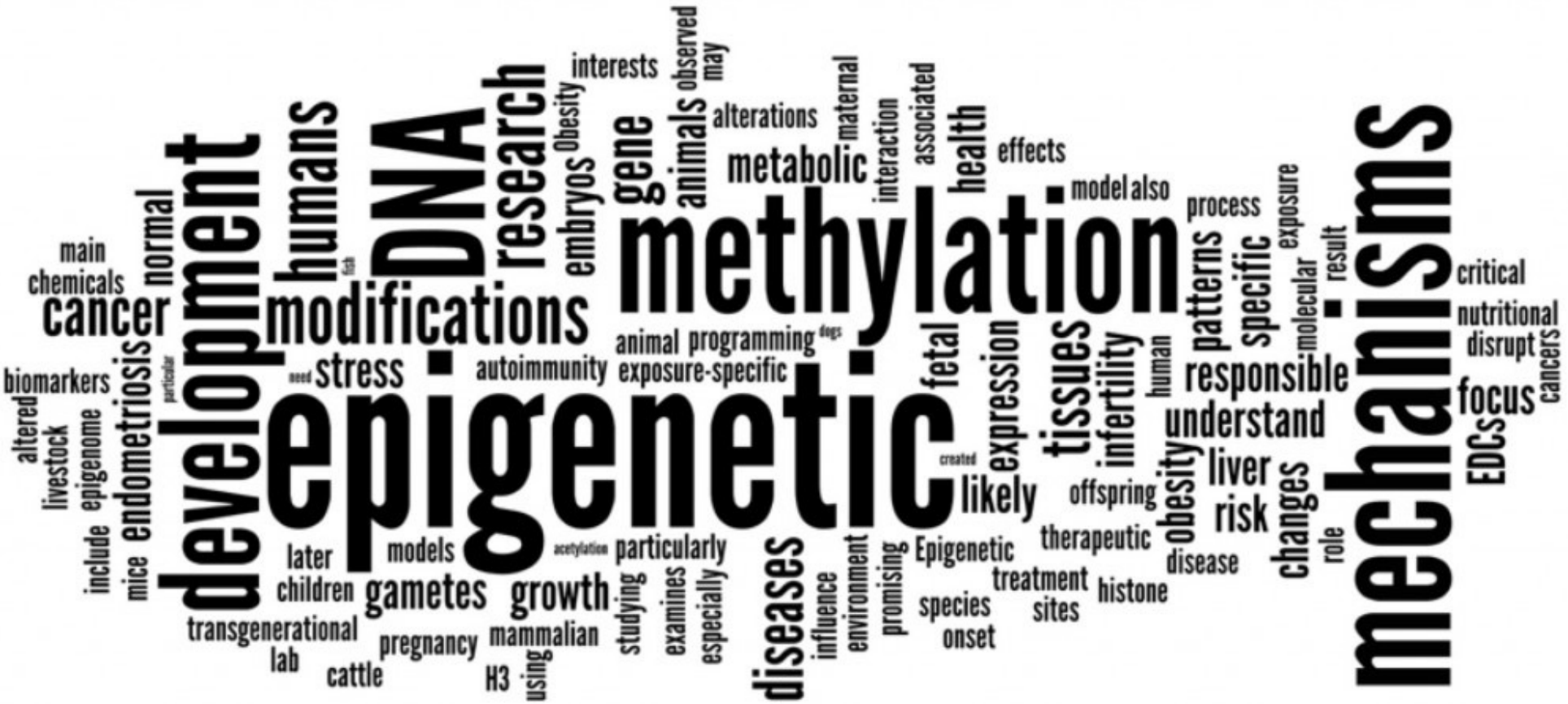
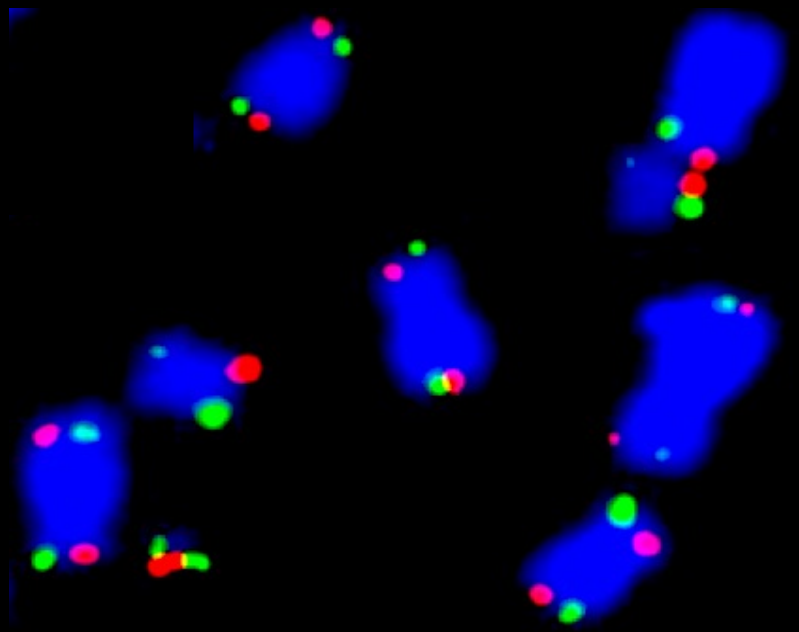
EPIGENETICA
CON LABORATORIO



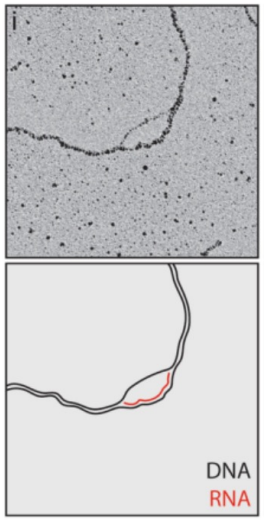
ncRNA and Genome Instability Laboratory

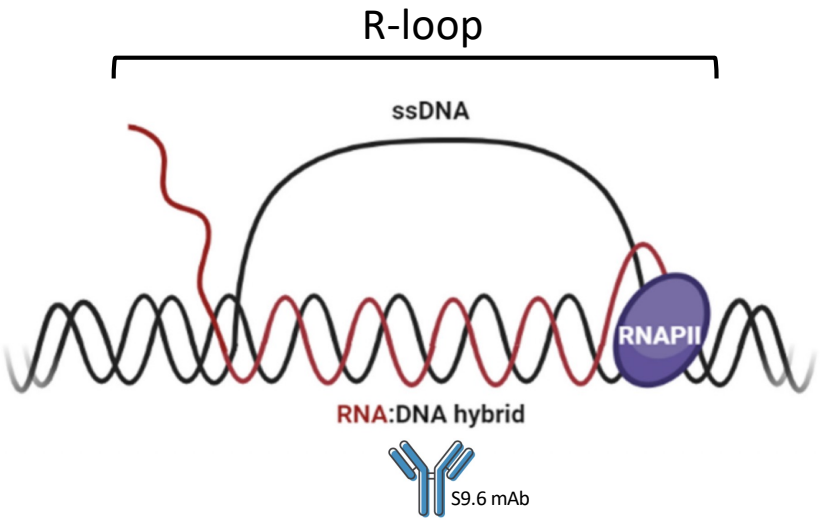


Alessandro Ferrando, PhD student
Simone Giaquinto, PhD Student
Andrea Parlante, PhD Student
Alessandra Poma, Borsista post-laurea
Davide Cacciapaglia, Master student
Erika Zampa, Master student
Gabriele Cechutti, Master student

Ed C11
Second floor
Lab 244-247

R-loops are atypical nucleic structures





- *<5% of genome*
- *in cis (transcription)*
- *in trans (recombinase)*
- *GC rich regions*
- *repeats*
- *G-quadruplex regions*
- *long genes (>800kb)*

Benifical role
»scheduled R-loops«

Immunoglobulin class-switch recombination

DNA replication

Activation/Termination of transcription

Chromatin structure

Genome instability
«unscheduled R-loops»

Mutagenesis/Recombination

Replication stress

DNA breaks

Cancer and Neurodegeneration

Antineoplastic therapies

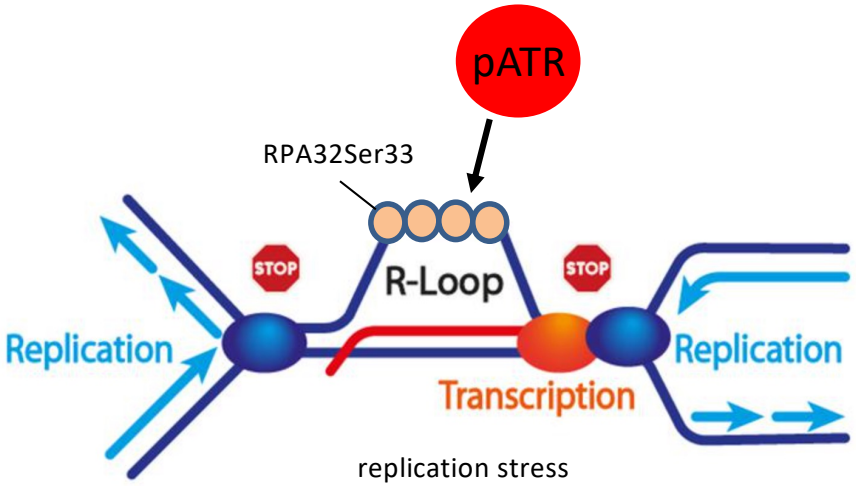
Stoy et al. 2022; Methods in Molecular Biology
Modified from: Uruci et al. 2021; International Journal of Moleculr Sciences

Role of R-loops in controlling genome stability in cancer cells

R-loops are connected to tumorsuppressors, oncogenes and therapy

Replication/Repair factors
BRCA1, BRCA2
FANCA, I, D2, L, M
ATAD

Helicases/Translocases
DDX1, 5, 9, 19, 21, 23
BLM, WRN, RTEL,
FANCM, SMARCL1
SETX, ACQ,



Topoisomerase
TOP1, TOP2A

DNA damage kinases
ATR, ATM

Nucleases
RNaseH1, RNaseH2
CtIP, XPG, FEN1
XRN2, XPF

Chromatin Modifiers
SWI/SNF, INO80
mSin3, SIRT7, EZH2,
ATRX, DAXX

RNA processing factors
U2AF1, SRSF2
THO/TREX, SFPQ
ASF/SRSF1, SF3B1

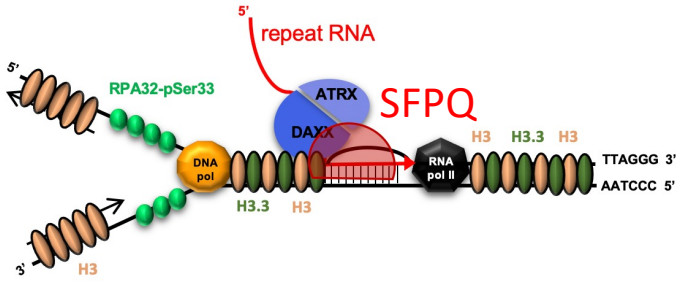
Termination factors
XRN2, SETX
MYCN

Other oncogenes that drive R-loops
Cyclin E, H-RAS, p53, MDM2,
Ring1B/RNF ESR1

Neoplastic agents that drive R-loops:
Campothecin →Topoisomerase
Etoposide → Toposiomerase,
ATR inhibitors
Crosslinking agents

1. MECHANISMS OF R-LOOP RESOLUTION IN CANCER CELLS

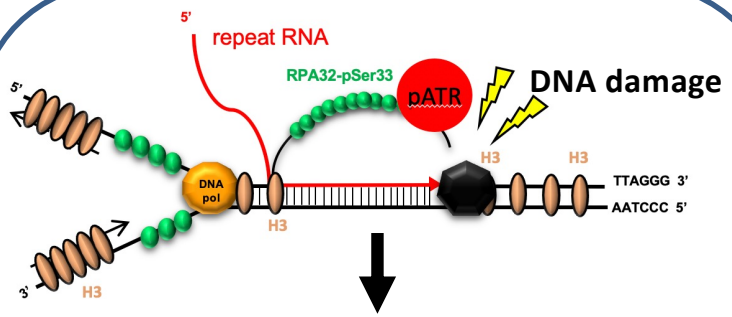
SFPQ stabilizes repetitive DNA by targeting R-loops and recruiting DAXX dependent H.3. histone chaperon activity



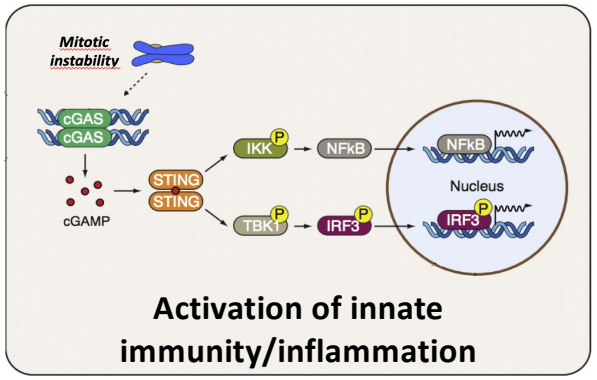
Stabilization of repeats exhibiting R-loop structures

DAXX: H3.3 histone chaperon activity

ATRX: DNA helicase activity



Instability of repetitive elements



Activation of innate immunity/inflammation

Modified from: Zierhut and Funabiki, 2020

Trends in Cell Biology

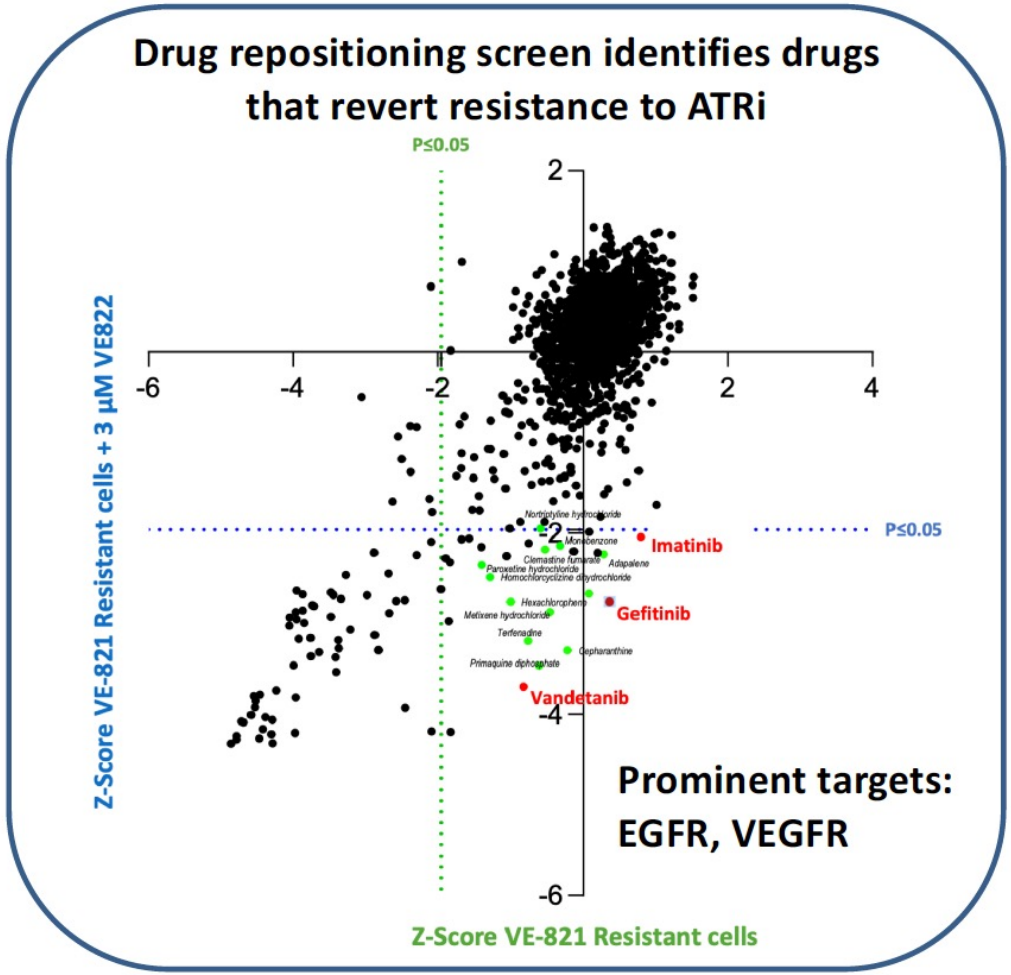
TME

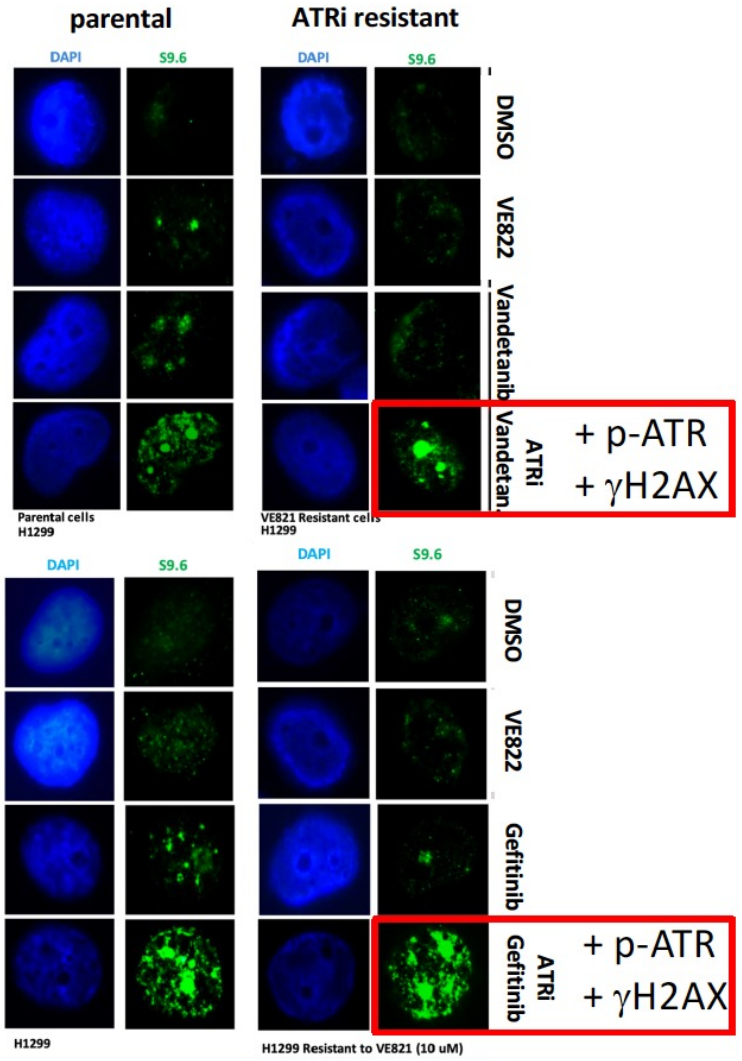
Tumor Suppression?

Tumor Promotion?

MASTER STUDENT POSITION AVAILABLE

2. *RELEVANCE OF RNA:DNA HYBRID MANAGEMENT FOR THERAPY EFFICIENCY AND THERAPY RESISTANCE*





2. *RELEVANCE OF RNA:DNA HYBRID MANAGEMENT FOR THERAPY EFFICIENCY AND THERAPY RESISTANCE*

FUTURE OUTLOOK:

- **DRIP seq under repositioning conditions to map R-loop landscape in resensitizised cells**
- **Identify RNAs that engage in R-loop formation to drive damage**
- **Characterization of singalling pathways that connect Vandetanib/Gefitinib to R-loop resolution machineries**
- **Investigate impact on pathways that imact on the TME: main target cGAS/STING**

Nature 2022

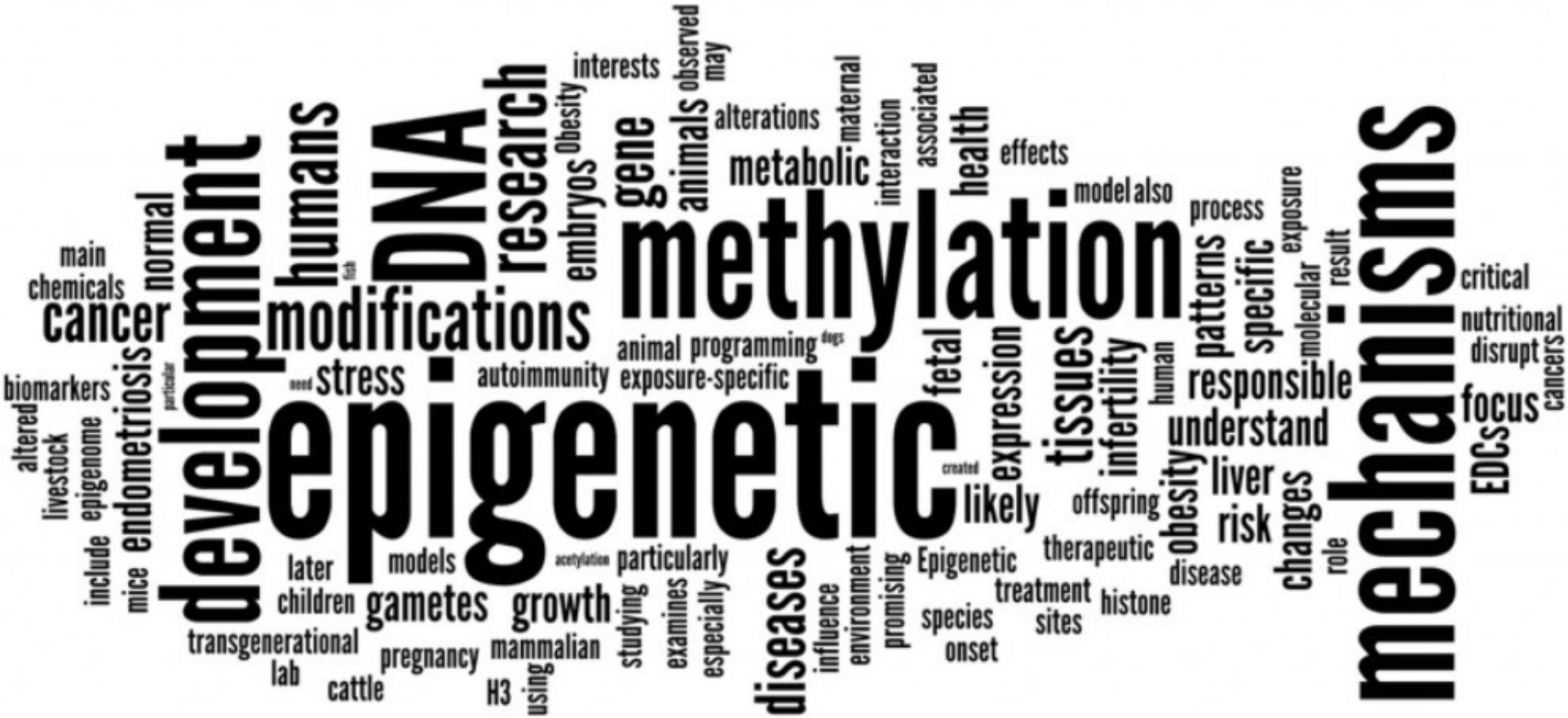
Article

R-loop-derived cytoplasmic RNA–DNA hybrids activate an immune response

https://doi.org/10.1038/s41586-022-05545-9
Received: 30 June 2021
Accepted: 8 November 2022

Magdalena P. Crossley[1,8], Chenlin Song[1,8], Michael J. Bocek[1], Jun-Hyuk Choi[1,6,7], Joseph Kousorous[1], Ataya Sathirachinda[1], Cindy Lin[2,3,4], Joshua R. Brickner[1], Gongshi Bai[1], Hannes Lans[5], Wim Vermeulen[5], Monther Abu-Remaileh[2,3,4] & Karlene A. Cimprich[1]✉

EPIGENETICA
CON LABORATORIO



General things

Commissione didattica – Genomica Funzionale

Prof. Stefan SCHOEFTNER
Coordinatore
- *Regolamento didattico, offerta didattica e supervisione programmi degli insegnamenti, budget didattica, SMA, SUA, pratiche studenti, orientamento, piani di studio (referente), vademecum per gli studenti (in concerto con rappresentanti)*

Prof.ssa Roberta BULLA
Vice coordinatore, segretario CD
- *passaggi da altre università,* ***referente tirocinio, orientamento***

Prof. Guidalberto MANFIOLETTI
- ***coordinatore doppio diploma****;* ***referente per l'internazionalizzazione***

Prof. Francesco NAPOLETANO
- *ammissioni, organizzazione lauree, raccolta laboratori per tirocini***Prof. Francesco NAPOLETANO**

Rappresentanti studenti
Chiara PERUGINI
Alessio PUNTIN
Paola TREVISIOL

Andrea CELANT
Eric DUGAN

Tutor Genomica Funzionale
Juri BERETTA: tutorgf@units.it
- Riferimento studenti per pratiche "inizio" e "fine" internato, convenzione con istituzione ospitante (tirocinio)
- Supporto Internazionalizzazione (doppio diploma, international week)

INTERAZIONE CON didattica.dsv@units.it, COMMISSIONE DIDATTICA e TUTOR GF
Esclusivamente: e-mail: @units.it; oggetto chiaro, corso di laurea, testo chiaro

General things

Pratiche studenti – approvazine in Consiglio di Corso di Studio

ATTENZIONE: Deadline consegna pratiche: giorno 20 di ogni mese

Approvazione in CdCdS: prima settimana di ogni mese (eccetto: gennaio, agosto)

ELEZIONE RAPPRESENTANTI – 3° o 4° settimana di marzo

Lecture and Laboratory Schedule

Lecture: 6CFU
Laboratory: 1CFU

Monday:
13:00 – 15:00 Aula I, Ed. C1

Tuesday:
11:00 – 13:00 Aula I, Ed. C1

Thursday:
15:00 – 17:00 Aula A, Ed. A

	lunedì	martedì	mercoledì	giovedì	venerdì
08:00-09:00	SPERIMENTALI E BIOINFORMATICI Silvano Piazza Aula 1_A Edificio Q		PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula 1C Edificio H3	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula L Edificio C1	
09:00-10:00	SPERIMENTALI E BIOINFORMATICI Silvano Piazza Aula 1_A Edificio Q	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula I Edificio C1	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula 1C Edificio H3	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula L Edificio C1	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula A Edificio A
10:00-11:00	SPERIMENTALI E BIOINFORMATICI Silvano Piazza Aula 1_A Edificio Q	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula I Edificio C1	RIGENERAZIONE TISSUTALE - MOD. BIOL. MOLECOLARE CHIARA COLLESI Aula 1_A Edificio Q	RIGENERAZIONE TISSUTALE - MOD. ISTOLOGIA GIOVANNI SORRENTINO Aula A Edificio A	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula A Edificio A
11:00-12:00	RIGENERAZIONE TISSUTALE - MOD. BIOL. MOLECOLARE CHIARA COLLESI Aula Z Edificio G	EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula I Edificio C1	RIGENERAZIONE TISSUTALE - MOD. BIOL. MOLECOLARE CHIARA COLLESI Aula 1_A Edificio Q	RIGENERAZIONE TISSUTALE - MOD. ISTOLOGIA GIOVANNI SORRENTINO Aula A Edificio A	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula A Edificio A
12:00-13:00		EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula I Edificio C1	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula I Edificio C1	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula A Edificio A	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula A Edificio A
13:00-14:00	EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula I Edificio C1			BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula A Edificio A	
14:00-15:00	EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula I Edificio C1	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula 1C Edificio H3	COMUNICAZIONE SCIENTIFICA IN LINGUA INGLESE DONATO RAMANI Aula 5B Edificio H2bis		
15:00-16:00	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula F Edificio C1	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula 1C Edificio H3	COMUNICAZIONE SCIENTIFICA IN LINGUA INGLESE DONATO RAMANI Aula 5B Edificio H2bis	EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula A Edificio A	
16:00-17:00	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula F Edificio C1	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula 1C Edificio H3	COMUNICAZIONE SCIENTIFICA IN LINGUA INGLESE DONATO RAMANI Aula 5B Edificio H2bis	EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula A Edificio A	
17:00-18:00		BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula 1C Edificio H3			

Start of lectures:
03.03.2025

End of lectures:
30.04.2025

Laboratory
05.05.2024 → 30.05.2025
(2 Turni)

Student Representatives:
1. **Eric Dugan**
2. **Andrea Celant**

Interaction:
Institutional e-mail: @units.it

Lecture and Laboratory Schedule

Lecture: 6CFU
Laboratory: 1CFU

Monday:
13:00 – 15:00 Aula I, Ed. C1

Tuesday:
11:00 – 13:00 Aula I, Ed. C1

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	lunedì	martedì	mercoledì	giovedì	venerdì
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12:00-13:00		EPIGENETICA CON LABORATORIOLezione Stefan SCHOEFTNER, Francesca BORTOLOTTI Aula I Edificio C1	TECNOLOGIE MOLECOLARI E CELLULARI DANIELE SBLATTERO Aula I Edificio C1	BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula A Edificio A	PROTEOMICA CON LABORATORIOLezione RICCARDO SGARRA Aula A Edificio A
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17:00-18:00		BIOLOGIA DEL CANCRO CON LABORATORIOLezione FIAMMA MANTOVANI Aula 1C Edificio H3			

Tight program:

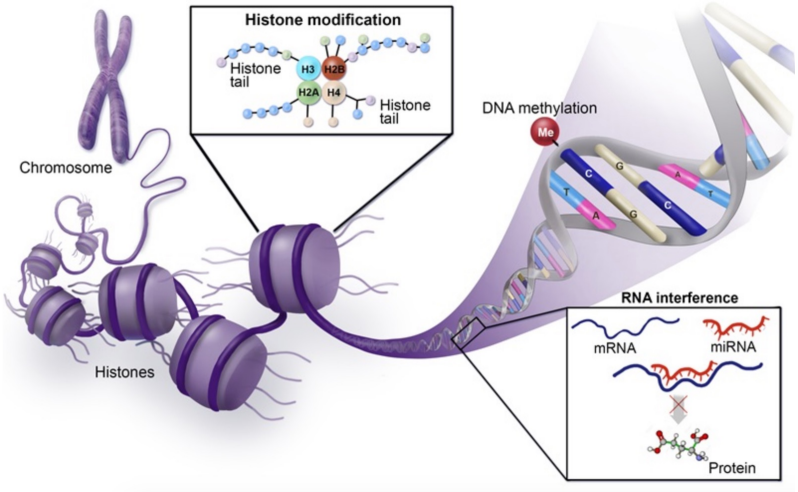
Occasional exchange of lecture slots between
- Epignetica con Laboratorio
- Proteomica con Laboratorio
- Biologia del Cancro con Laboratorio

lezione 1	17 Marzo
lezione 2	28 Marzo
lezione 3	31 Marzo
lezione 4	11 Aprile
lezione 5	14 Aprile
lezione 6	28 Aprile
lezione 7	12 Maggio
lezione 8	26 Maggio

Occasional use of Tuesday slot Tuesday 17:00 – 18:00

Lecture and Laboratory Schedule

Lectures (6CFU)



Mechanisms of epigenetic gene regulation and impact on genome stability, gene expression controls, development and disease

Laboratory course (1CFU)

Joint laboratory course (3CFU, 2 Turni), 03.05 – 30.05.2024

- PROTEOMICA CON LABORATORIO (Prof. Riccardo Sgarra; 1CFU)
- BIOLOGIA DEL CANCRO CON LABORATORIO (Prof.ssa Fiamma Mantovani; 1 CFU)
- EPIGENETICA CON LABORATORIO (Prof.ssa Francesca Bortolotti, 1 CFU)

Goal of laboratory analysis: 10 Days

Goal of laboratory analysis: Complete experiment and data analysis related to the induction of genome instability by altering epigentic regulation

- Knock-down of epigenetic regulator
- Analysis of knock-down efficacy by western blotting and statistical data analysis
- Immunoflorescence experiments on R-loop abundance and induction of DNA damage
- Determination of cell viability
- Analysis if genome instability on metaphase chromosome spreads

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.

