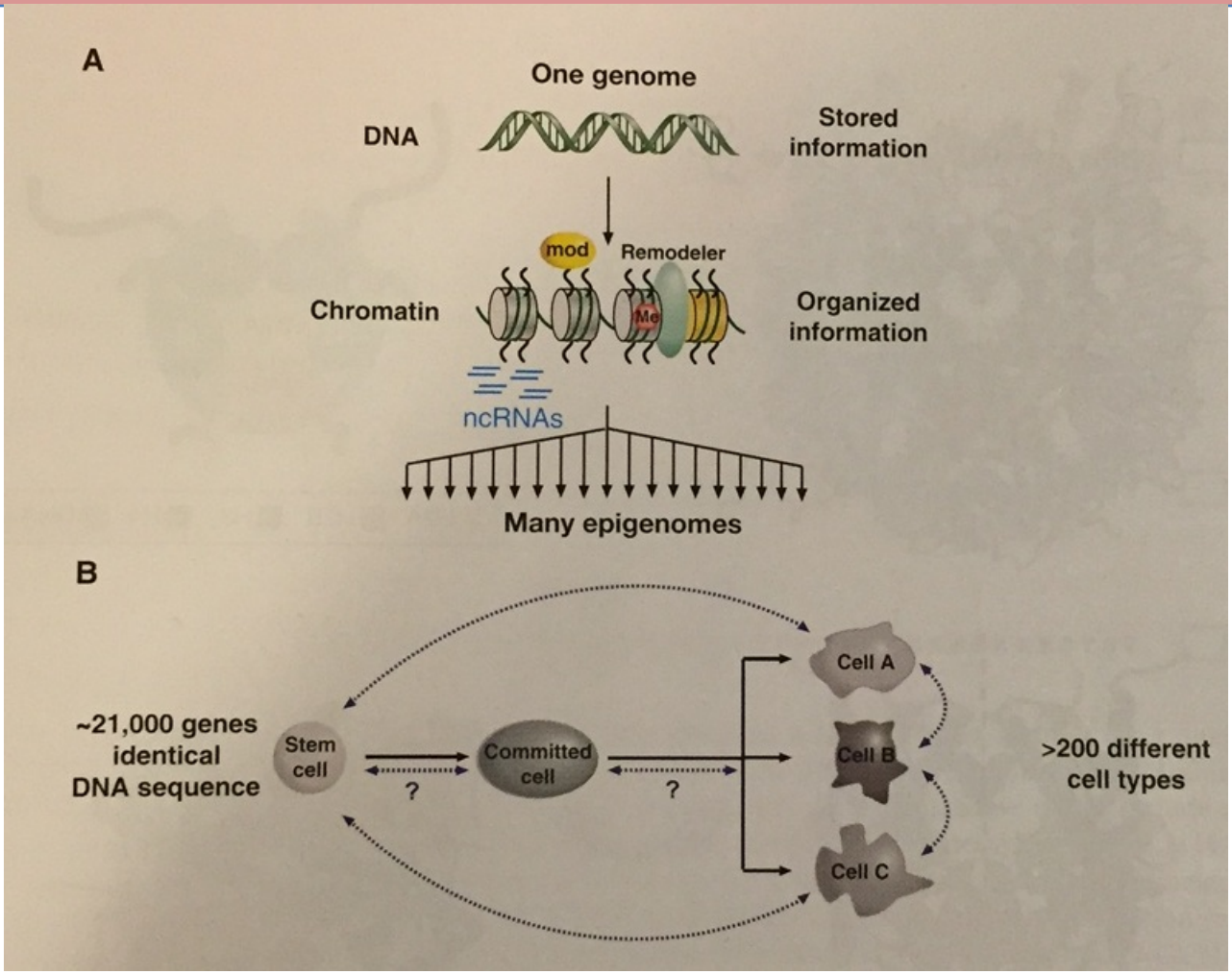
Figure 3. Genetics versus epigenetics. Genetics: Mutations (red stars) of the DNA template (green helix) are heritable somatically and through the germline. Epigenetics: Variations in chromatin structure modulate the use of the genome by (1) histone modifications (mod), (2) chromatin remodeling (remodeler), (3) histone variant composition (yellow nucleosome), (4) DNA methylation (Me), and (5) noncoding RNAs (ncRNAs). Marks on the chromatin template may be heritable through cell division and collectively contribute to determining cellular phenotype.

can be reversed

Epigenetic code is erased after fertilization (DNA methylation)

Differences between individuals Possible (life-style, nutrition, external Effects)

DEFINING EPIGENTICS



Epigenetics controls the use of our DNA

Alterations of the Epigenetic code change Gene expression and change the identity of the cell
→Such changes can come from the Developmental programs, disease, environment, metabolism, mutations in epigenetic regulators, etc

The human body contains more Than 200 different cell types That share the same information (exception: B and T cells)
Each cell type has a characteristic Gene expression profile that is controlled and maintained (inherited or epigenetic memory) by an defined epigenetic profile

Holliday 1994: Epigenetics is the nuclear inheritance which is not based on differences in DNA sequence

More mechanistically: Epigenetics is the sum of the alterations to the chromatin template that collectively establish and propagate different patters of gene expression (transcription) and silencing from the same genome

WHAT IS EPIGENETICS ??

Waddington 1950
…Epigenetics is the study of processes that categorize all of the developmental events leading from the fertilized oozyte to the mature organism – that is, all of the regulated processes that, beginning with the genetic material, shape the final product

Riggs,1996; Riggs and Porter 1996:
…the study of mitotically heritable changes changes in gene function that cannot be explained by changes in in DNA seqeunce"

+

Berger 2009
…the initiation of a new epigenetic state involve a transient mechanism Separate from the one required to maintain it

+

Holliday 1994:
Epigenetics is the nuclear inheritance which is not based on differences in DNA sequence

+

Epigenetics is the sum of the alterations to the chromatin template that collectively establish and propagate different patters of gene expression (transcription) and silencing from the same genome