2. LABORATORY EXERCISE

Evaluation code of laboratory reports: As maximum, one additional point(increments: 0.0, 0.25, 0.5, 0.75, 1.0 points) will be assigned according tothe quality of laboratory reports prepared by individual students.

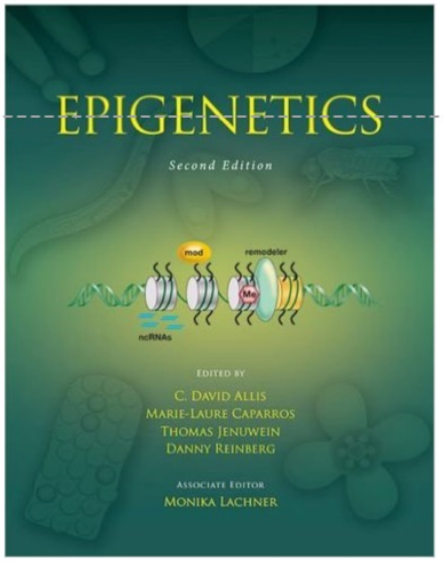
Students are asked to contact the teacher if not possible to attend the entire the laboratory program. Depending on the individual situation, the lack of laboratory attendance can be compensated by additional questions during the written exam.

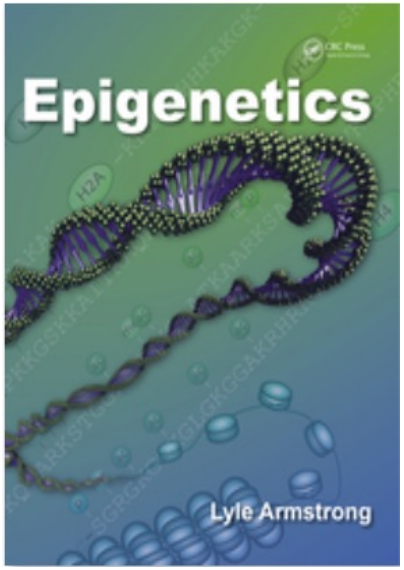
3. EXAMPLE FOR THE CALULATION OF THE "VOTO FINALE"

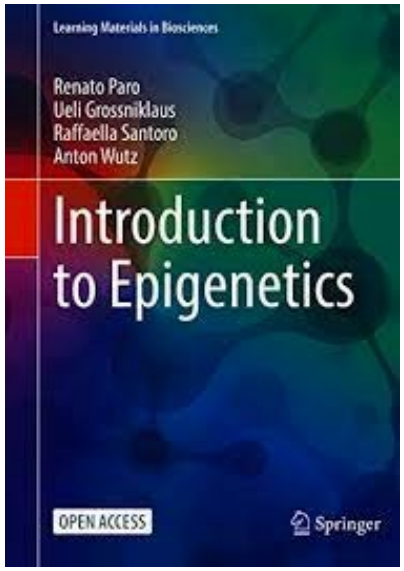
Result written exam: 29/31
Lab-reports: 1 point

29/31*30=28,0645
28,0645 + 1 = 29

Materiale didattico AA2024-2025







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

R. Paro et al., Springer; 2021
Introduction to Epigenetics, Learning Materials in Biosciences
https://doi.org/10.1007/978-3-030-68670-3_1

MSTeams:

Book Chapters
PPT slides
Publications
Recording of lectures
Teamcode:
twl5fby

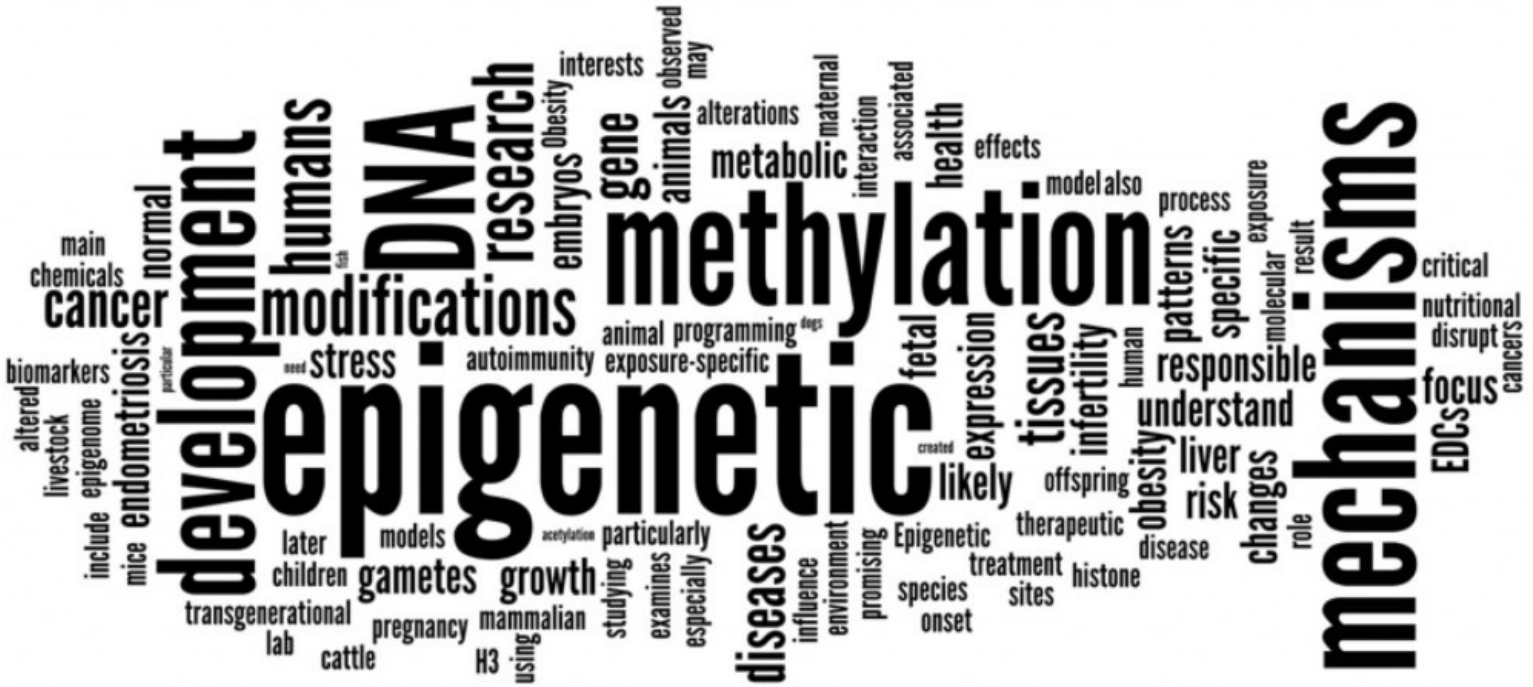
WHAT IS YOUR EXPECTION ON THE LECTURE….

WHAT DO YOU THINK YOU SHOULD LEARN….

Form groups, discuss 5-7 minutes, individuate 2-3 of your goals

Choose speaker of group

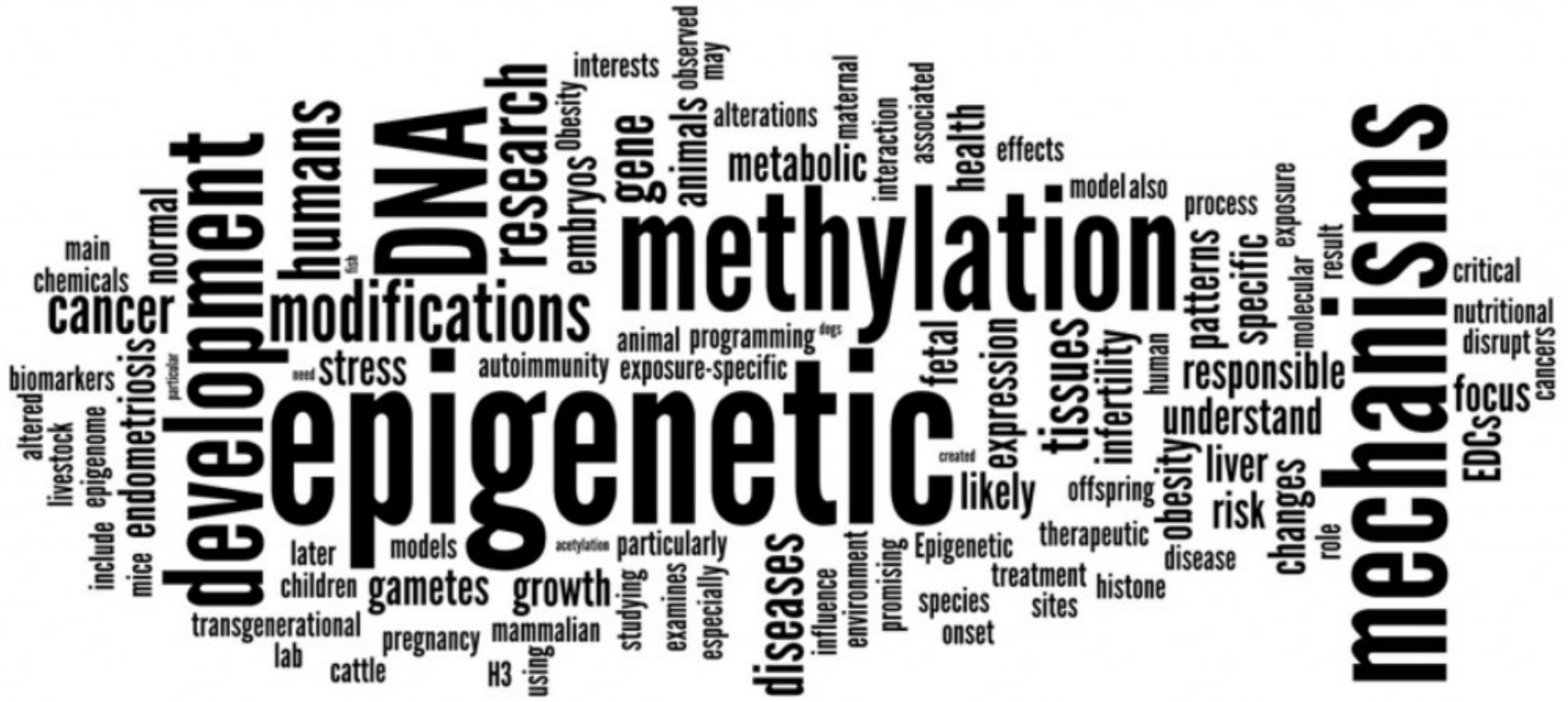
Present result



WHAT IS YOUR EXPECTION ON THE LECTURE….

WHAT DO YOU THINK YOU SHOULD LEARN….

MS TEAM CODE: 129v6qv



PROGETTO PRO-BENE-COMUNE

PROmozione del BENEssere della COMunità UNivErsitaria

Un progetto finanziato dal MUR nell'ambito dell'avviso «PRO-BEN», con l'obiettivo di promuovere **e diffondere il benessere psicofisico**, contrastare i **fenomeni di disagio psicologico ed emotivo** e prevenire le dipendenze patologiche della **popolazione studentesca universitaria e nelle AFAM.**

PRO BENE COMUNE

Ministero dell'Università e della Ricerca

PRO-BEN

UNIVERSITÀ DEGLI STUDI DI TRIESTE

survey multicentrica sulla salute psicofisica

25 min

non è obbligatoria;

1. potrà essere fatta sia da chi non ha mai compilato in precedenza la survey del progetto, sia da chi l'ha fatto l'anno scorso (cosa buona per avere dati longitudinali),

2. **nel rispetto delle norme sulla privacy i dati saranno pseudonimizzati (quindi si può rassicurare tutt* sul fatto che non riusciremo a risalire ai loro nominativi)**,

Inquadra il QR-code e partecipa alla nostra indagine!

...in alternativa, utilizza questo link:
https://dsvunits.qualtrics.com/jfe/form/SV_3JVFIafYxOgCUwC

Partecipando, ci aiuterai a individuare percorsi, pratiche e strumenti utili a promuovere i fattori che favoriscono il benessere psicofisico e a contrastare quelli che contribuiscono al disagio psicologico ed emotivo.

Non importa se è la prima volta che compili questa survey o se ne hai già completata una simile in precedenza: in entrambi i casi, ci aiuterai a comprendere lo stato di benessere della popolazione studentesca universitaria e come questo cambia durante il percorso accademico.

PER AGGIORNAMENTI

SULLE INIZIATIVE IN CORSO

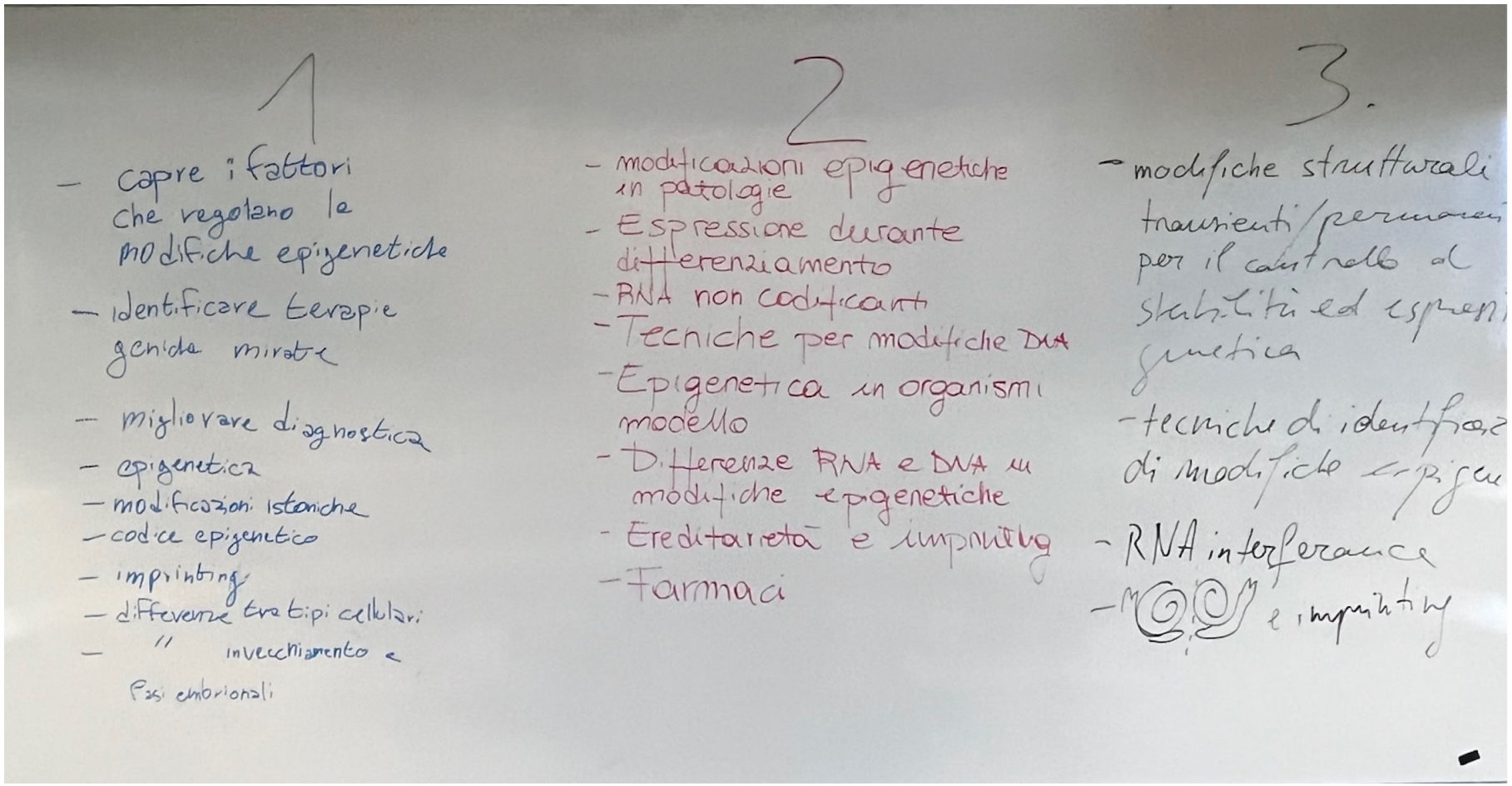
DEL PROGETTO **PRO-BENE-COMUNE 2.0:**

Corso Moodle
PRO-BENE-COMUNE 25-26

Canale Whatsapp
Progetto UNITS

WHAT IS YOUR EXPECTION ON THE LECTURE….

WHAT DO YOU THINK YOU SHOULD LEARN….



Lecture Program AA2023-2024

Goal of the lecture:

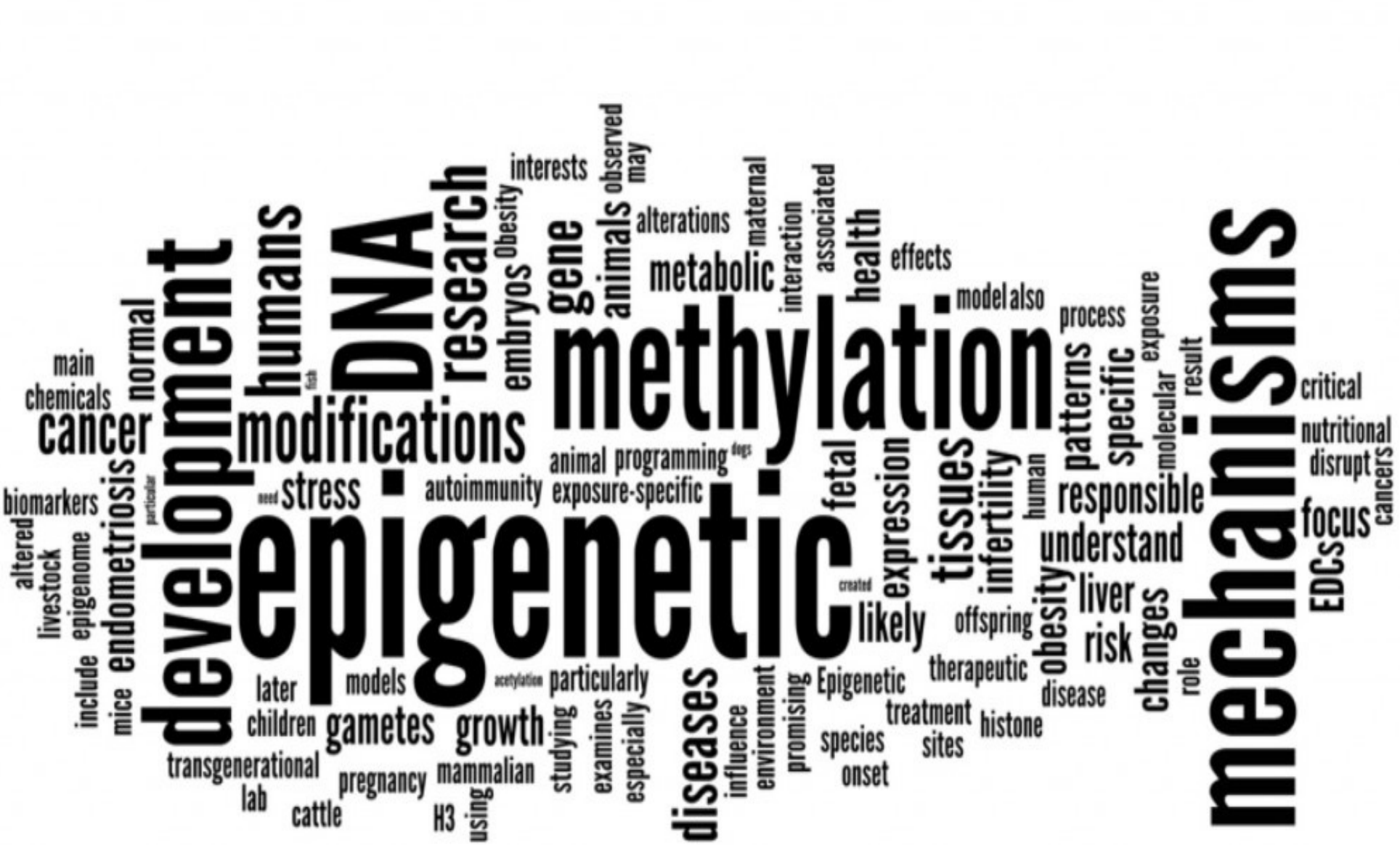
- **Overview on aspectes of epigenetics involving protein and RNA factors**
- **Detailed knowledge of selected epigentic regulatory pathays**
- **Integration of epigentic processes in development and disease**
- **Capacity to understand and interpret experimental data in scientific publications**
- **Capacity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

(for details check syllabus):

Lecture Program AA2025-2026

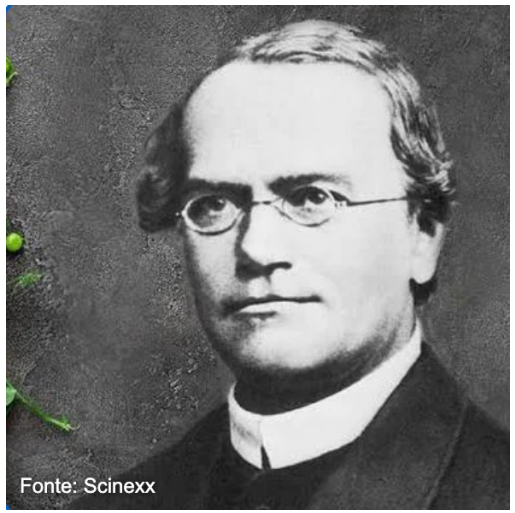
1. Introduction in epigentics and groudbreaking discoveries
2. Chromatin organization in Pro- and Eukaryotes
3. Histone acetylation and Deacetylation
4. Histone and DNA methylation
6. Structural and functional coordination of DNA and histone mehtylation
7. Maintenance of Epigenetic Information
8. Histone Variants in Cell Physiology
9. RNAi and Heterochromatin assembly
10. Position Effect Variegation, heterochromatin formation and gene silencing in Drosophila
11. Polycomb and Trithorax group proteins
12. Epigenetics in Human Disease

EPIGENETICS

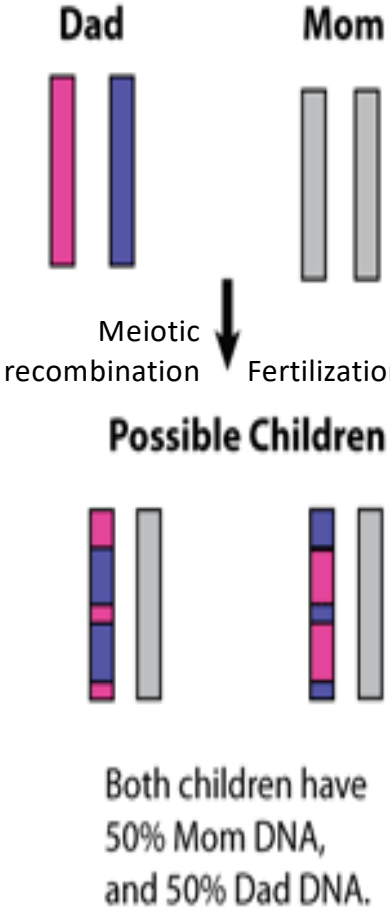


GENETICS ← → EPIGENETICS

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



GENETICS: DNA based



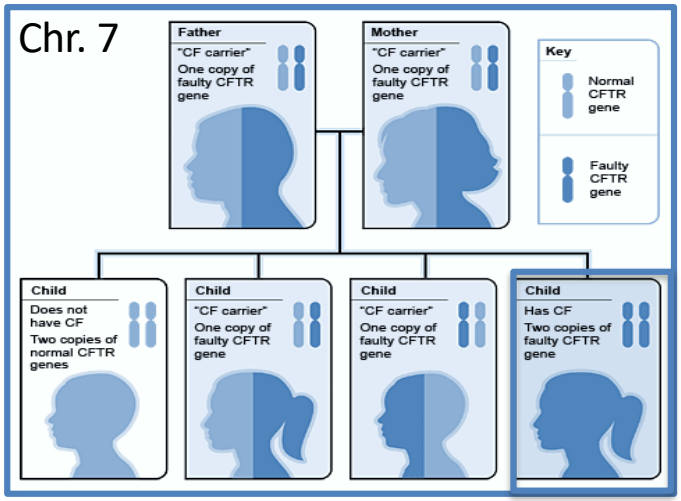
No discrimination: Mom is also doing meiotic recombination.

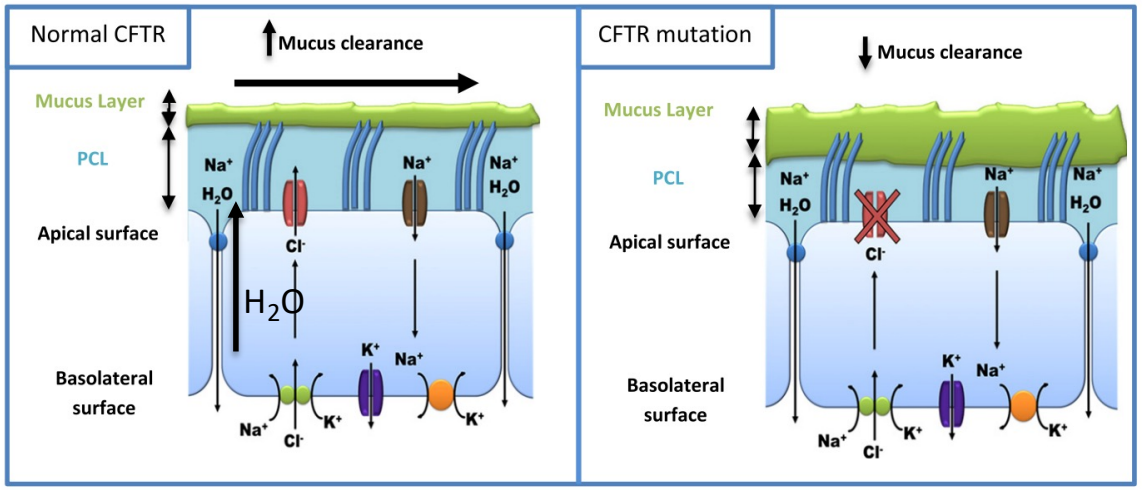
Genetic inheritance

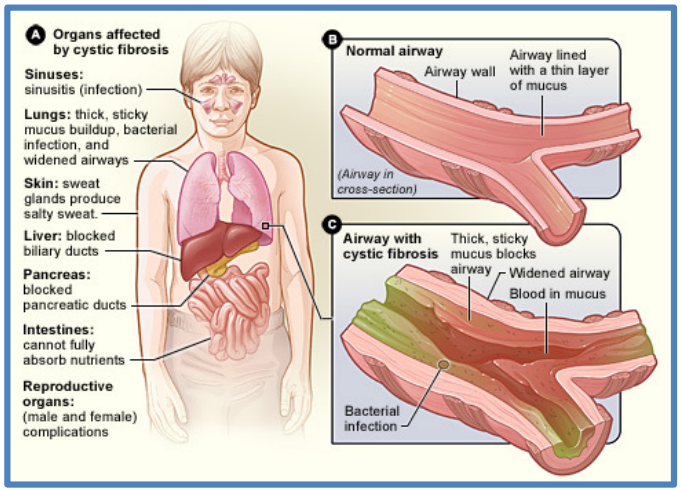
GENETICS ← → EPIGENTICS

Genetic inheritance in the context of diseaseof Cystic fibrosis

Autosomal Recessive CTCF mutation is inherited







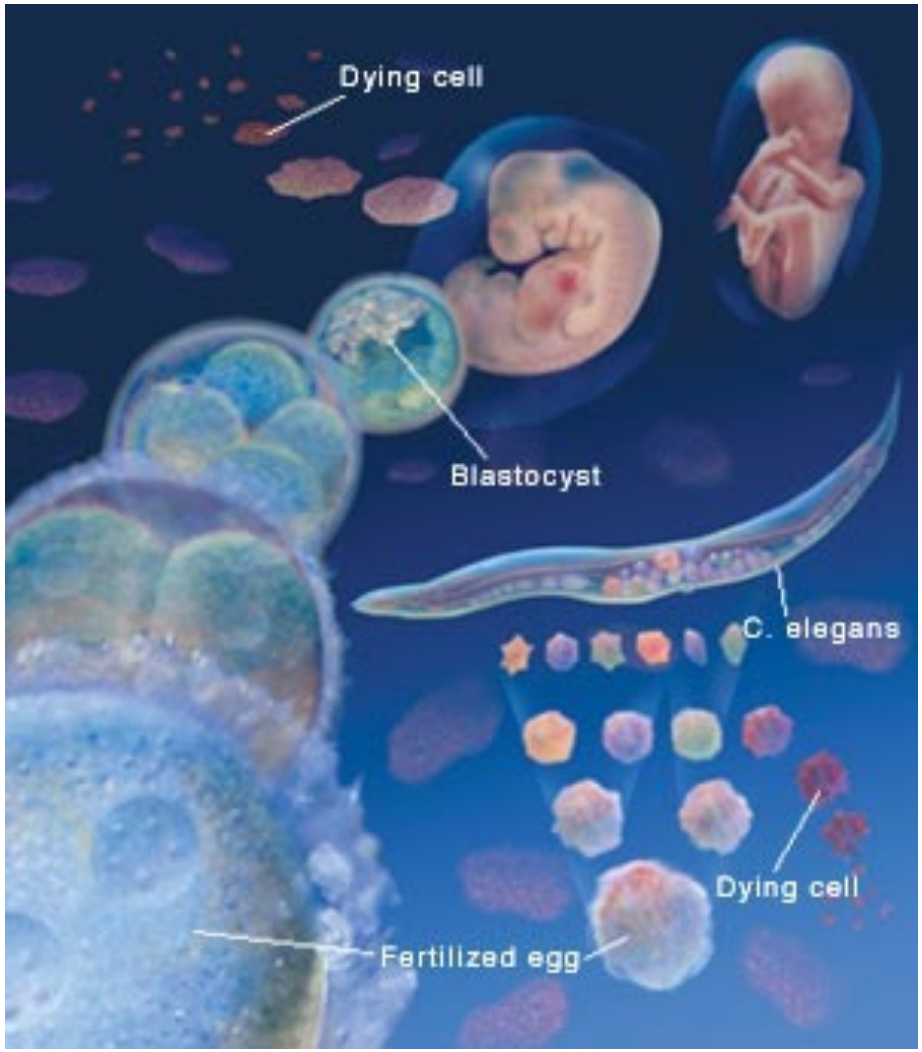
The mutation alters the secondary and tertiary structure of the protein, so that chloride channels fail to open in response to elevated cAMP in epithelial cells. Defective expression, trafficking or function of CFTR leads to impaired secretion of chloride and an increase in sodium absorption. This causes depletion of the airway surface liquid and, in turn, to defective mucociliary action and reduced mucus clearance. This encourages bacterial colonisation, recurrent infections, chronic inflammation and irreversible damage to the airway epithelium

However: phenotype limited to selected tissue

CFTR gene in other tissue types not relevant

GENETICS ← → EPIGENETICS

What is Epigenetics?….a scientific term in evolution



KEY OBSERVATION IN BIOLOGY:

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
However: different tissues are formed….

KEY QUESTION IN EARLY 1900:

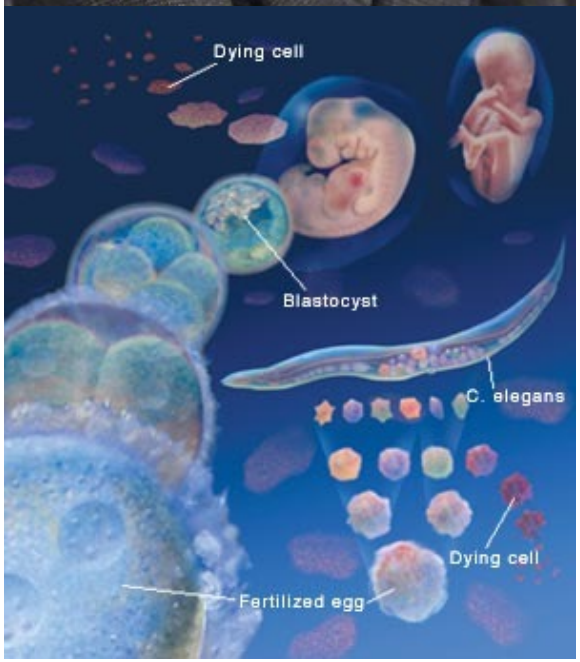
What molecules within the chromosomes carry the genetic information?

How do they direct the developmental program?

How is the information transmitted and maintained during cell division?

GENETICS ← → EPIGENTICS

Conrad Hall Waddington:
British embryologist; 1950



What is Epigenetics?….a scientific term in evolution

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

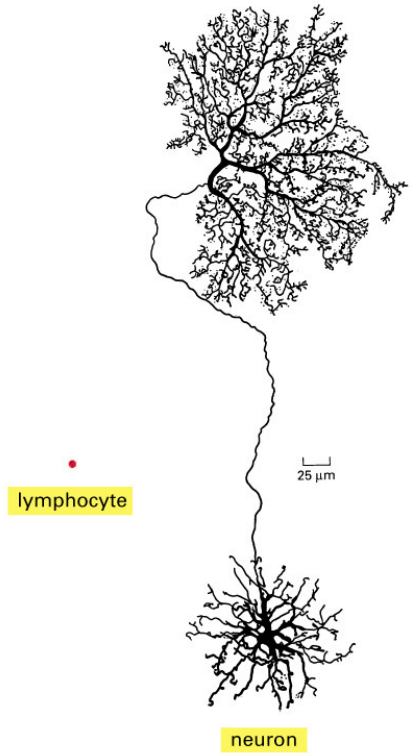
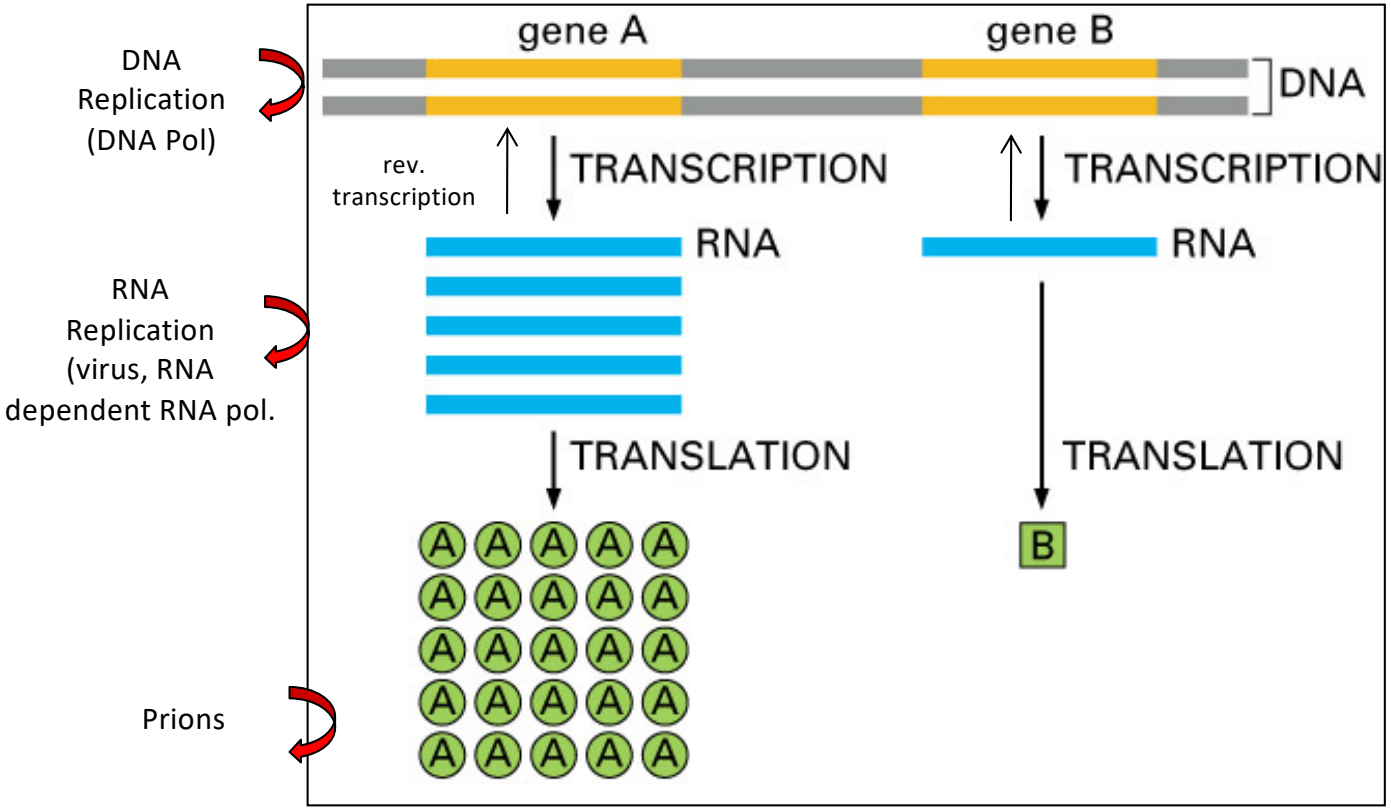


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

Epigentics is compatible with 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.



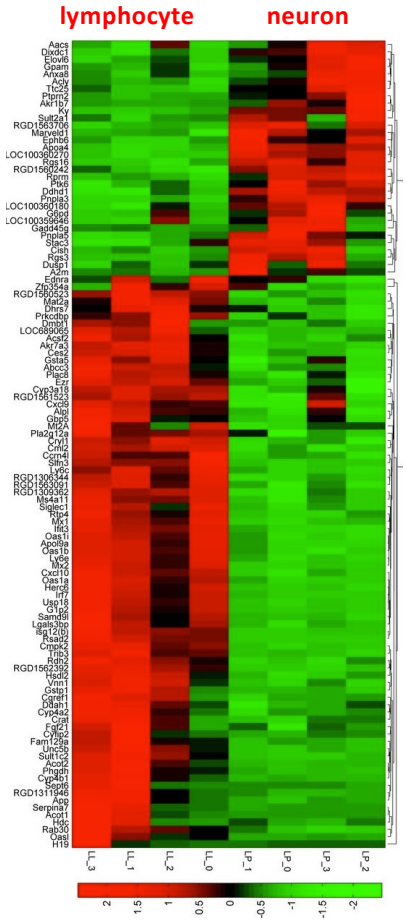
Gene expression control for cell specification and development

GENOME
coding and
non-coding genes

1 Genome →

>200 different cell types

Specific gene expression programs



Cell function

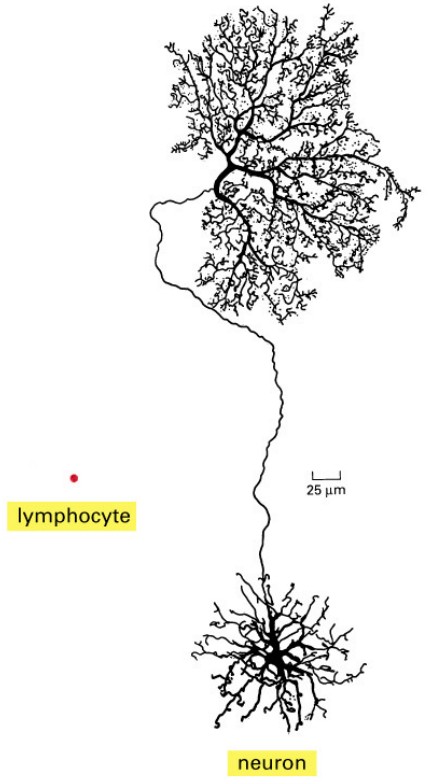


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

Genetic information is used in a highly controlled manner

Epigentics is compatible with the central dogma

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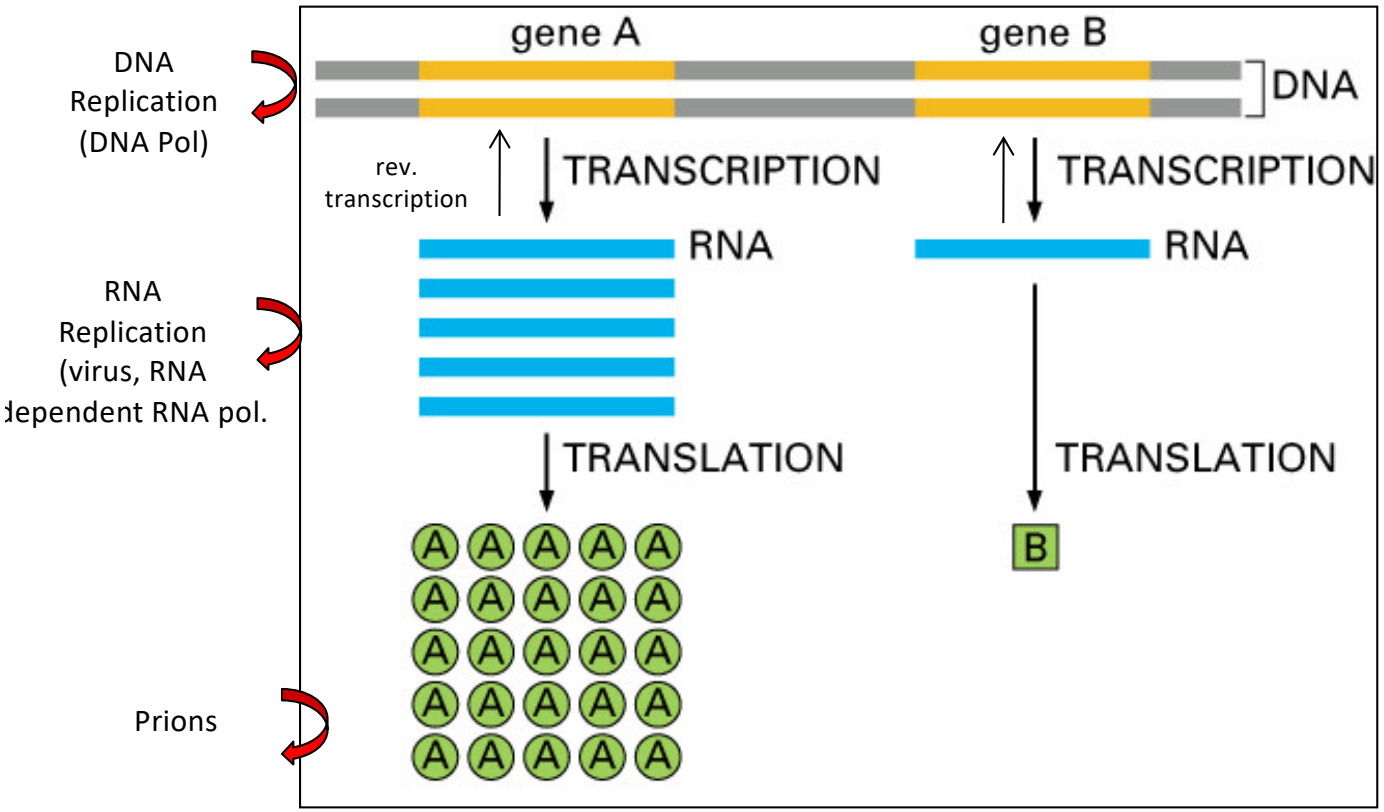


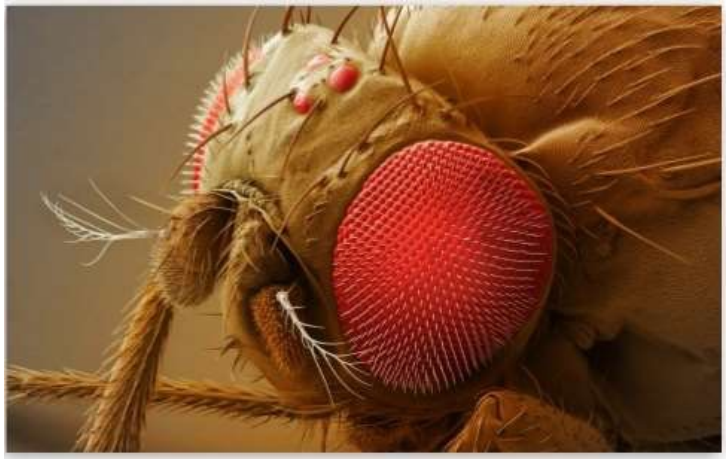
Epigenetics impacts on different levels of gene expression:

1. **Chromatin structure**
2. **RNA regulatory pathways (miRNAs,lncRNAs, siRNAs)**
3. **Genome stability**

Can we make observations in nature/organisms that can be used as an argument that epigenetic processes exist may function on a molecular basis???

Can we attribute phenotypes to defects in epigenetics

Observations that cannot be explained by genetics…..
The drosophila eye – a role model system for genetic/epigenetic research



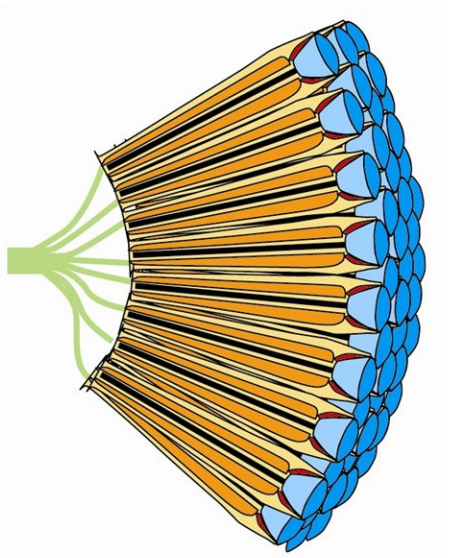
Drosophila compound **eye** is composed of 16.000 cells and contains a simple repetitive pattern of **700 to 750** of ommatidia.

Each ommatidium consists of 14 neighboring cells: 8 photoreceptor neurons in the core, 4 non-neuronal cone cells and 2 primary pigment cells.

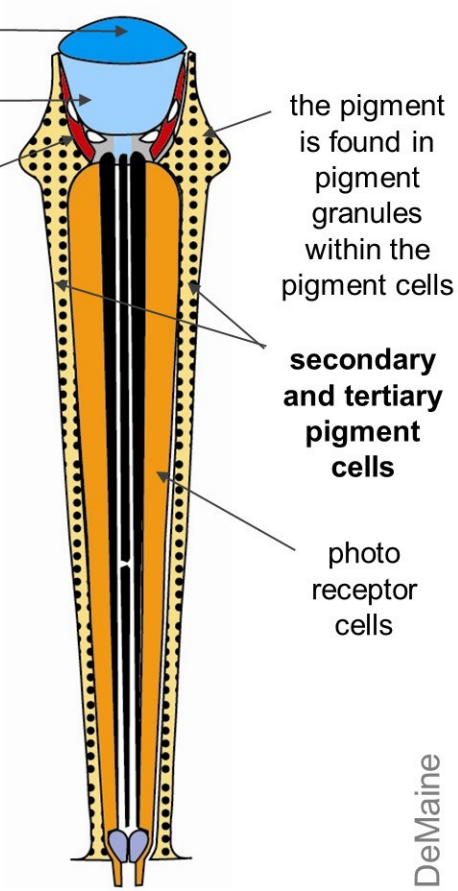
***Drosophila melanogaster* compound eye**

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

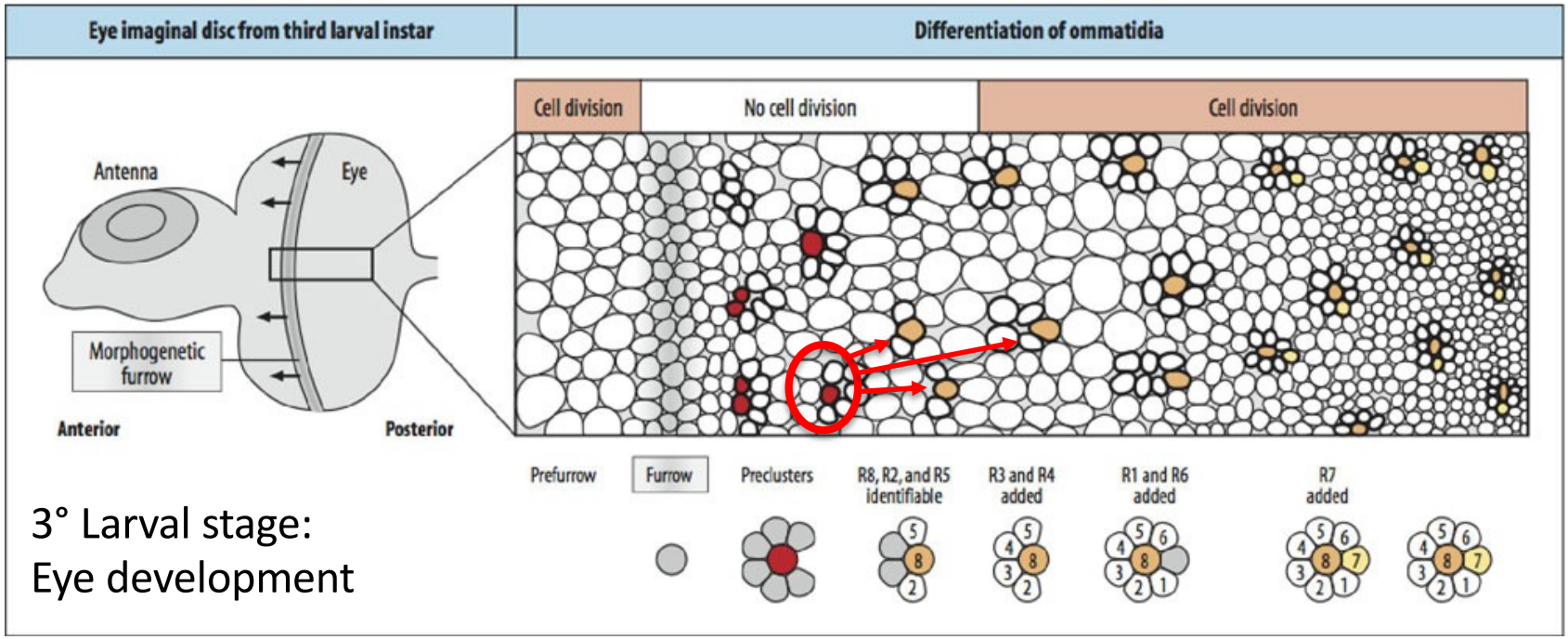


ommatidium

S. DeMaine

Observations that cannot be explained by genetics…..

Position effect variegation: Drosophila melanogaster



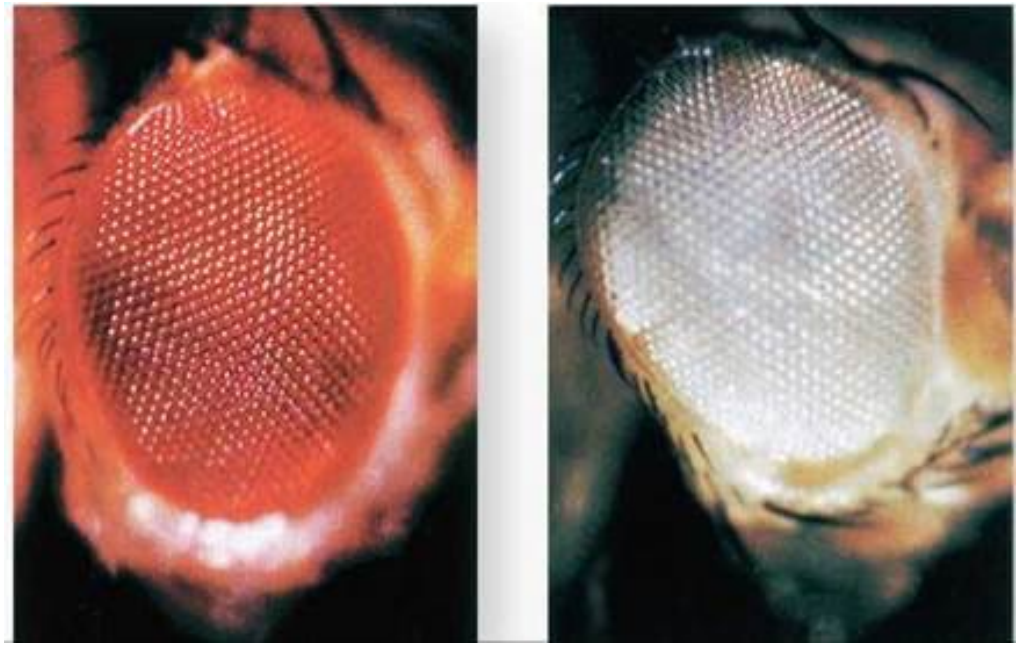
The Drosophila compound eye has 700 ommatidia (the photoreceptor organ), each which has 8 photreceptor neurons (R1-8), 4 cone cells (lens secretion) and **pigment cells**. Great model system to study a small group of cells in a tissue context.

- 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.

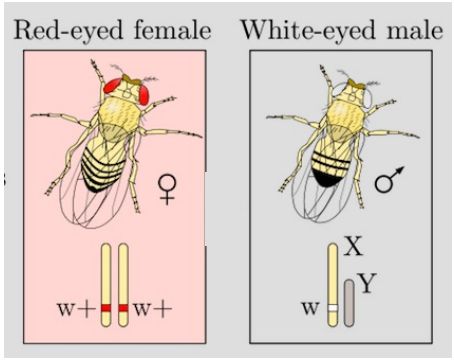
Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

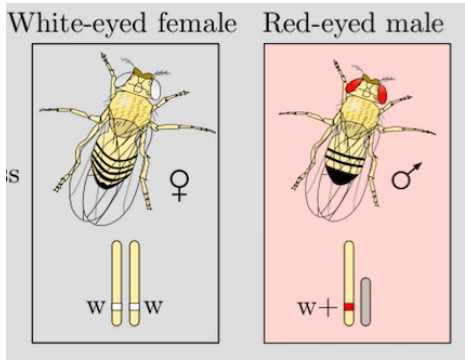
Wild-type

Genetic *white* mutation (X linked)



Thomas and Lilian Morgan 1910



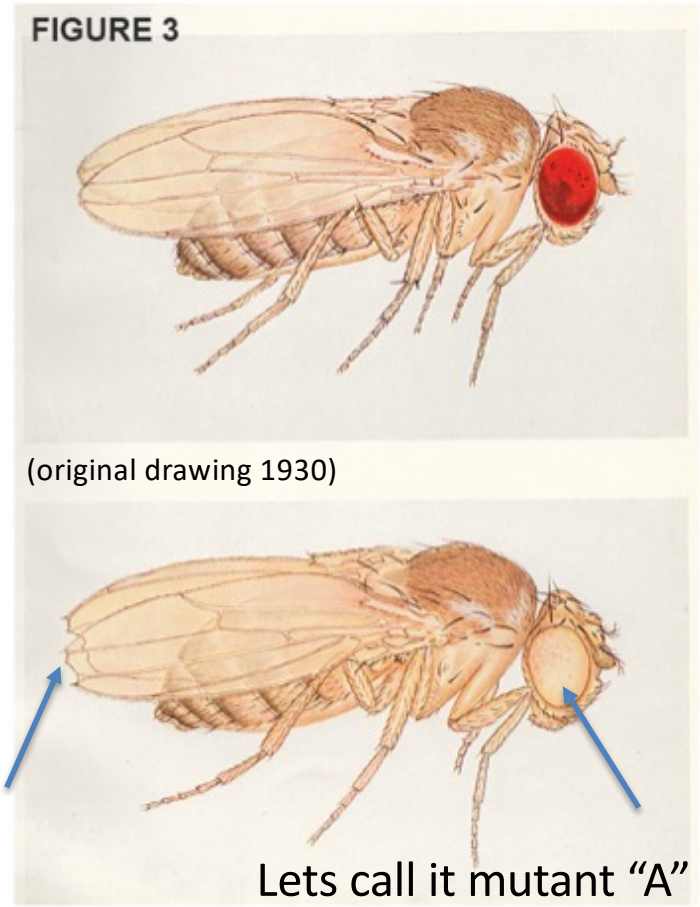


The protein coded by the **white gene (X-inked)** functions as an ATP-binding cassette (ABC) transporter. It carries the precursors of the red and brown eye color pigments, guanine and tryptophan, into the developing eyes during pupation. The white gene is located on the X chromosome

Mutation is stable over generations w^-/w^-: always white eyes

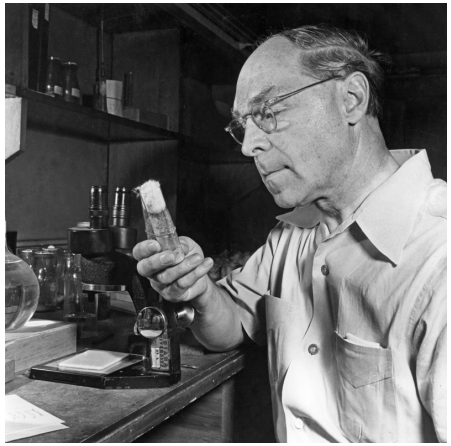
Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

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



(original drawing 1930)

Wild-type



A new **screen** identified a new white-like mutation that is combined with a wing malformation

Lets call it mutant “A”

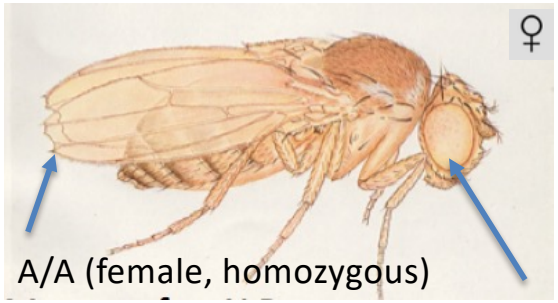
Mutant fly after X-Rays:
- X-linked mutation
- Notched wings (ali dentellate)
- White eyes (“White allele”)

→ different spectrum of phenotype compared to Thomas and Lilian Morgan)
→ Precise genomic context (on DNA level) not knwon

(Note: in the genome, the white allele is located closely to the notch allele)

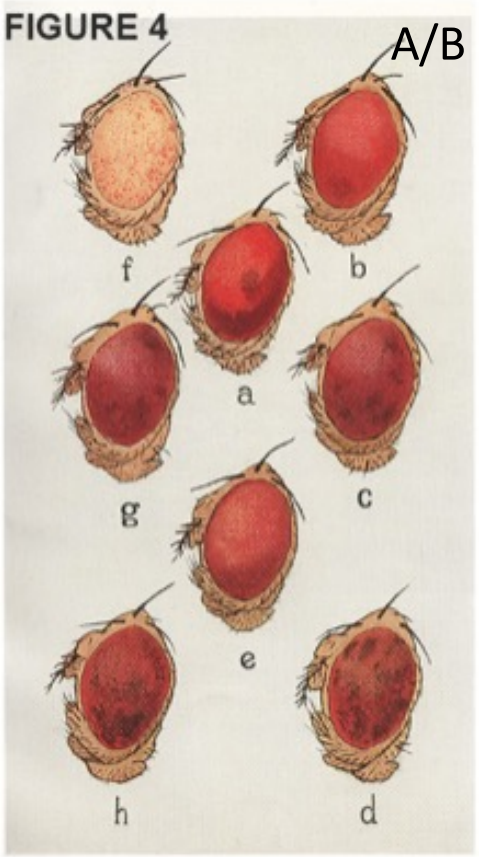
Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

New white mutation (Muller) : let' call it "mutant A"



Crossing with:

offsprings



FEMALE FLIES:
"Mottled eye phenotype" no stable phenotype

2 different white mutations:
1 allele Mutant A
1 allele Mutant B (classic null mutation)

We know that Mutant B is a classic germline mutation that gives the *white* phenotype

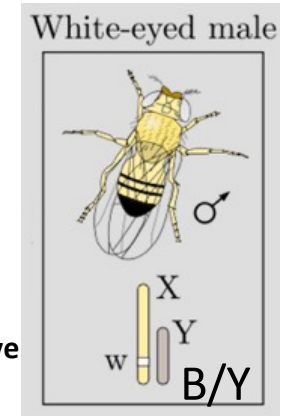
Mutated allele A is inherited but shows phenotypic variation in offsprings
(reversible phenoptype of red pigmentation)

Variegation phenotype must come from the mutant A allele

Mutant A does not correspond to a classic genetic phenotype

OTHER STRAIN CARRYING A DEFINED GENETIC MUTATION CONFERING "WHITE" EYE PHENOTYPE (*white allele;*)
CLASSIC X-linked mutation

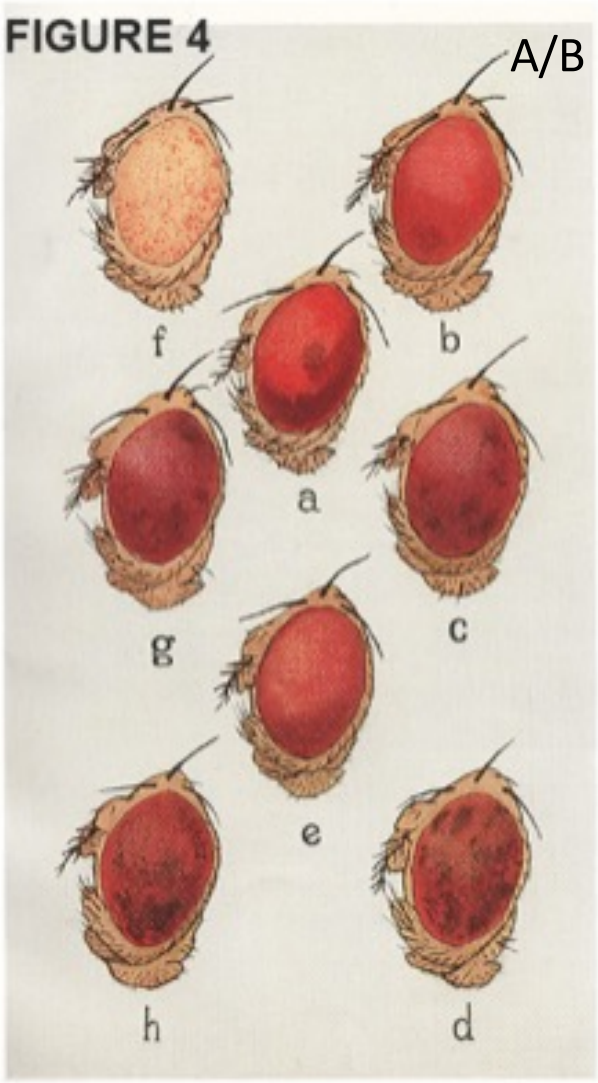
let's call it mutant "B"
Note: this mutation passes phenotype reliably in successive generations



(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…..



THINK…..

How can you explain the different pigmentation?

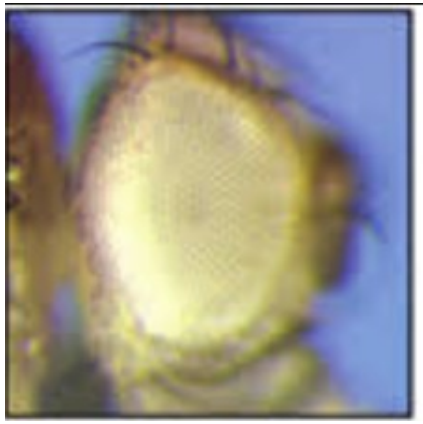
Why is the pigmentation it mottled (areas of bright or dark read)

What type of mutation can explain – mechanistically -the phenotype?

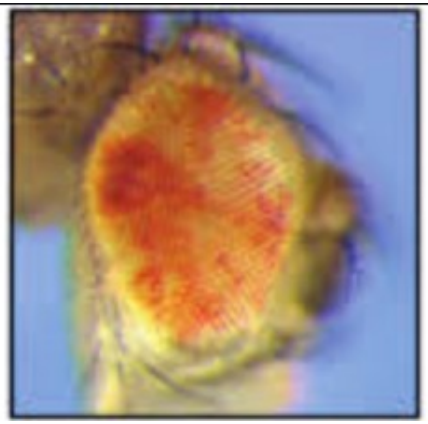
Observations that cannot be explained by genetics…..
Muller 1930

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

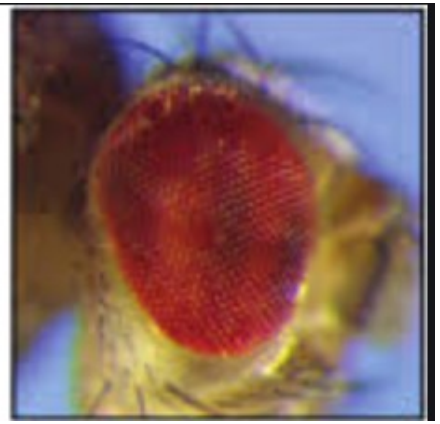
Omatides enable to observe genetic but also non-genetic events that define gene expression



***white*: mutant**



White: functional/mutant
???? What has happenedd???

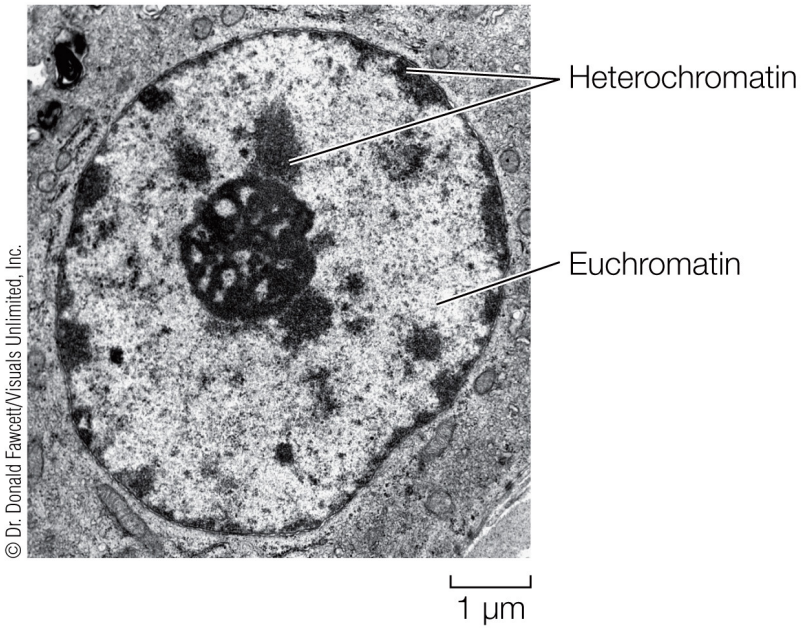


White: functional

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.

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

WHAT IS HETEROCHROMATIN??



LIFE: THE SCIENCE OF BIOLOGY 11e, In-Text Art, Ch. 16, p. 352

what is heterochromatin?

Heterochromatin is a tightly packed form of DNA found in the nucleus of eukaryotic cells. It's one of the two main types of chromatin (the other being euchromatin) and is typically more condensed and less transcriptionally active. This means that the genes located in heterochromatic regions are generally not expressed as much as those in euchromatin.

Key characteristics of heterochromatin:

- **Density:** Heterochromatin appears denser under a microscope due to its tightly packed structure.
- **Location:** It is often found at the periphery of the nucleus and in regions like the centromeres and telomeres of chromosomes.
- **Gene Expression:** Genes within heterochromatin are usually silenced or expressed at lower levels.
- **Types:** There are two main types of heterochromatin—constitutive and facultative. Constitutive heterochromatin is always in the heterochromatic state, whereas facultative heterochromatin can switch between being tightly packed and more relaxed, depending on the cell's needs.

It's an essential component of the cell's genetic material, playing roles in chromosome stability, regulation of gene expression, and protection of the genome from transposable

Message Copilot

more questions, feel free to ask!

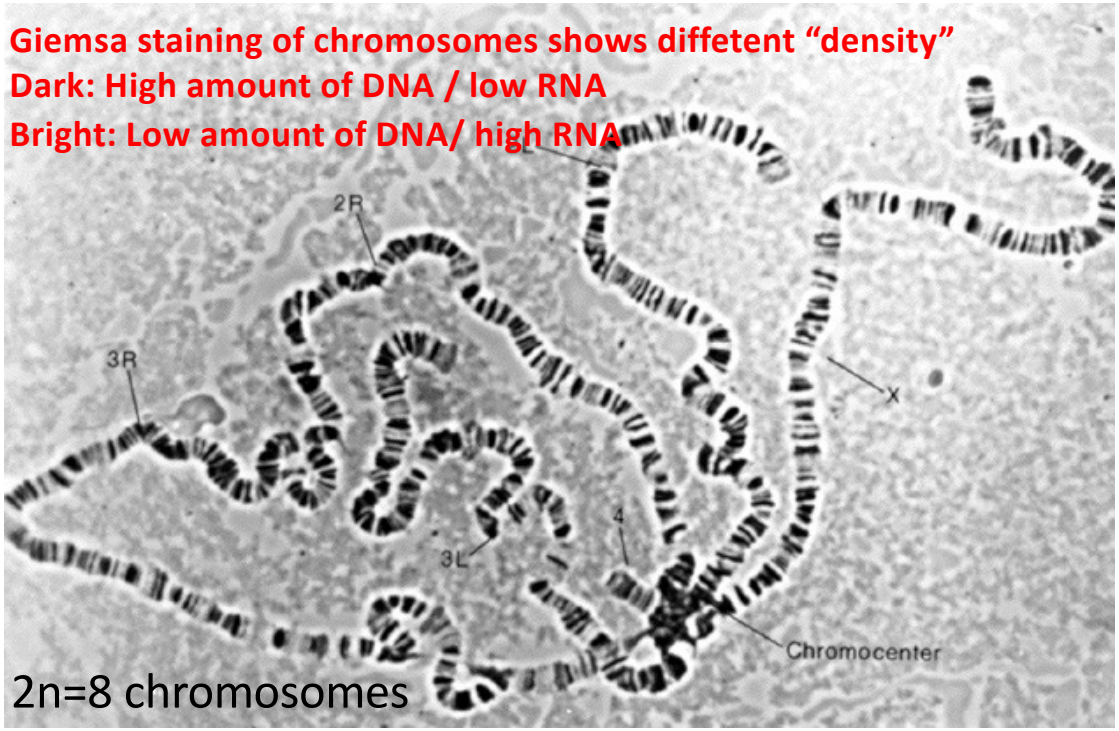
Position-effect variegation (PEV) is a variegation caused by the silencing of

a gene in **some cells** through **its abnormal juxtaposition with**

heterochromatin via rearrangement or transposition.

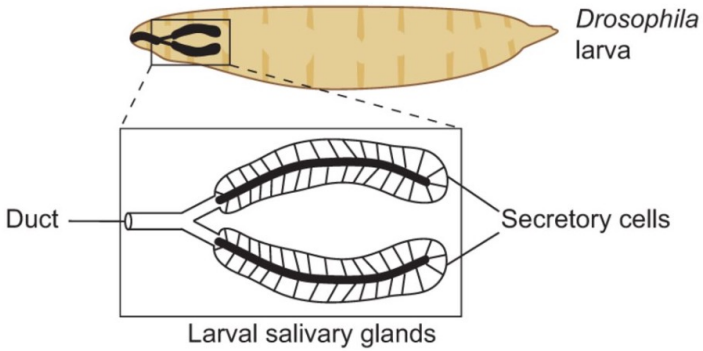
Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

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



2n=8 chromosomes

Drosophila melanogaster larvae

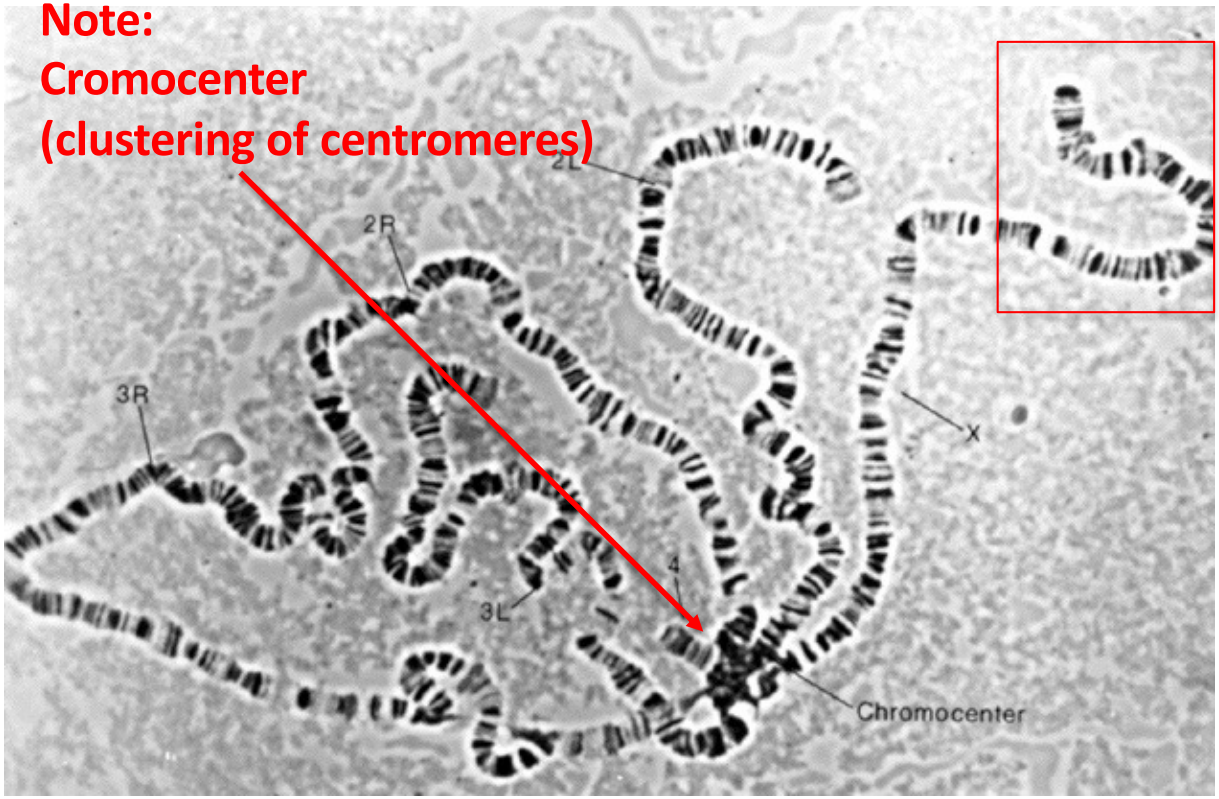


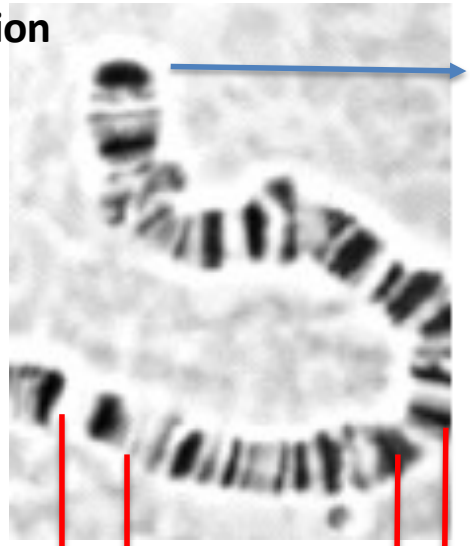
Chromosome 1 (X/Y Chromosome): These are the sex chromosomes, with males having one X and one Y, and females having two X chromosomes.
Chromosome 2 (2L and 2R): This autosome is divided into two arms, the left arm (2L) and the right arm (2R).
Chromosome 3 (3L and 3R): Similar to chromosome 2, this autosome also has two arms, the left arm (3L) and the right arm (3R).
Chromosome 4: This is the smallest autosome and is often referred to as the "dot chromosome" because it appears small and dot-like under the microscope.

Polythene chromosomes are generated by **endoreduplication**
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 aligned 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…..

Genomic DNA is organized into regions with high and low compaction





Polythen chromosomes are generated by endoreduplication

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.

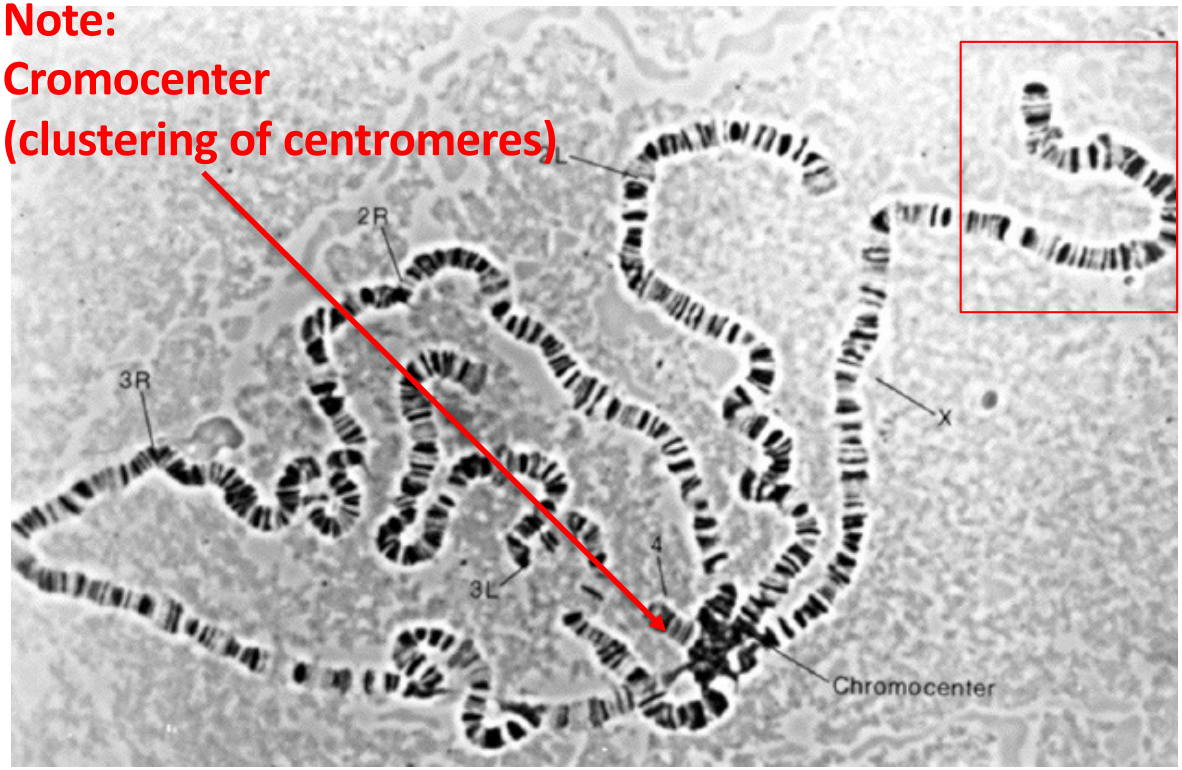
They are produced when repeated rounds of DNA replication without cell division; forms a “giant” chromosome. Thus polytene chromosomes form when multiple rounds of replication produce many sister chromatids which stay aligned together.Polytene chromosomes, at interphase, are seen to have distinct thick and thin banding patterns. These patterns were originally used to help map chromosomes, identify small chromosome mutations, and in taxonomic identification. Now they are used to study the function of genes in transcription.

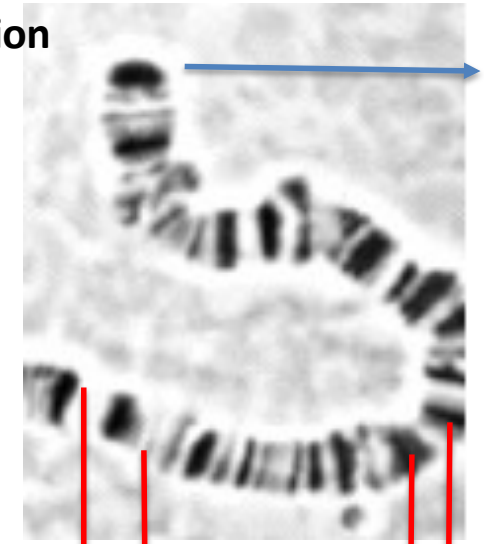
In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "salivary gland chromosomes".

Observations that cannot be explained by genetics…..

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

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

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

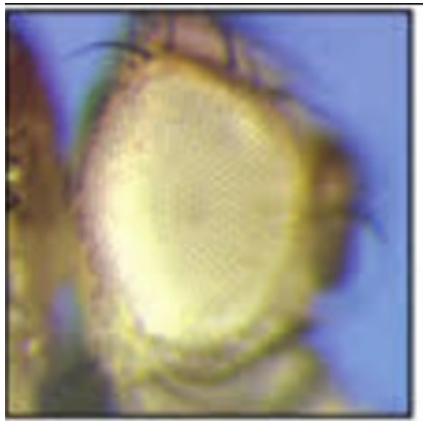
CHROMATIN = DNA + all proteins + RNAs directly or indirectly associated with DNA

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

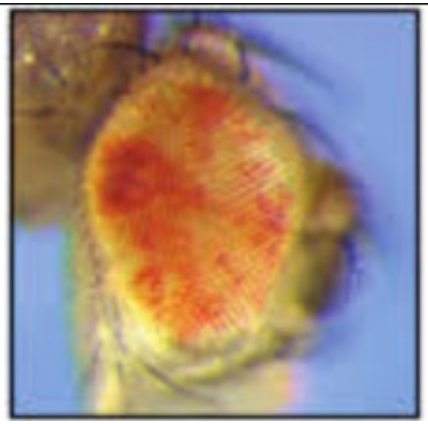
Observations that cannot be explained by genetics…..
Muller 1930

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

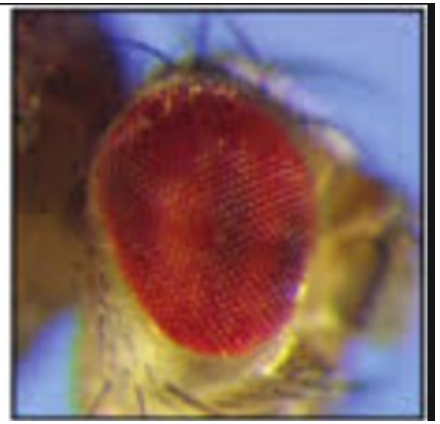
Omatides enable to observe genetic but also non-genetic events that define gene expression



***white*: mutant**



White: functional/mutant
???? What has happenedd???

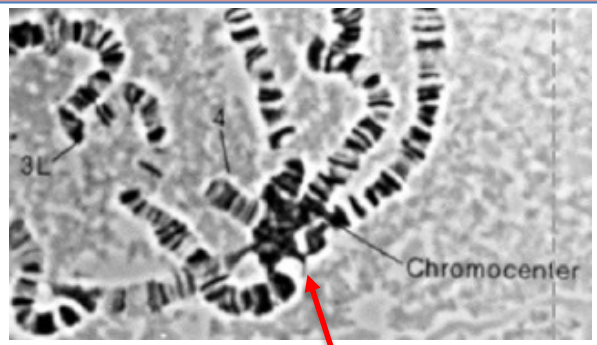


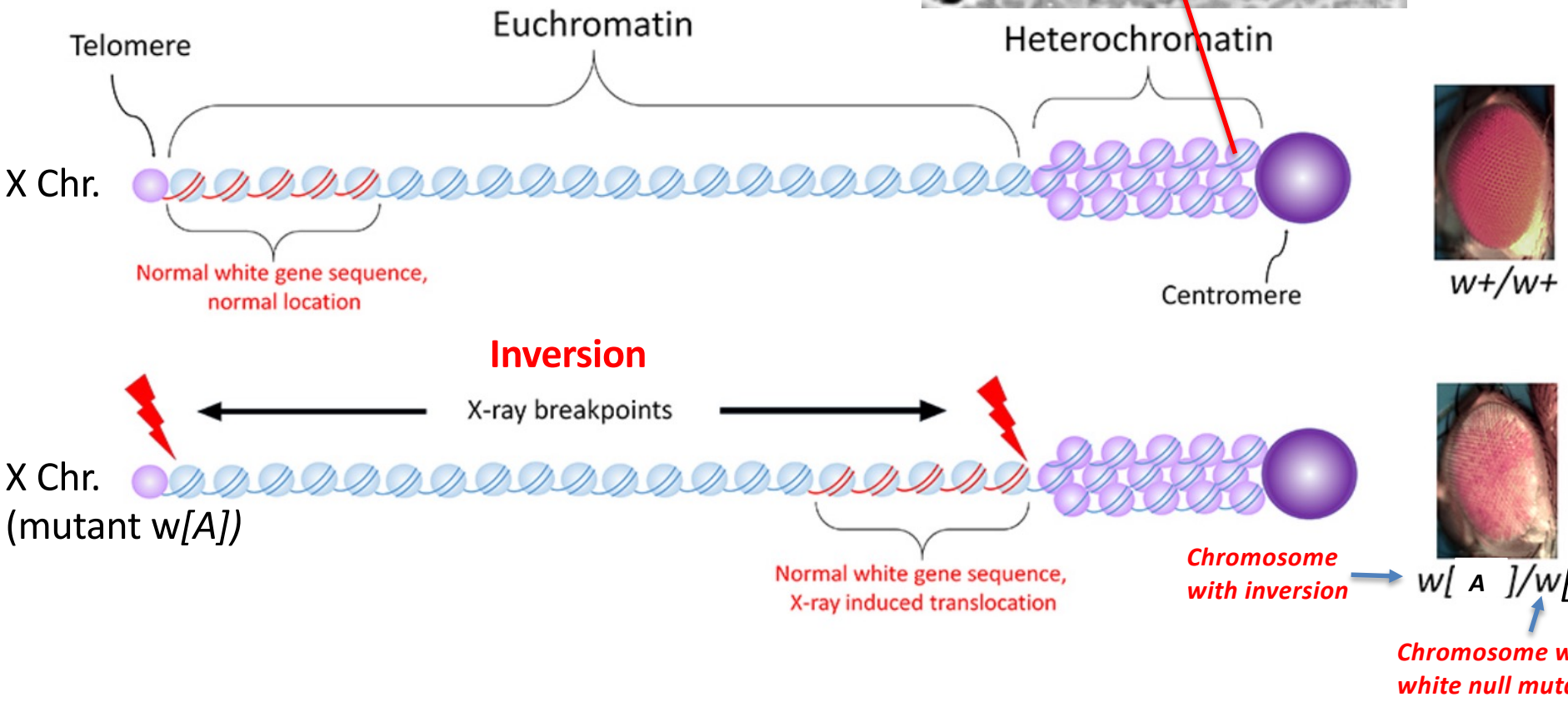
White: functional

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.

Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

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.



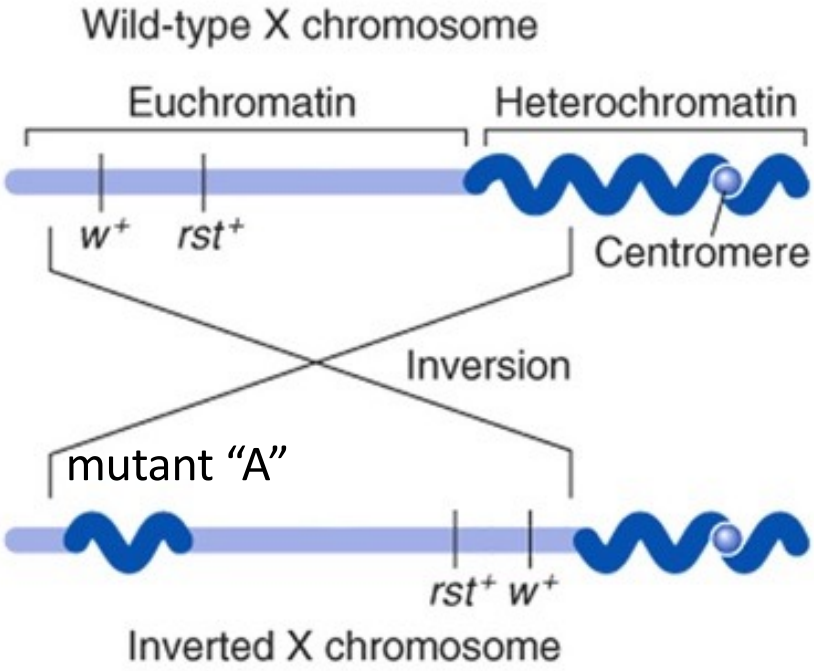


Note: Flies survived X ray treatment and show normal development → all genetic information is present

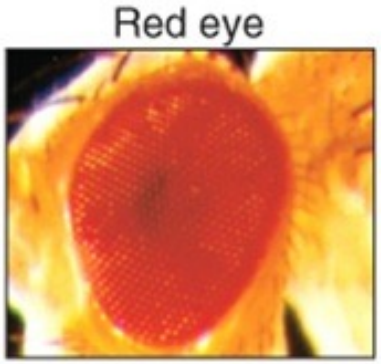
Observations that cannot be explained by genetics…..
2. The drosophila eye – a role model system for genetic/epigenetic research

Position-effect variegation

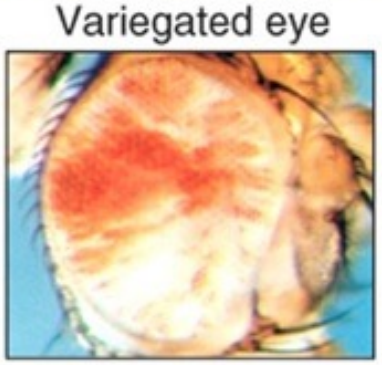
mutant "A" isolated by Muller



Insertion of white gene into compacted DNA (heterochromatin)



Normal fly:
w^+ :red pigment expressed



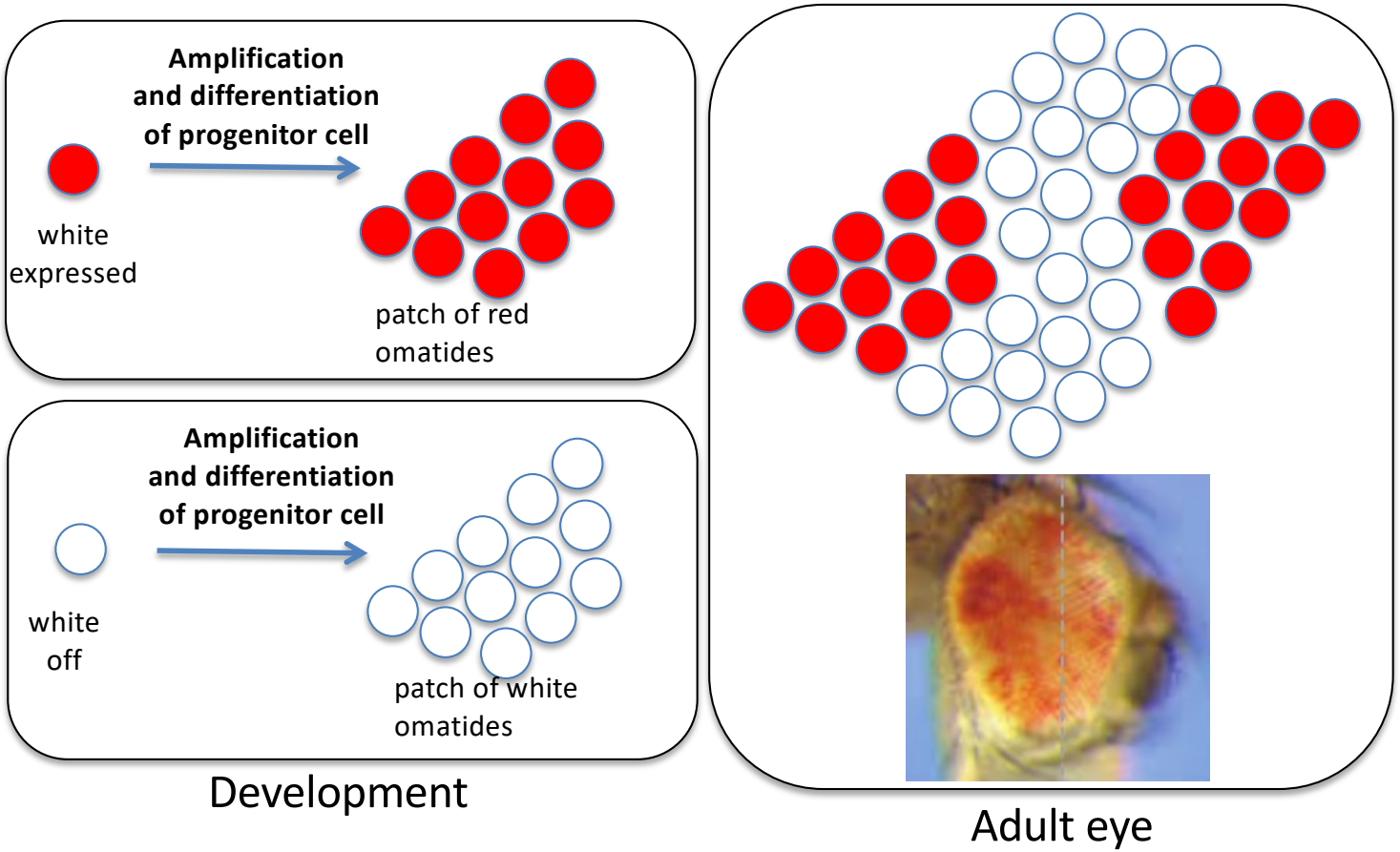
Genome rearrangement:
All genes are present but white gene
is close to the centromere
White gene is still wild type but no longer expressed in all ommatidia

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?

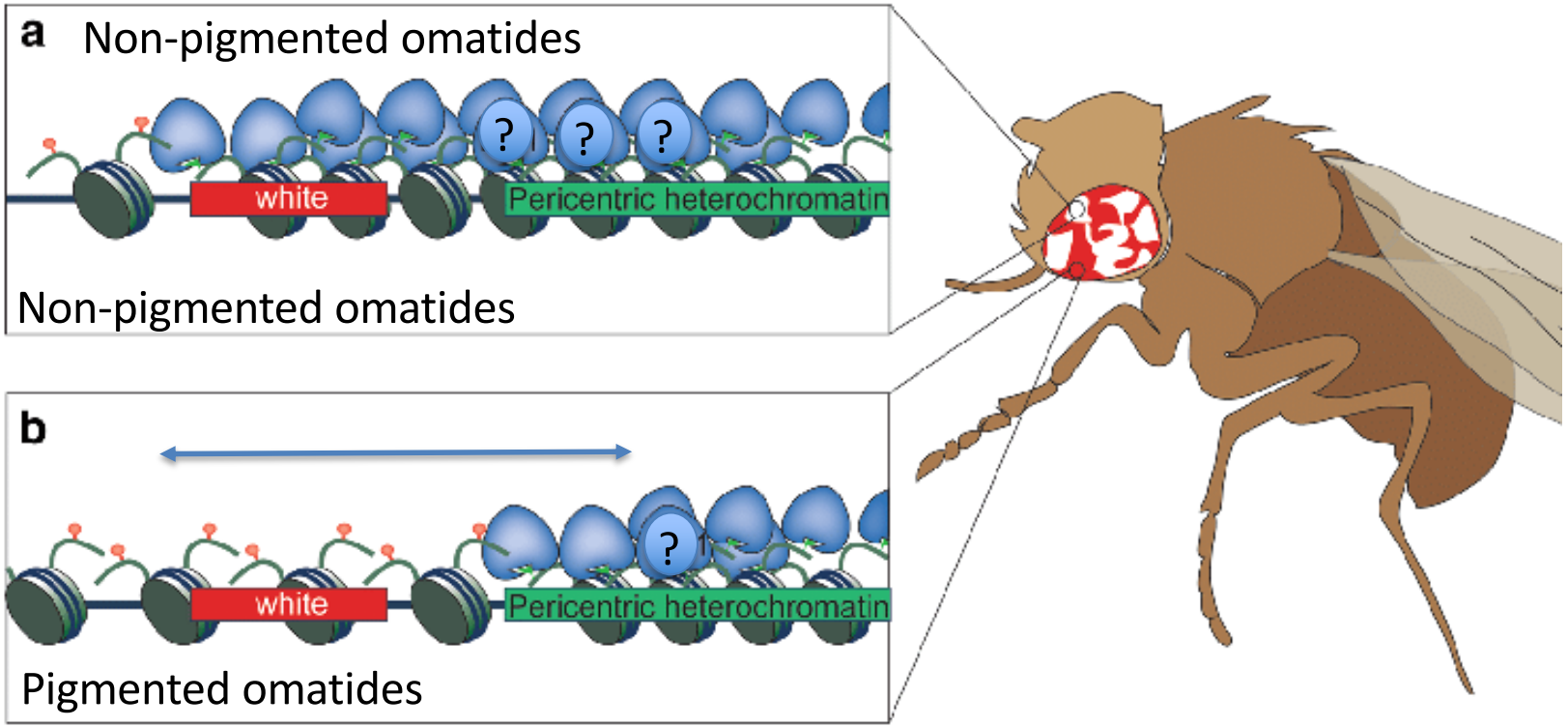
Variegated expression of white gene in progenitor cells



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

How is differential white gene expression acheived?



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

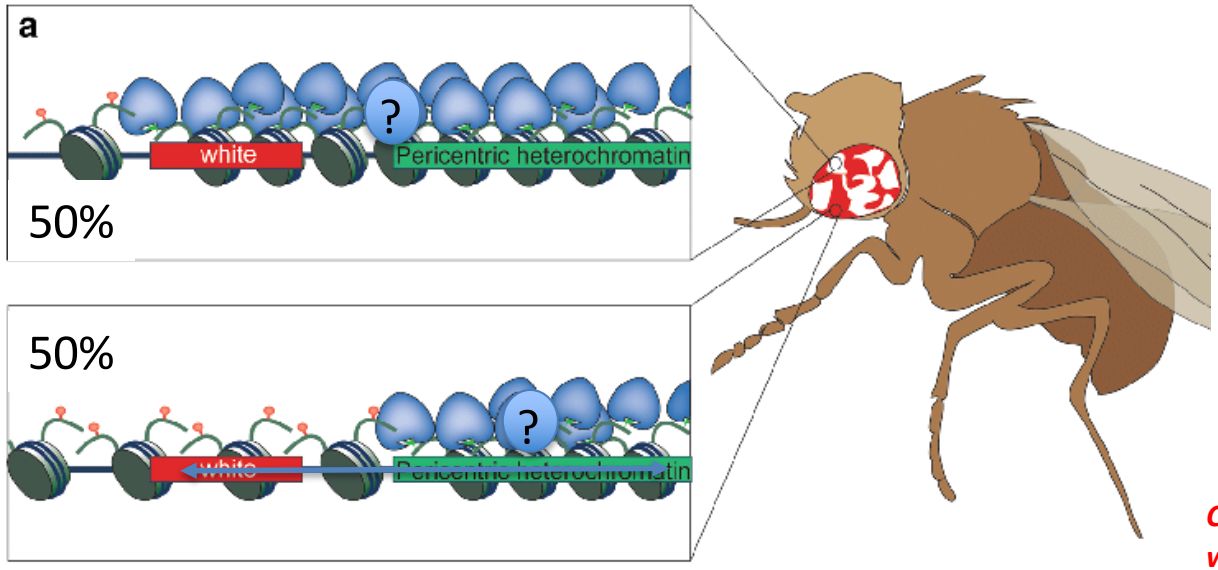
? .....factors that build heterochromatin

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

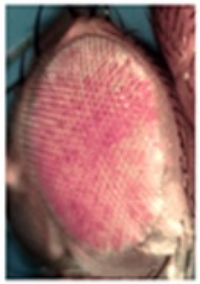
} Should act as **modifiers of PEV**

Genetic screens to identify modifiers of PEV

Non-pigmented omatides



Pigmented omatides



w[A]/w [B]

Chromosome with inversion

Chromosome with white null mutation

Perform forward genetics - Mutagenesis screens

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

Pigmented/non pigmented = 50%/50%

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

→ *Mutagenesis*

Strain X: Pigmented/non pigmented = 3/1

Strain Y: Pigmented/non pigmented = 1/5

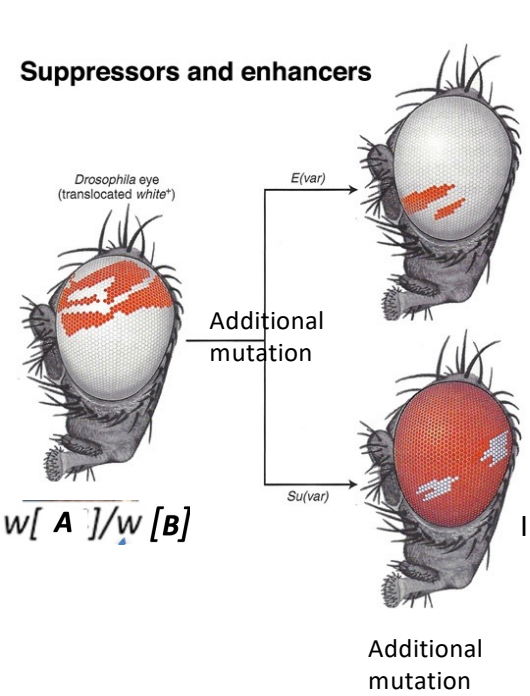
Strain Z: Pigmented/non pigmented = 1/2

Strain A: Pigmented/non pigmented = 1/1

Visual inspection of Mutant offspring strain: Check for flies that do not have partental patterning (low number of mutations)

Observations that cannot be explained by genetics…..

Mutations in certain genes can suppress or enhance PEV: example: HDAC1, HDAC3 mutations

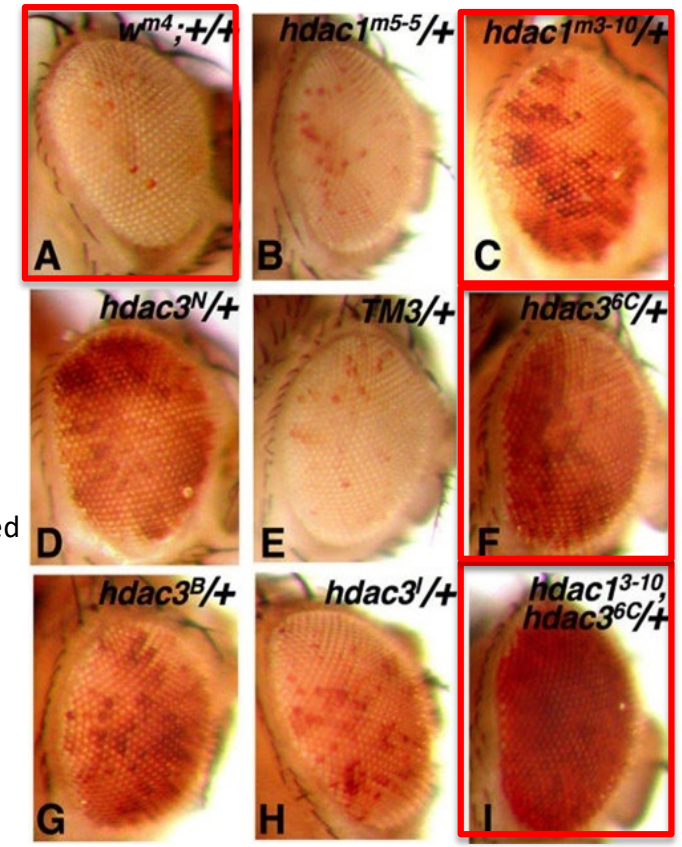


E(var)

tighter/spread hetechromatin

Su(var)

Impaired/retracted hetechromatin



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

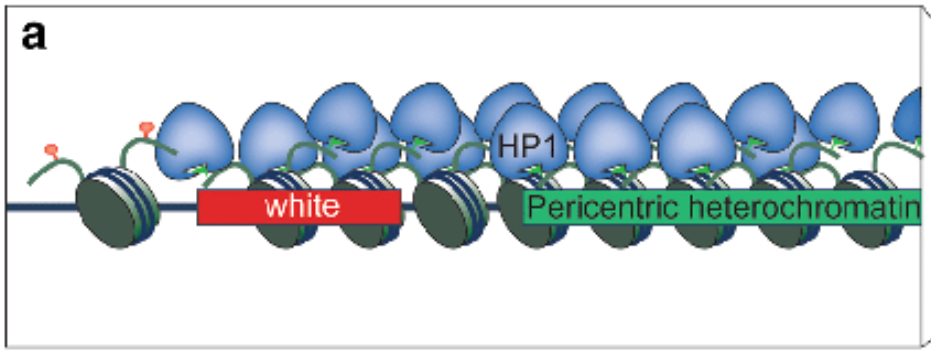
E(var):Enhancers of Variegation: when mutant, white gene is **repressed** in higher number of omatides = less pigmentation: gene encoded a factor that **antagonizes chromatin compaction** around centromeres

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

Observations that cannot be explained by genetics…..

Mutations in certain genes can suppress or enhance PEV: example: HDAC1, HDAC3 mutations

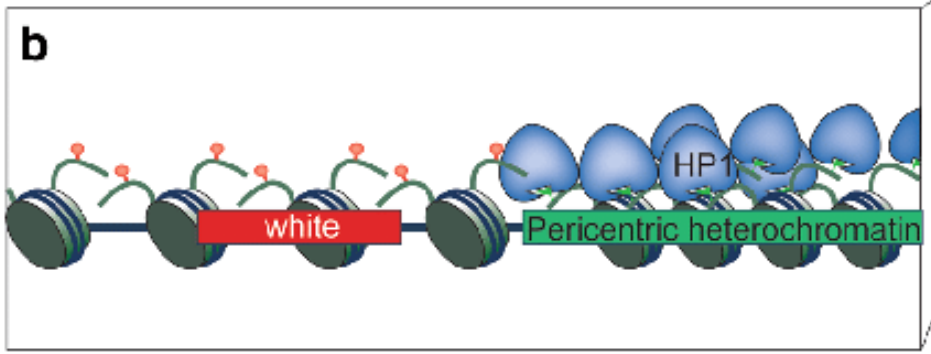
de-acetylation
→ ***low acetylation***
→ ***repressive chromatin***

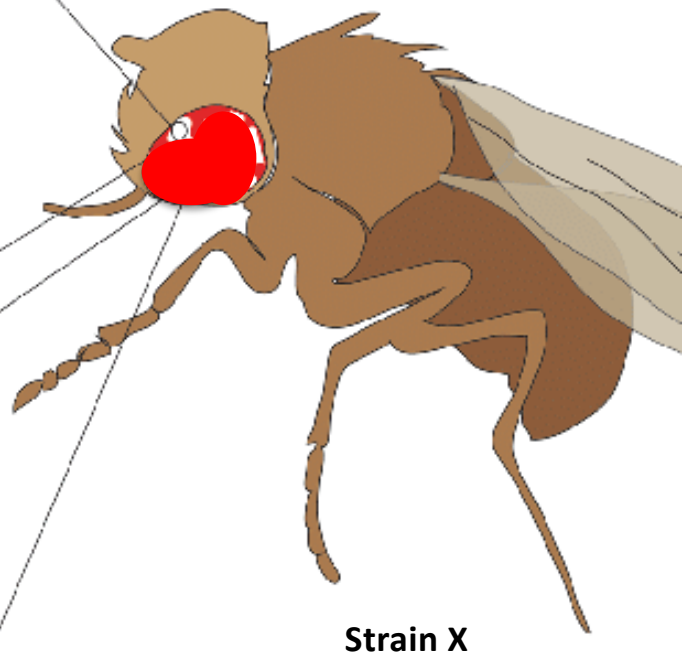


Acetylation

HDAC 1 mutant

HDAC 3 mutant





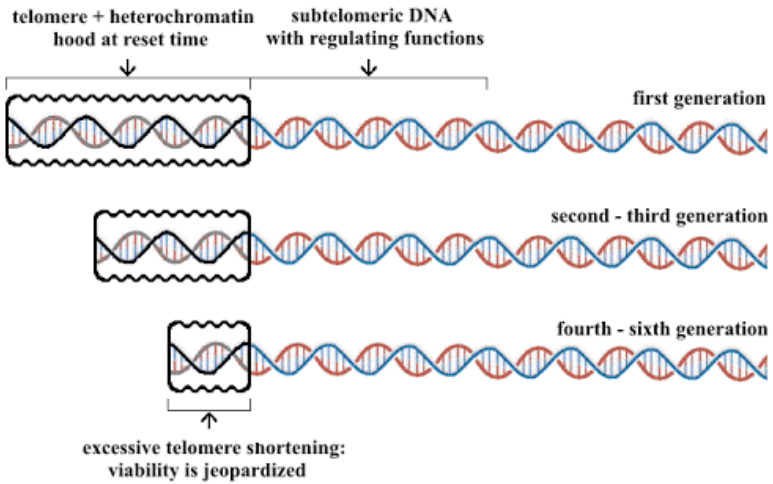
Strain X

less de-acetylation
→ ***more acetylation***
→ ***more active chromatin***

Strain A: Pigmented/non pigmented = 1/1 → **Strain X: Pigmented/non pigmented = 3/1**

Mutagenesis

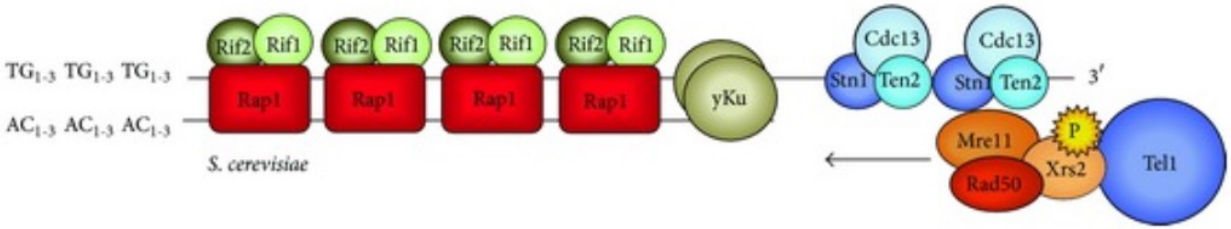
Observations that cannot be explained by genetics…..
3. S. cerevisiae telomeres – a role model system for genetic/epigenetic research



End-replication problem
Telomere shortenting

Telomere dysfunction - senescence

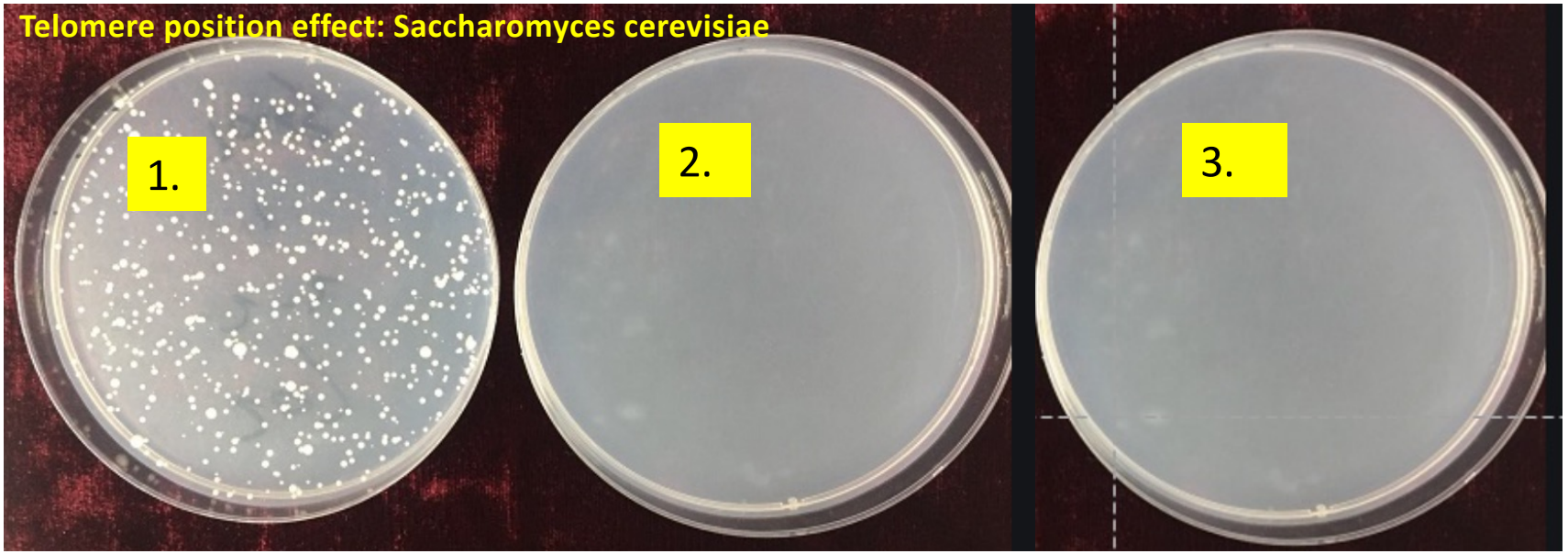
3’ telomere overhang



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…..
3. S. cerevisiae telomeres – a role model system for genetic/epigenetic research



Yeast	URA$^+$	URA$^-$	URA$^+$
	- Lack of Uracil - Lack of Uridne	- Lack of Uracil - Lack of Uridne	- Lack of Uracil - Lack of Uridine **+ 5-FOA (5-Fluoroorotic acid)**

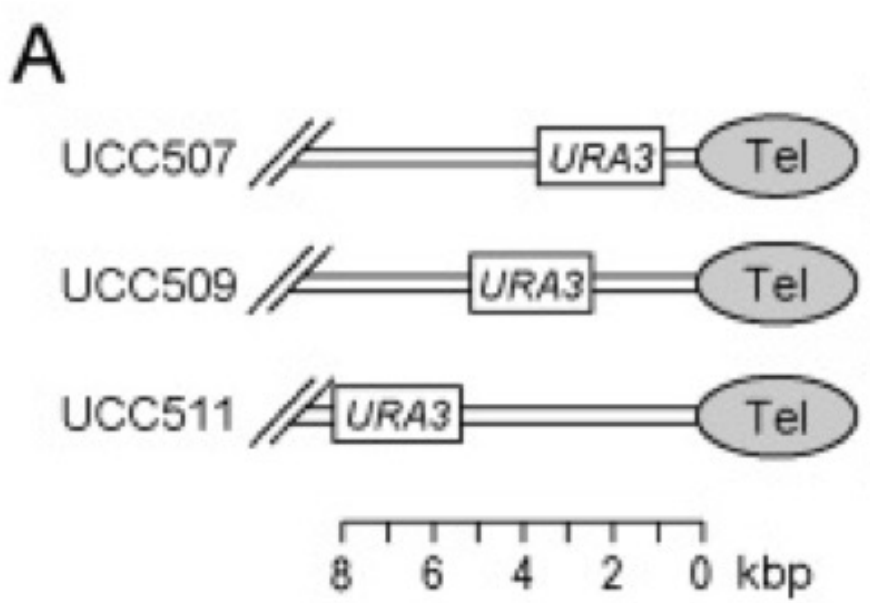
URA3 is a gene on chromosome V in Saccharomyces cerevisiae (yeast). URA3 is often used in yeast research as a "marker gene", that is, a gene to “label” chromosomes or plasmids. **URA3 encodes Orotidine 5'-phosphate decarboxylase (ODCase), which is an enzyme that catalyzes one reaction in the synthesis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

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

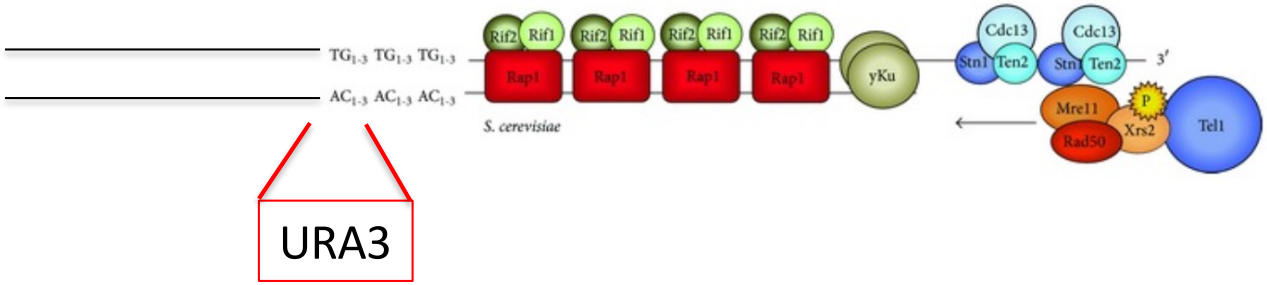


	Growth in medium without Uracil and Uridine	Growth in medium without Uracil and Uridine + FAO = sensitivity to FAO
UCC507	++	+
UCC509	++	++
UCC511	++	+++

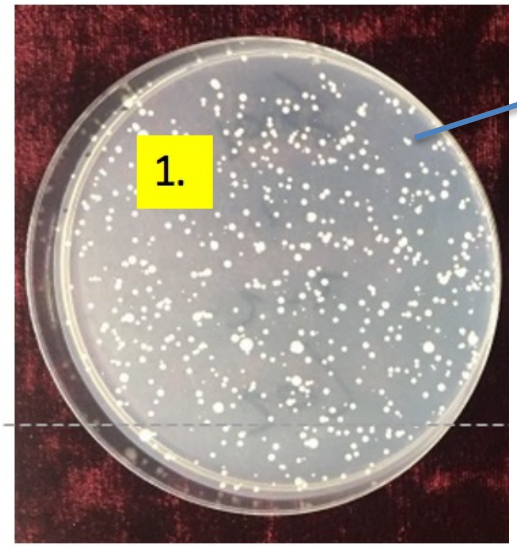
Observations that cannot be explained by genetics…..

Telomere position effect: Saccharomyces cerevisiae

Generation of S.cerevisae stains with subtelomeric insertion of URA3 gene
(endogenouse URA3 gene has been previously deleted)

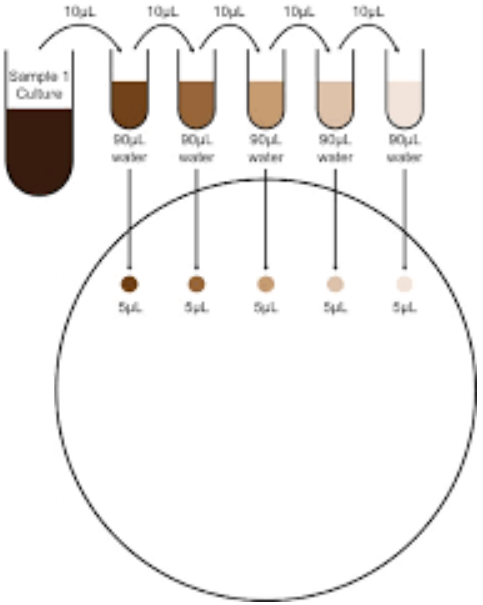


Spreading a suspension of yeast cells equally on plate



2. Amplification of colony in liquid culture

3. 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

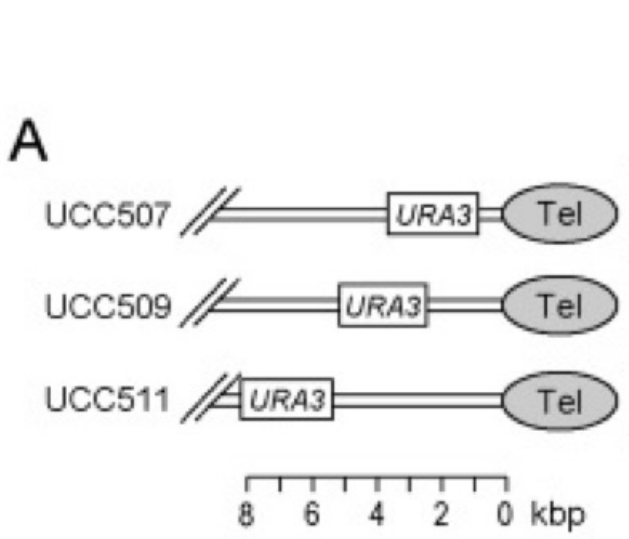
Concentration of plated cell suspension

Medium: no Uracil, no Uridne

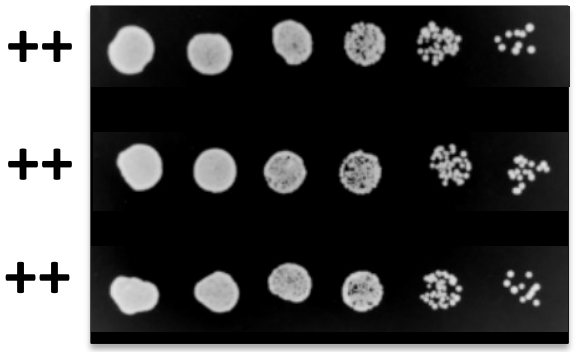
Observations that cannot be explained by genetics…..

Telomere position effect: yeast

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

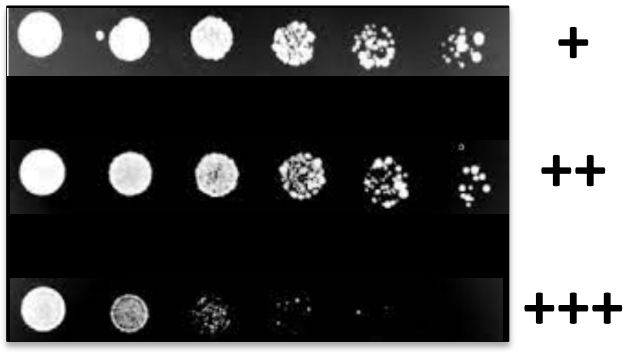


Growth in medium without Uracil and Uridine

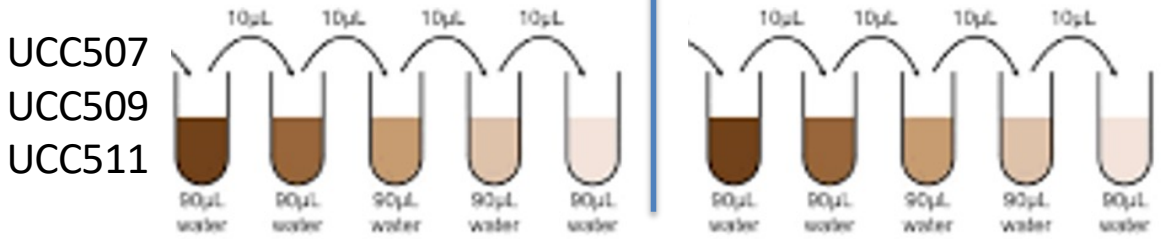


Viability

Growth in medium without Uracil and Uridine + 5-FAO



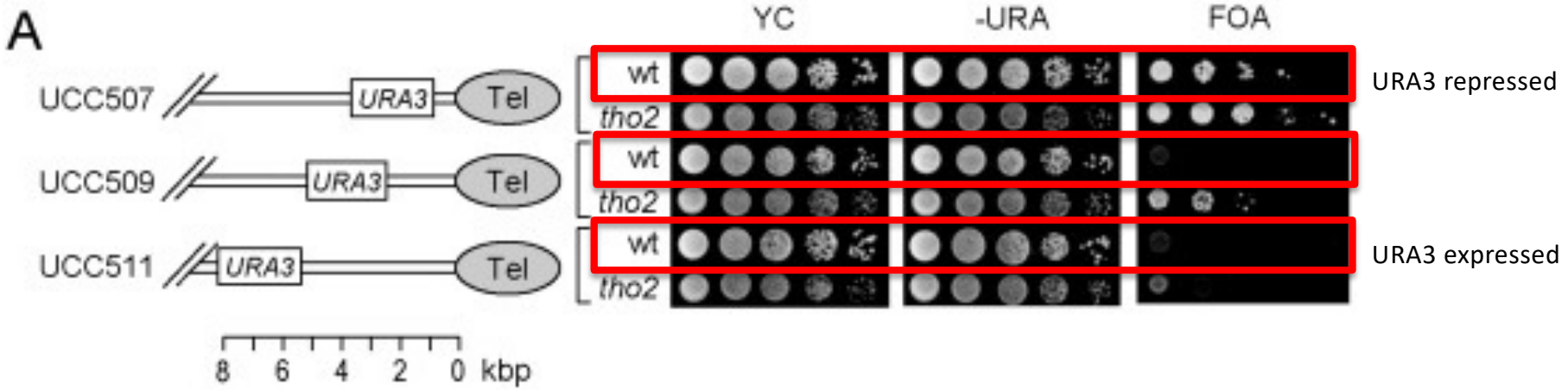
Sensitivity to 5-FAO



Observations that cannot be explained by genetics…..

Telomere position effect: yeast

Lets knock out a gene and observe a variegation of a pohenotype = FAO sensitivity



URA3 located far from telomere **UCC511**

All yeast cells grow (no TPE)

URA3 expressed

NO Colony formation

Medium +FAO

URA3 close to telomere **UCC507**

Variegated expression of URA3

Colony formation

patch of yeast with URA OFF

Medium +FAO

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

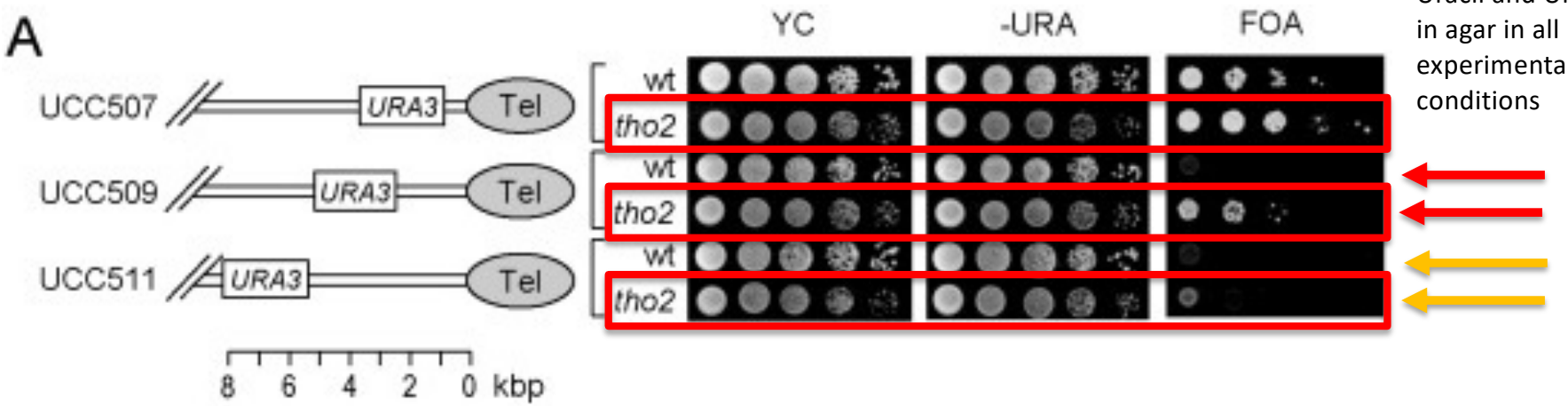
Important: Phenotype is stably during cell division

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

Observations that cannot be explained by genetics…..

Telomere position effect: Saccharomyces cerevisiae

Lets knock out a gene and observe a variegation of a pohenotype = FAO sensitivity



Uracil and Uridine in agar in all experimental conditions

Tho2 mutations cause a modification of the telomere position effect

→ Repressive effect on URA3 is enhanced in Tho2 loss of function cells
→ 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 (promotes heterochromatinization)

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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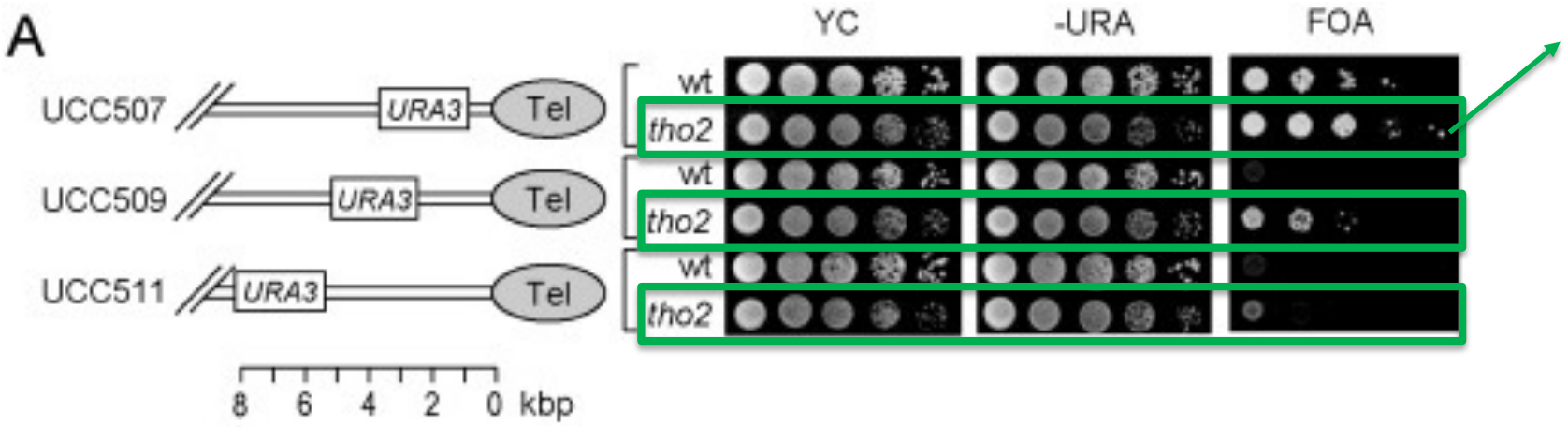
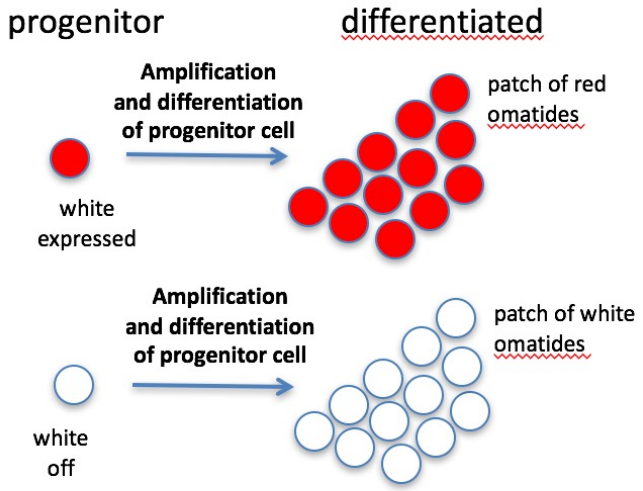
MODULATION OF GENE EXPRESSION IS PASSED ON TO DAUGHTER CELL

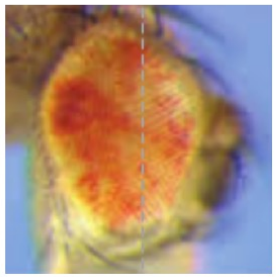
Position effect variegation (Telomere position effect): Saccharomyces cerevisiae:



Single colony consist of millions of single cells with same feature

Position effect variegation: Drosophila melanogaster:





Pachy appearance

.....Marker has been artificially located into a heterochromatic region with already established epigenetic regulation

….epigentic context is subjected to cell – to – cell variation

....however, ”epigentic information” in individual cell will be passed on to next generation of cells

= Epigenetic inheritance or Maintenance of epigenetic information

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

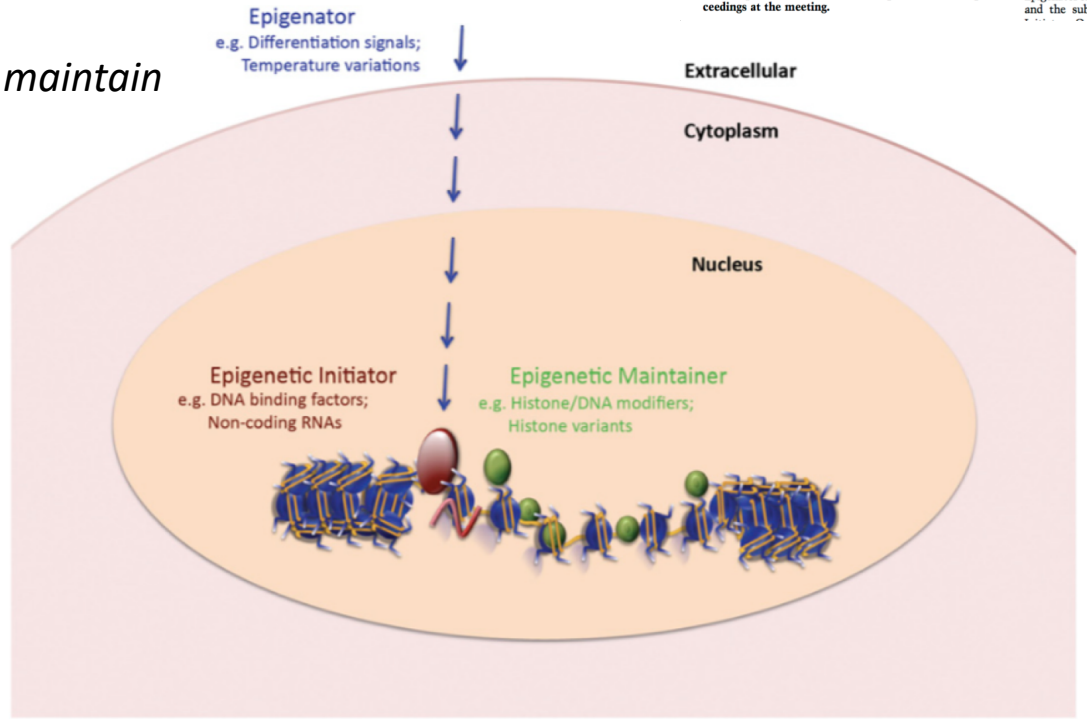


Figure 1. The epigenetic pathway. Three categories of signals are proposed to operate in the establishment of a stably heritable epigenetic state. An extracellular signal referred to as the "Epigenator" (shown in blue) originates from the environment and can trigger the start of the epigenetic pathway. 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 into all different cell types of an organism

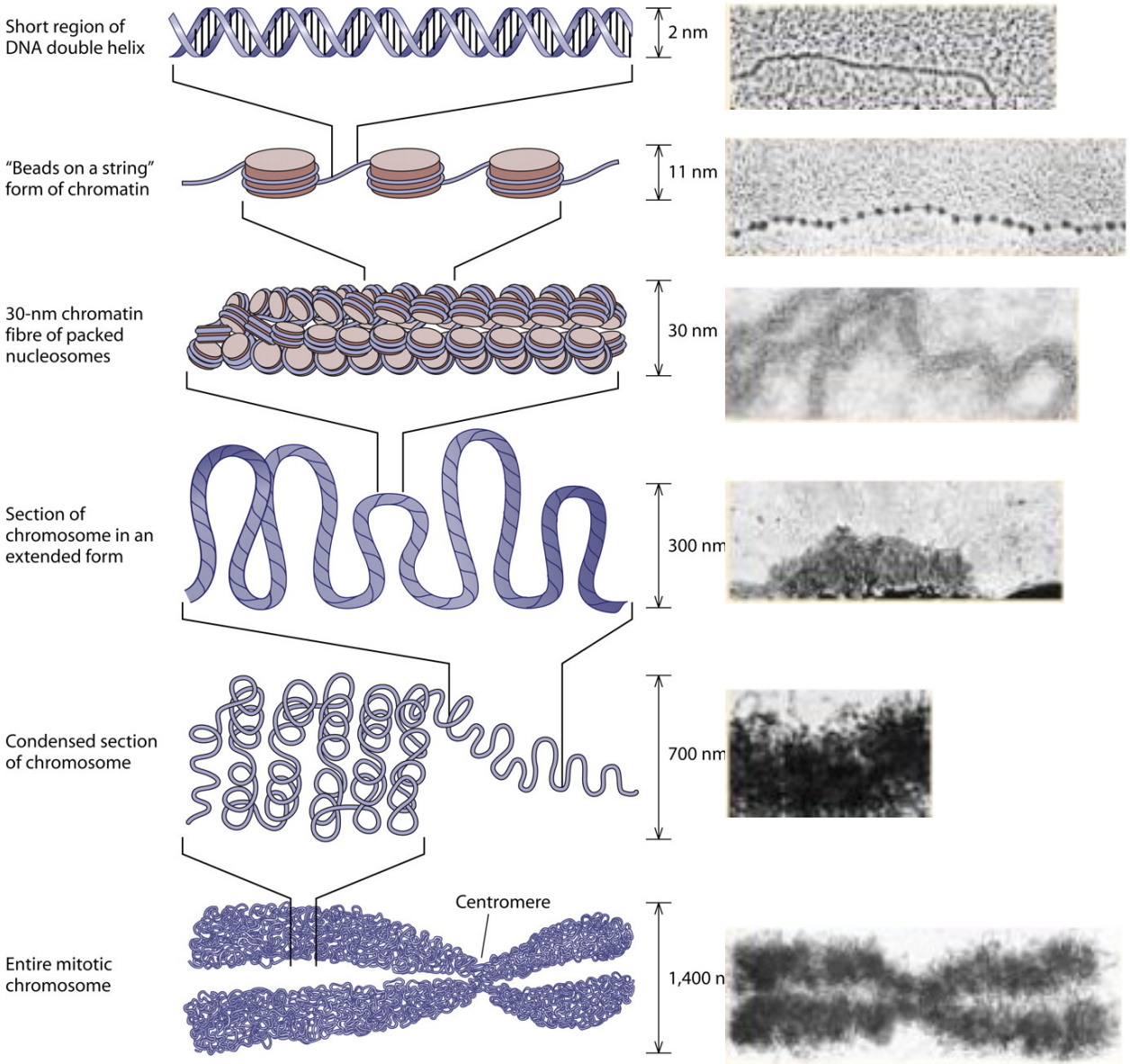
Histone genes were first cloned in the 1980ies

The DNA is not a naked molecule in the nucleus.

DNA is bound to proteins

DNA+DNA-bound proteins and RNA = CHROMATIN

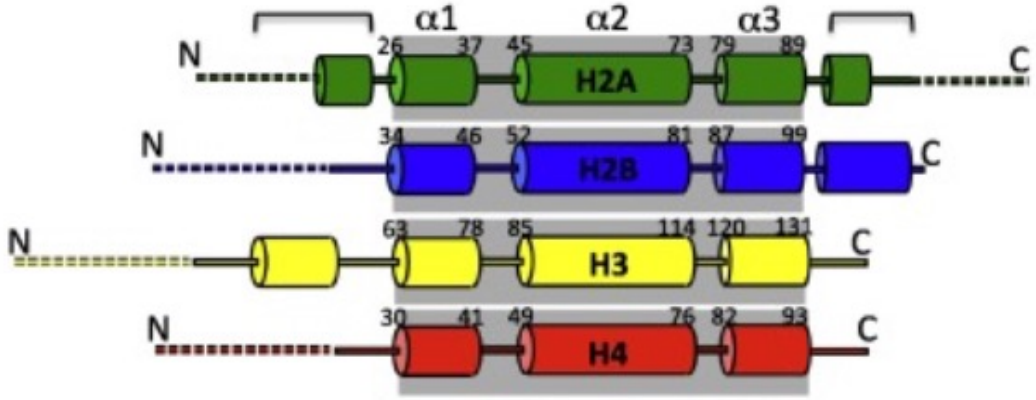
Chromatin is organized at different levels



The “packaging” of genetic information is essential for gene expression

Histones have common protein domain organizaion: Histone fold domain

eukaryotes



11-15kDA

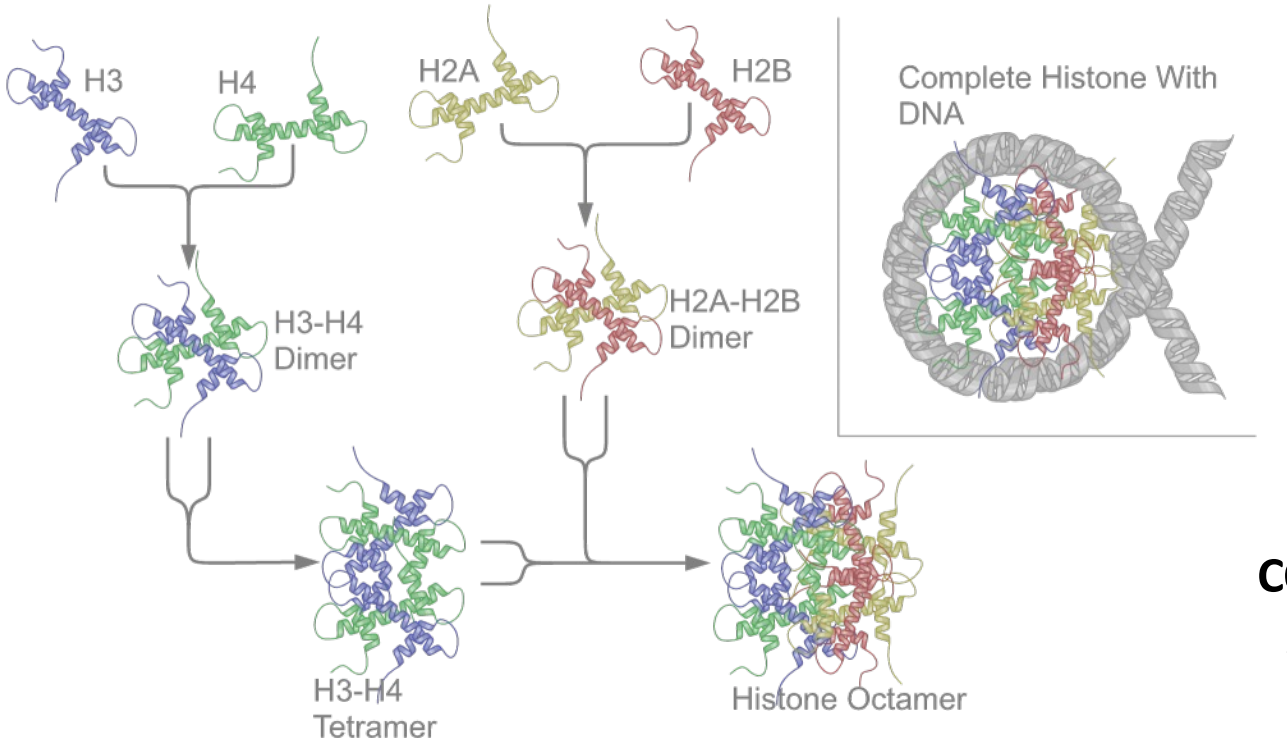
Top: Schematic showing secondary structure of the cannoncal histone proteins that from the major nucleosome type (core histone):

- 3 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/MAINTAIN EPIGENETIC INFORAMTION

TPE, PEV: higher order gene regulation –
but not the presence of additional regulatory DNA elements

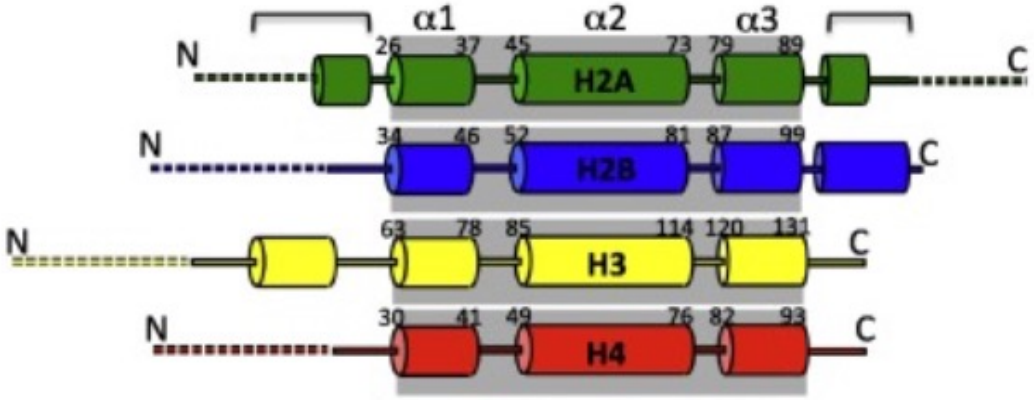
How can it be tested if histones have an important role in regulating gene expression???

QUESTION: What parts in canonical histone proteins may hold specific information??

Histones have common protein domain organizaion: Histone fold domain

11-15kDA

eukaryotes



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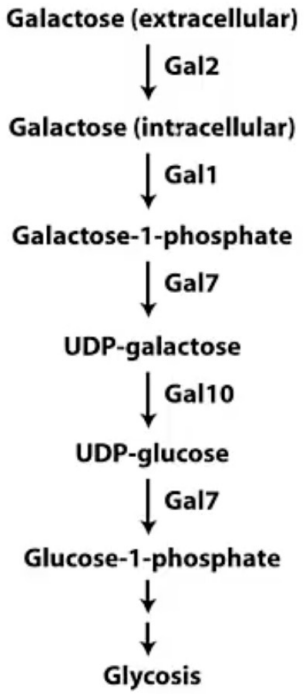
Histone tails are essential for epigenetic information

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

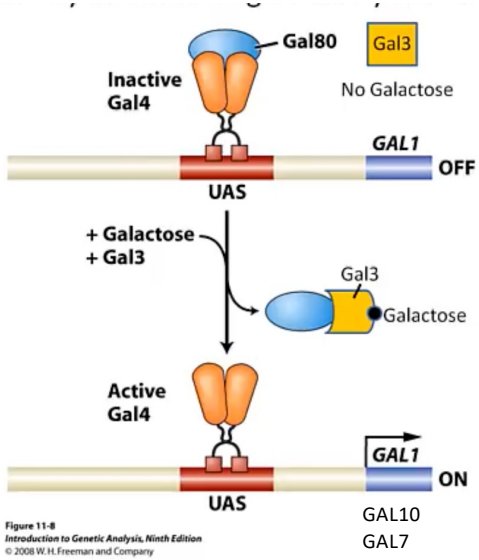
S. cerevisiae; model system: the Galactose pathway

Required to process Glactose to Glucose-1-phosphate
and render it accessible to glycosis. Processing controlled by several genes that are repressed when galactose is absent



Activation of the Galactose pathway

…Galactose bind to Gal3 to block the action of the Gal4 repressor protein. Activation of GAL1, GAL2, GAL7, GAL10



- Gal80 binds to Gal4 and represses it
- Gal3 binds to the allosteric effector (binds galactose)
- When Gal3 binds galactose, it increases affinity for Gal80, inhibiting it from inhibiting Gal4

Question: *are histones involved in the activation of GAL1, 2, 7, 10?*

QUESTION: DO HISTONES CONTRIBUTE TO GENE REGULATION ?

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

David Allis Lab: THE MODEL SYSTEM TO EXPLORE THIS QUESTION:

CONCEPT: Yeast strain:

Step 1: insert an extra copy of a histone H4 gene (wt or mutant) that is controlled by the **Galactose-inducible GAL1 promoter** into the S. cerevisiae genome

System: Upon addition of Galactose, yeast grown in media containig galactose (and lack Glucose), galactose activates the GAL promoter

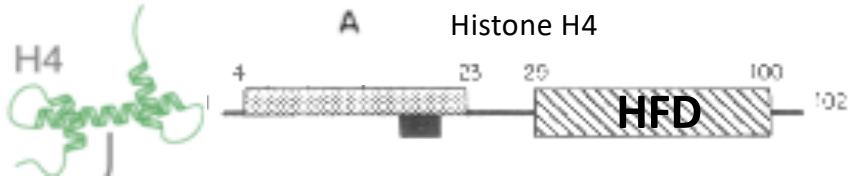
Step 2: delete both copies of the endogenous histone H4 gene and grow cells in medium with galactose.

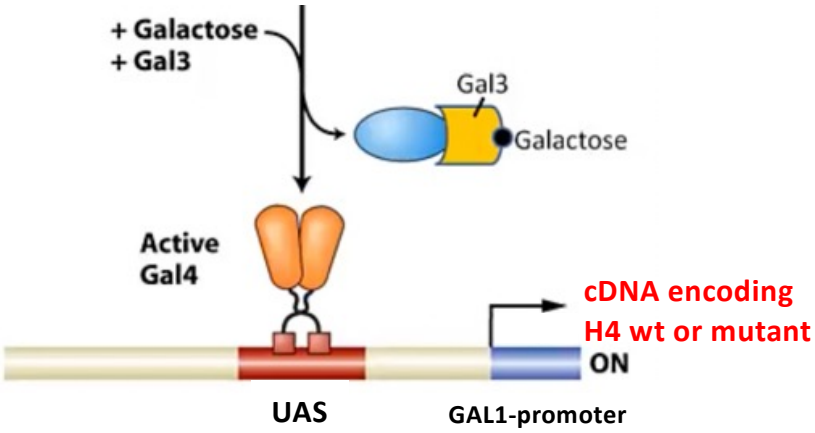
- H4 constructs assure viability
- Question: Effect of mutant H4 versions on the activation of gene expression?

--> Test if S.cerevisiae is able to activate the Galactose pathway (Gal1, Gal2, Gal 7, Gal 10)

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.

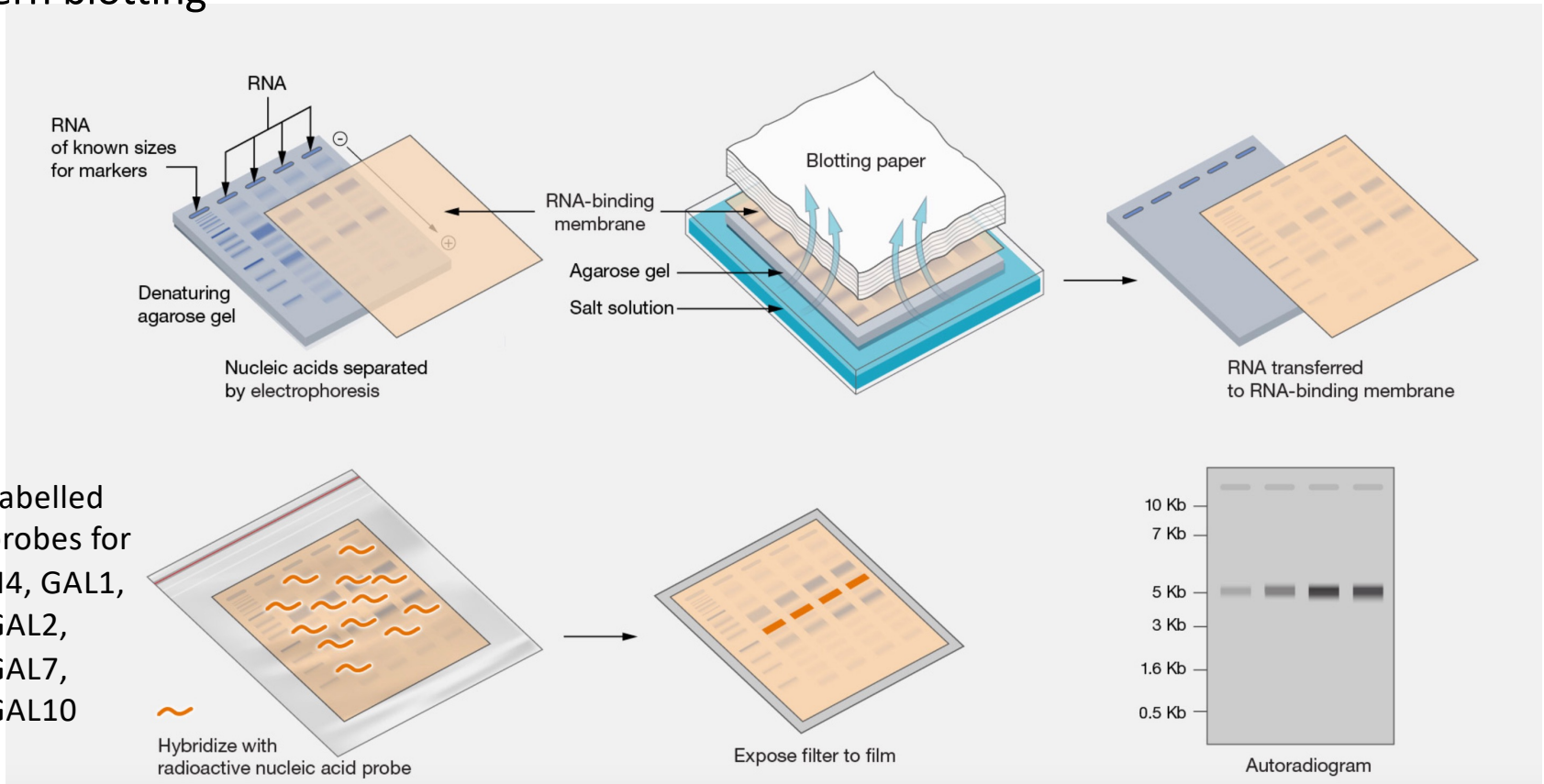




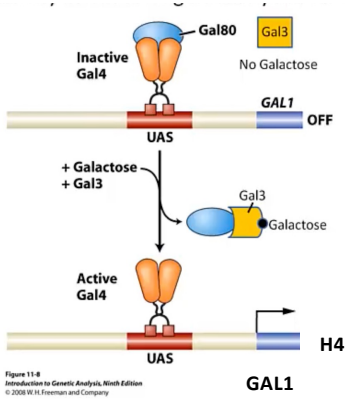
Question: is a particular region of H4 required for the expression of GAL1, 2, 7, 10?

Histone tails are essential to control gene expression

Northern blotting



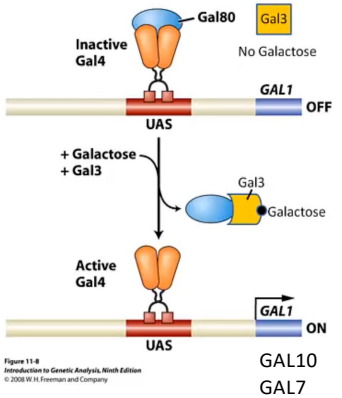
Events at
the H4 rescue
constucts



H4 mutant versions / wt version

GAL1
promoter

Events at
the endogenous
GAL1, GAL2,
GAL7, GAL10
promoters



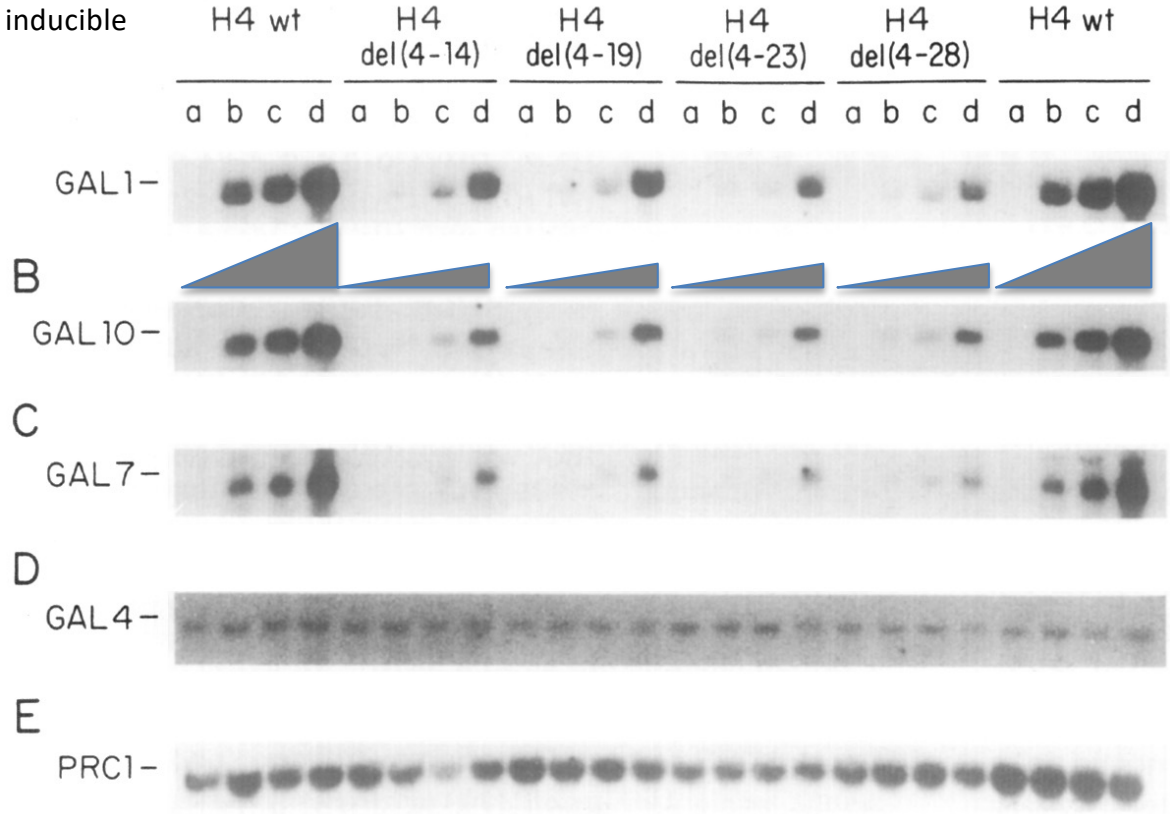
- Gal80 binds to Gal4 and represses it
- Gal3 binds to the allosteric effector (binds galactose)
- When Gal3 binds galactose, it increases affinity for Gal80, inhibiting it from inhibiting Gal4

GAL10
GAL7
GAL2

Histone tails are essential to control gene expression

REPLACE GLUCOSE FOR GALACTOSE AND MEASURE GALACTOSE PAHTWAY ACCTIVITY:

READOUT: Northern blot – gene expression analysis



Yeast cells were treated with Galactose (at different concentrations; a, b, c, d) to induce:

1. H4 (wt or mutant) expression.
2. Monitor induction of GAL1, GAL10, GAL7 genes of the galactose processing pathway.

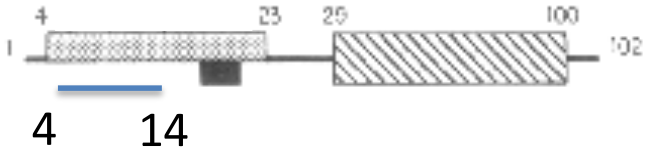
QUESTION: which H4 version is able to support gene activation GAL1, GAL10, GAL7?

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

NOTE:

- PRC1 and GAL4 are known to be not activate by galactose treatment
- a, b, c, d: increased amount of RNA loaded on gel

Cells with mutant H4 tails only inefficiently activate GAL1, GAL 10, GAL7, GAL4 expression



H4 TAIL CONTAINS INFORMATION TO ACTIVATE GENE EXPRESSION

Histones can be chemically modified

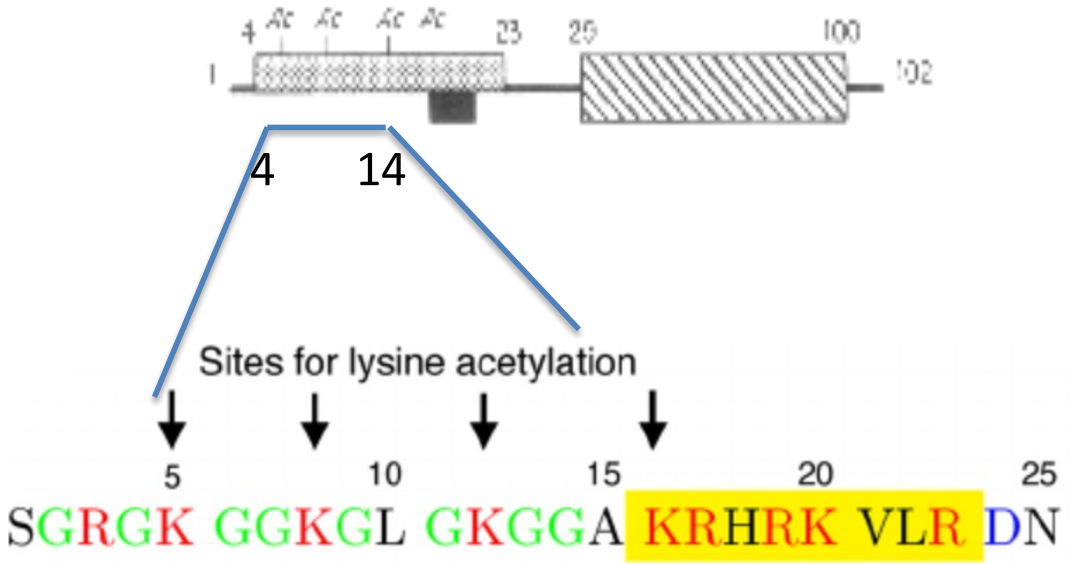
Observation: Histone tails are important for transcriptional actvation

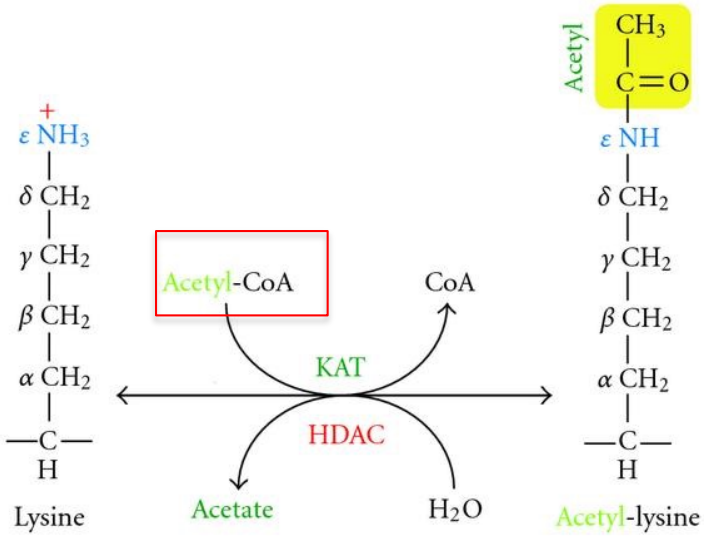
Problem: H4 is present in genes that are active and inactive genes

Hypothesis: Histone tails contain information that discriminates active form inactive genes

Observation from biochemists: Ammino-groups in lysines can be chemically modified *in vitro* using a abundantly present cofactor : Acetylation

H4 tail contains high proportion of lysine residues





Can cells chemically modify lysine residues in vivo??

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

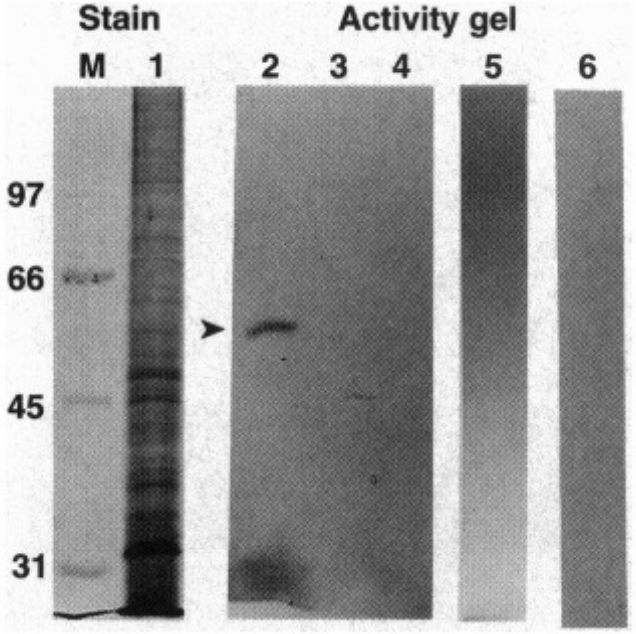
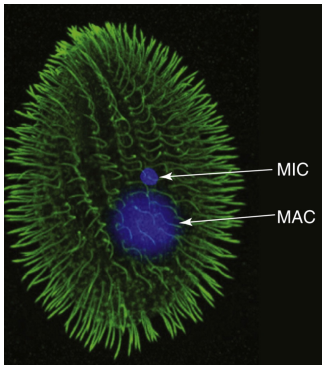


FIG. 1. A 55-kDa polypeptide specifically labels histones in an acetyltransferase activity gel assay. Crude macronuclear histone acetyltransferase activity was subjected to electrophoresis in SDS/8% polyacrylamide gels in which histones (lanes 2, 3, and 4), bovine serum albumin (lane 5), or no protein 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.

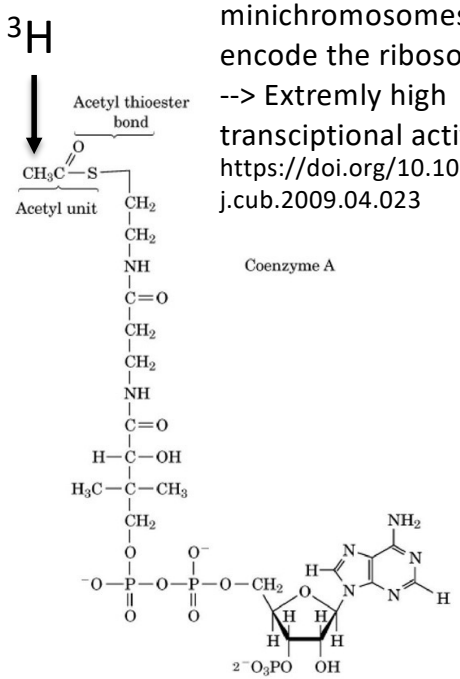
1. Make native Polyaclylamide gel with incorporated, purified histones (lanes 2, 3, 4) or BSA (lane 5)
2. Lane 3: extract boiled; Lane 4: N-ethylmaleimide – binds Cys
3. Take protein extract from Tetrahymena Macronuclei and load run on gel.
4. After stop of the gel the enzymatic assay will be performed: radioactive [^{3}H]-Acetyl-CoA (contains acetyl group – is a major enzymatic cofactor in cells) on top of gel.
5. After autoradiography, a band appears that marks acetylated histones that co-lolocalize with a “histone acetyltransferase” present in the Tetrahymena extract



Miconucleus: transcriptional inactive

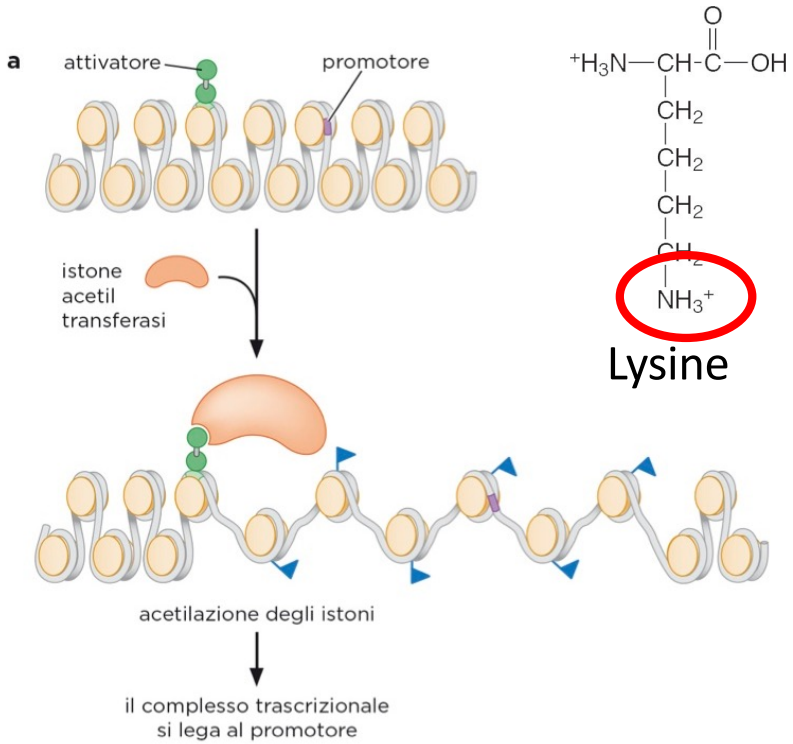
Macronucleus: transcriptional active; differentiate by a series of chromosomal rearrangements involving large scale DNA elimination, chromosome fragmentation, endoreplication, and gene amplification, resulting in a large nucleus containing ~45 copies of ~225 transcriptionally active chromosomes and ~9000 minichromosomes that encode the ribosomal RNAs --> Extremly high transciptional activity

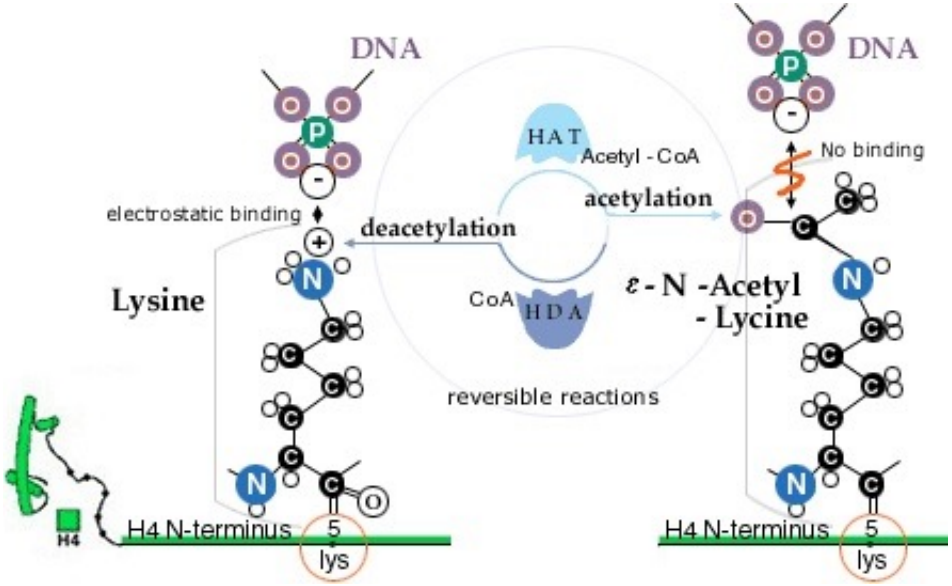
https://doi.org/10.1016/j.cub.2009.04.023



Tetrahymena is a genus of free-living, unicellular ciliate protozoa commonly found in freshwater environments.

Post-translational modifications can change the topology of chromatin





Simplyfied view: acetylated histone tails recruit proteins

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

Connecting histone acetylation to classic observations linked to epigentics

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

MODEL SYSTEM:

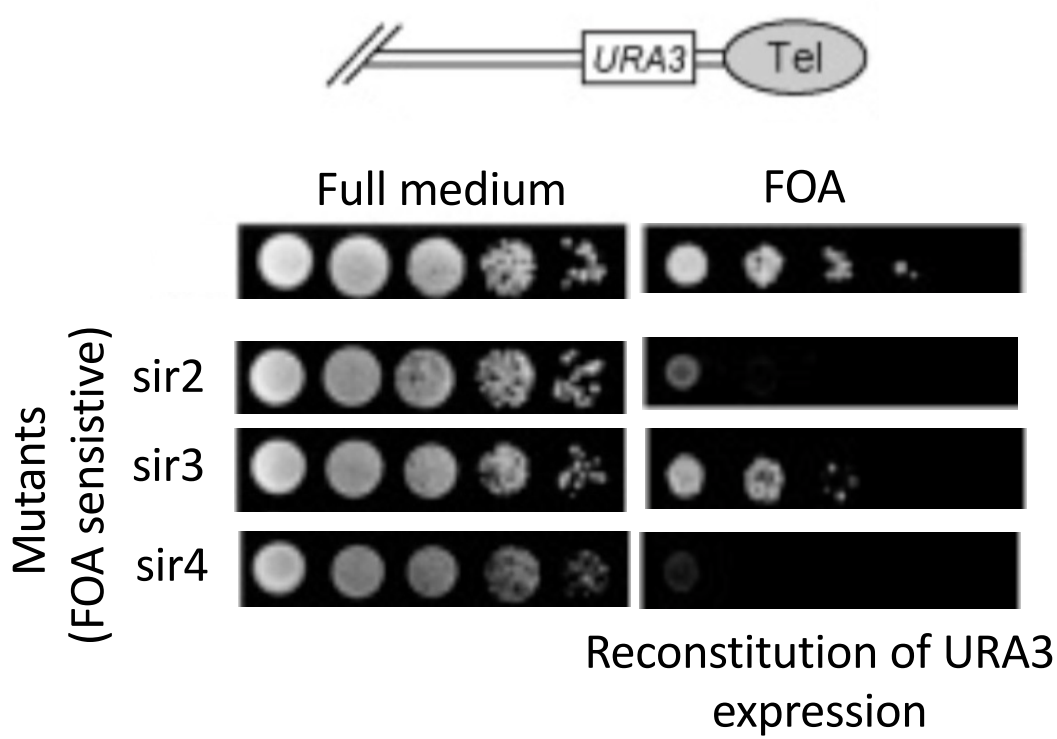
→ URA3 Reporter gene inserted into central position in chromosome: EXPRESSED

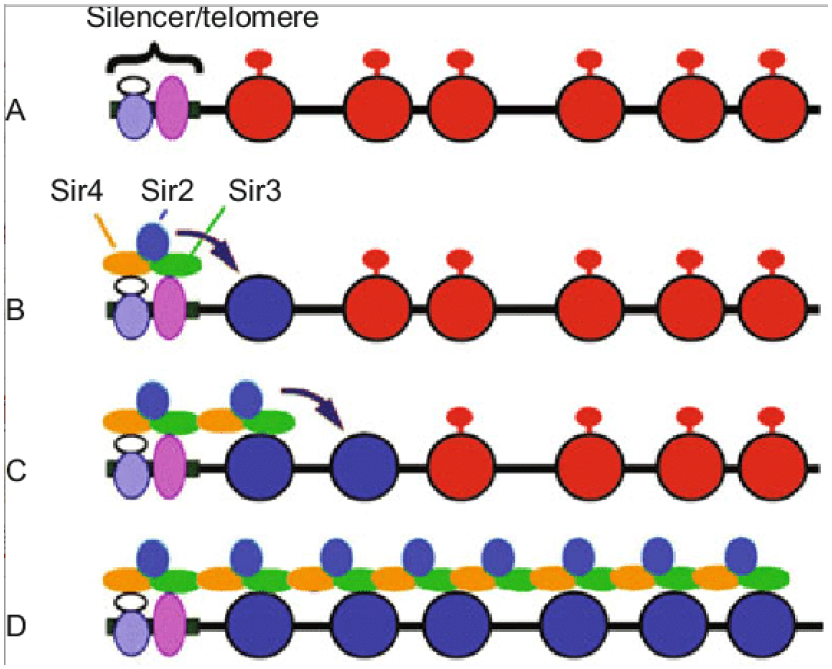
→ URA 3 Reporter gene inserted in proximity to chromosome ends: SILENT (see below)

→ make screed to identify S. cerevisiae mutants that release reporter from silencing

→ identify genes that are mutated

= SILENT INFORMATION REGUALTORS (SIR) GENES

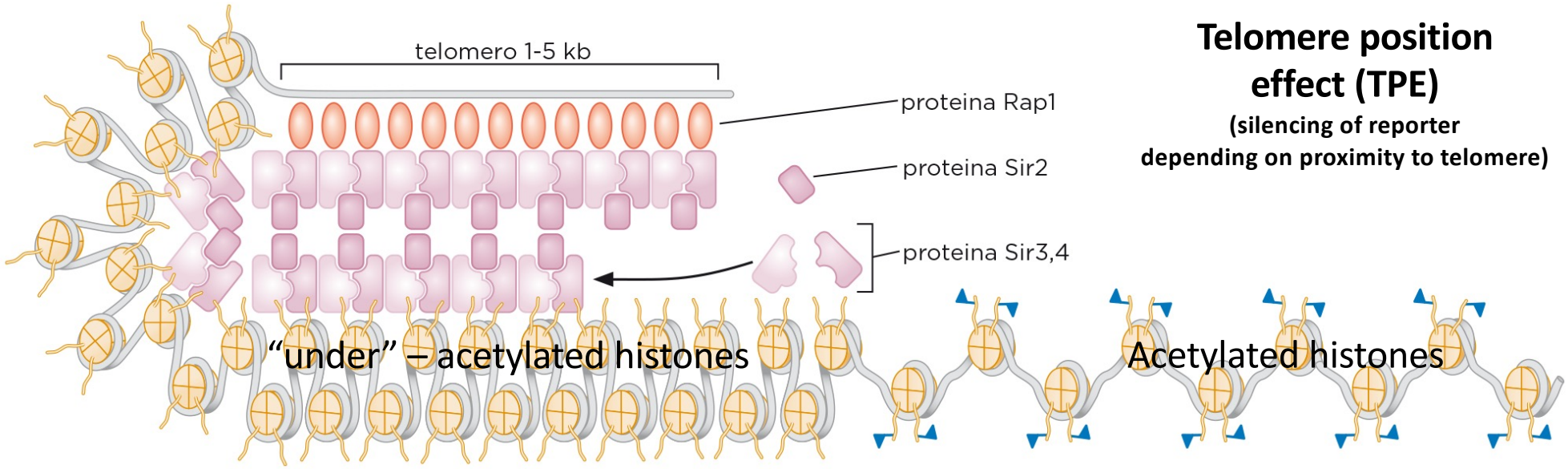




SIR2, 3, 4 form a protein complex that is present at chromosome ends

(representative image)

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



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

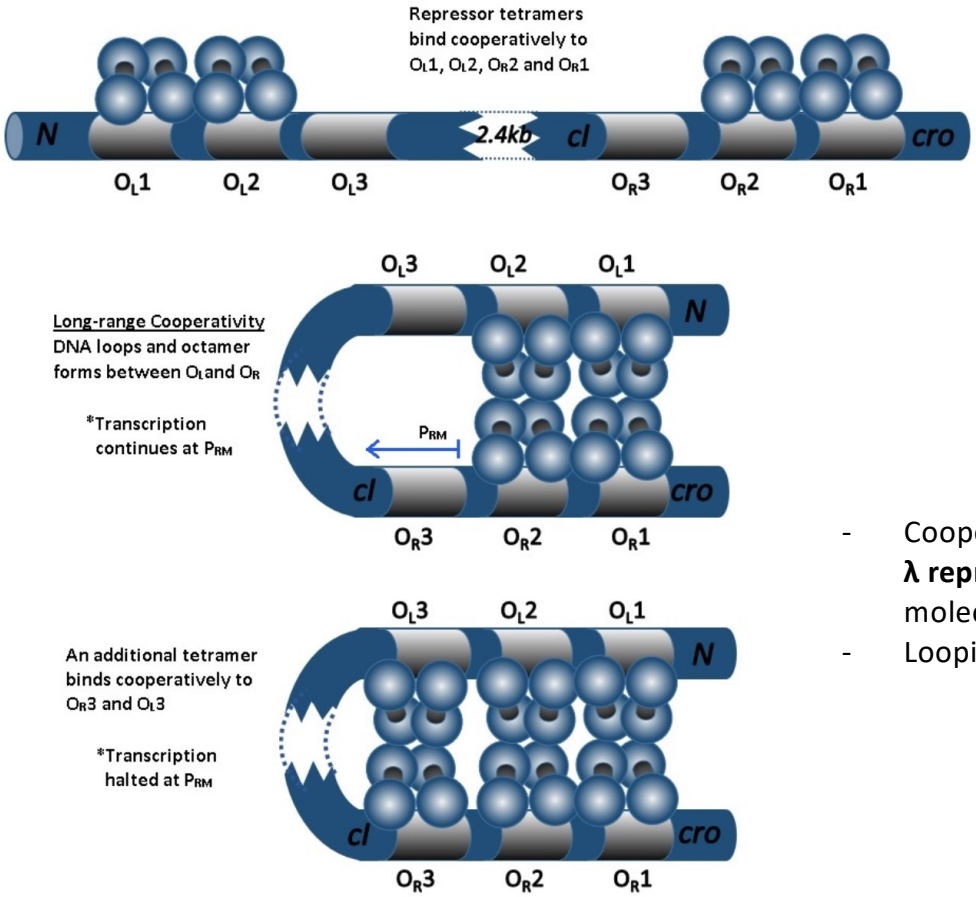
Mechansim

1. Rap1 specifically binds to telomeric repeat sequences ($G_{(2-3)}(TG)_{(1-6)}T$ consensus); telomere repeats are not organized in nucleosomes.
2. **Rap1 recruits the SIR complex (SIR2,3,4)** SIR binding is **co-operative** and Sir2,3,4 multimeres expands into subtelomeric region that containes 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 it **meets an antagonizing chromatin signature (active)**

Does cooperativty and loop formation sound familiar????

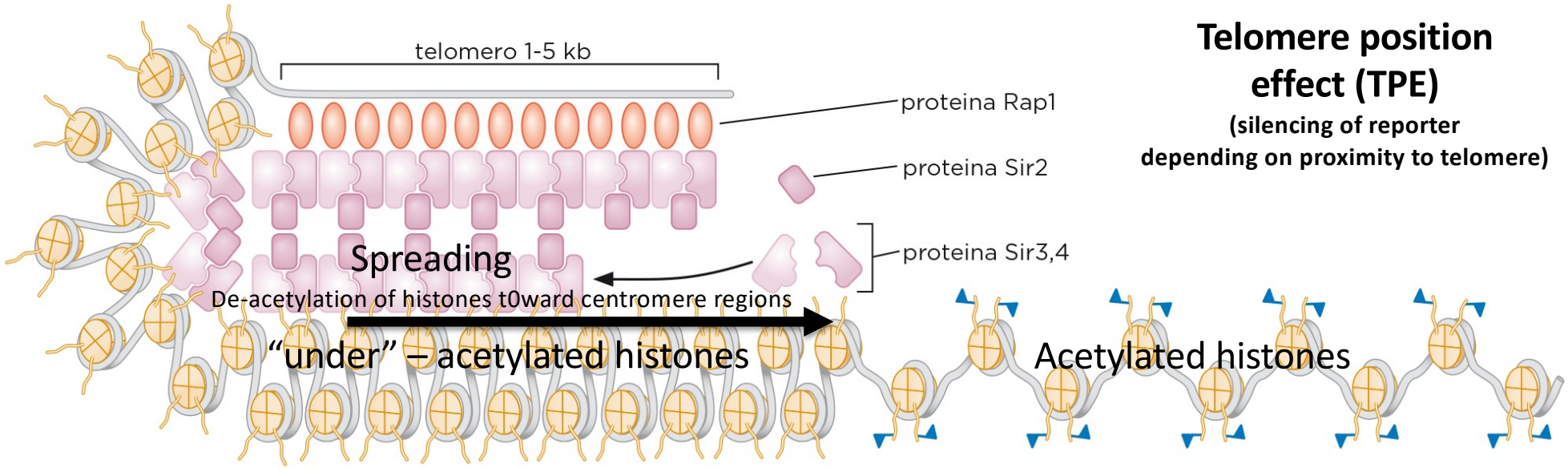
The **λ repressor** maintains lysogeny in bacteriophage λ by binding operator sites and blocking lytic gene expression (in particular via O_R1, O_L1

It also activates its own transcription, stabilizing the dormant state. This repression protects the host cell and ensures phage persistence until stress triggers repressor cleavage and entry into the lytic cycle.



- Cooperativy of **λ repressor** molecules
- Looping of DNA

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



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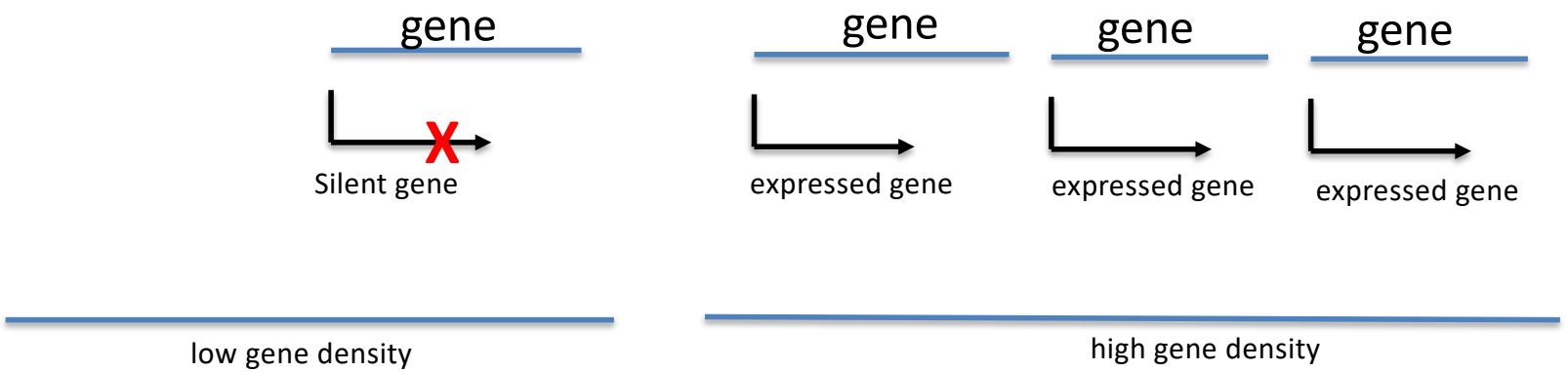


How is spreading of telomric heterochromatin controlled?

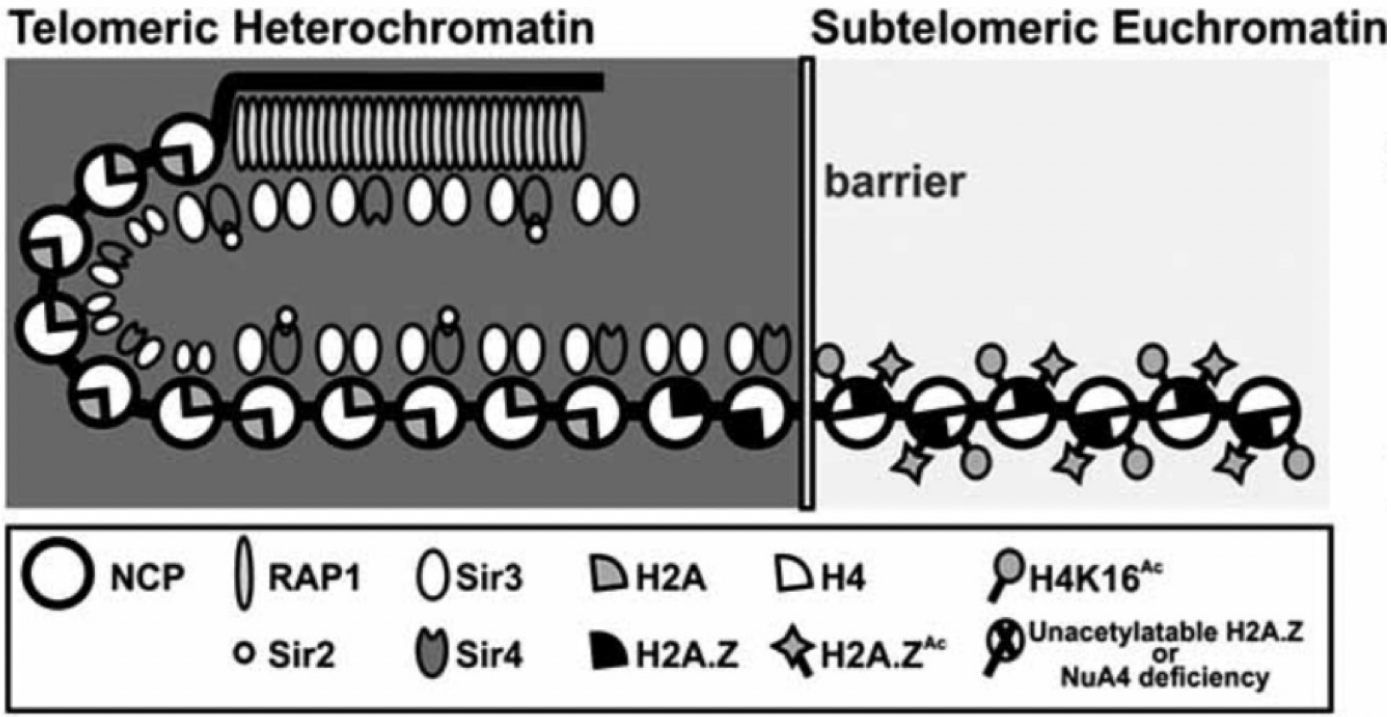
Post-translational modifications decide on gene expression
TELOMERE POSITION EFFECT AND ACETYLATION

HOW IS SPREADING REGUALTED??

Silencing complex spreads until meets a «barrier» at gene-rich regions
that are enriched for the H2A variant protein **H2A.Z** and histone **H4 acetylated at K16** (H4K16Ac)

H2A.Z and H4K16Ac are enriched in transcribed genes in subtelomeric euchromatin = strong information for active gene expression

Competition between repressive and active chromatin that meets its balance at the barrier



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

HOW IS SPREADING REGUALTED??

Impairing euchromatic chromatin code: spreading of heterochromatin (mutaions in H4 tails) → barrier shifts towards centromere)

Impairing euchromatic chromatin code: spreading of heterochromatin (mutation of H2AZ that marks active genes) → barrier shifts towards centromere

Impairing heterochromatin chromatin code: spreading of euchromatin (SIR2 mutations → barrier shifts towards repeats)

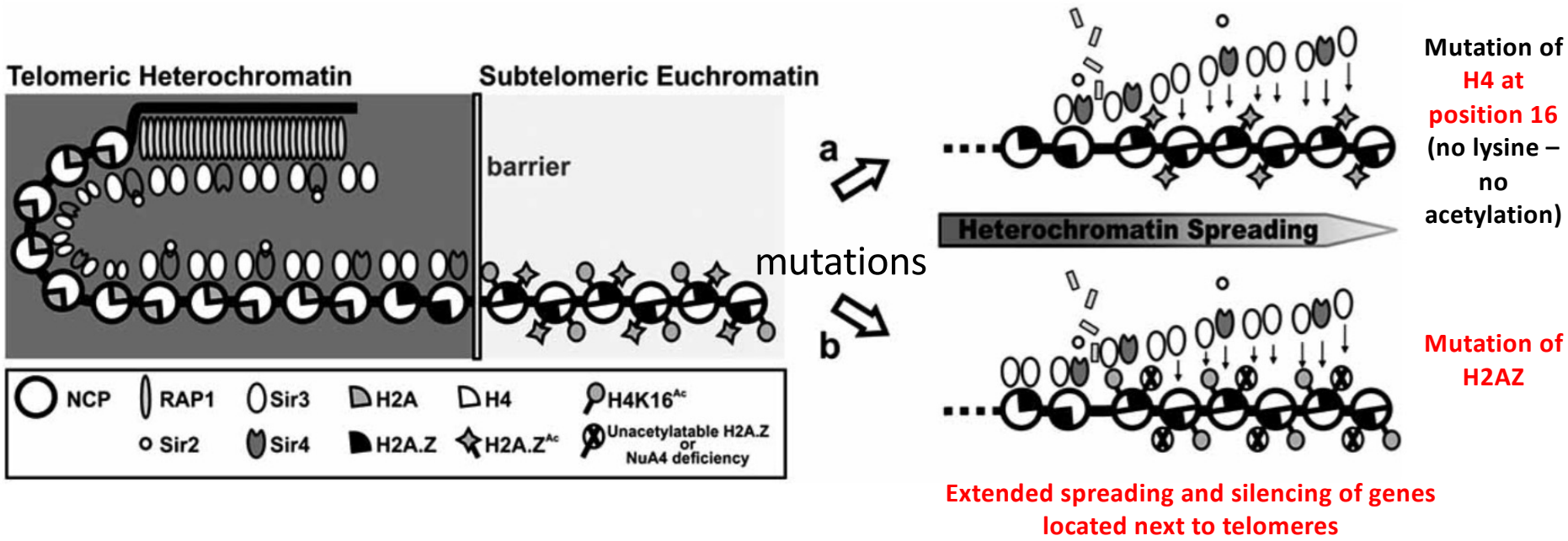


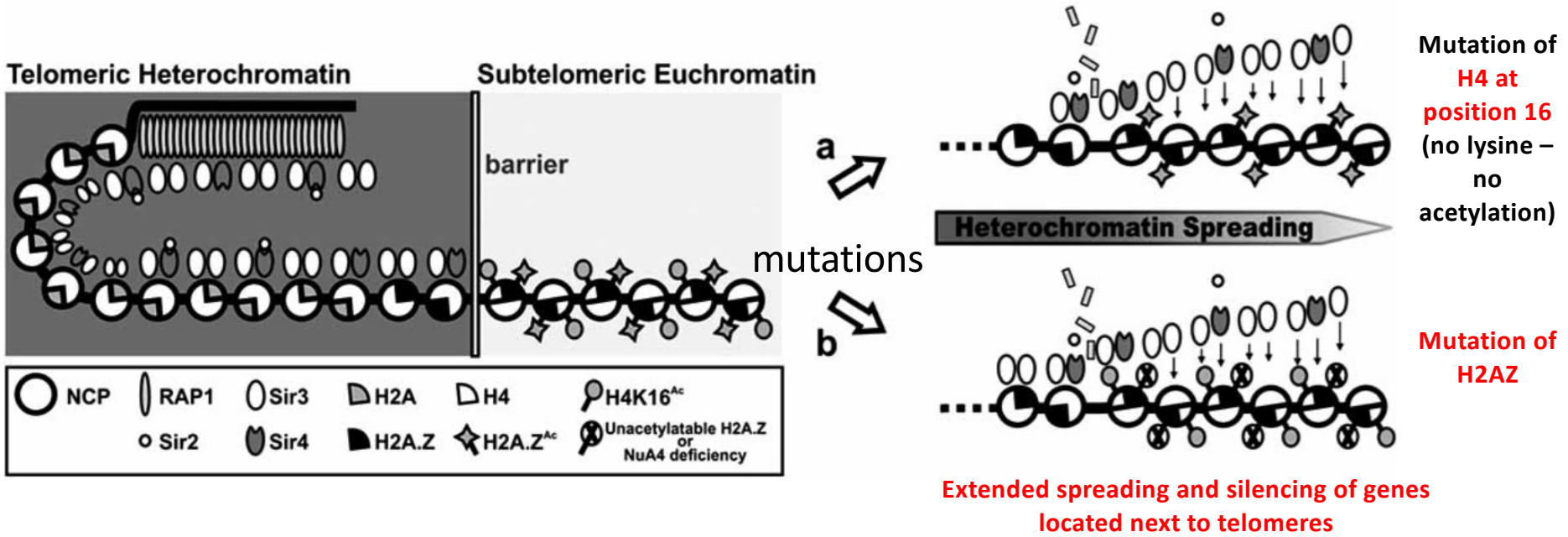
Fig. (2). A model for yeast telomeric heterochromatin adapted from [39]. Heterochromatin formation and maintenance involve the RAP-1 containing telosome, different Sir proteins and the interaction of these elements with histone H4. H2A.Z participates in preventing the spread of telomeric heterochromatin 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.

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

Activating and repressing regions can be in competition at barriers

→ Patchy phenotype in PEV in D- melanogaster
→ TPE in S-cerevisiae

Extension of chromatin domains can change



Mutation of H4 at position 16 (no lysine – no acetylation)

Mutation of H2AZ

Extended spreading and silencing of genes located next to telomeres

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.

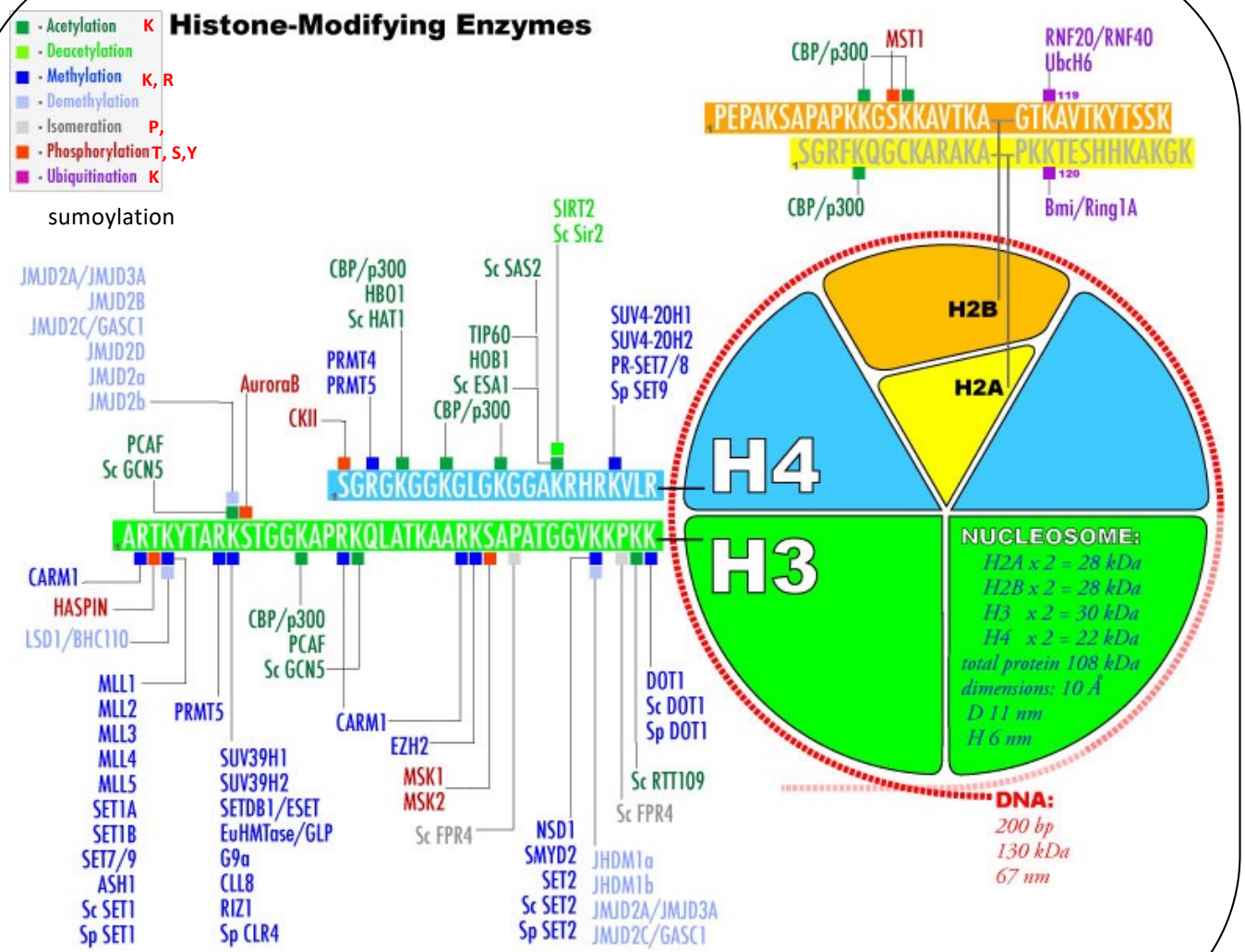
Chromatin is dynamic:

- Histone modifications and DNA methylation is central for chromatin status
- Chromatin can be remodelled

Histones and DNA can carry chemical modifications
=POST TRANSLATIONAL HISTONE MODIFICATIONS

POST TRANSLATIONAL HISTONE MODIFICATIONS

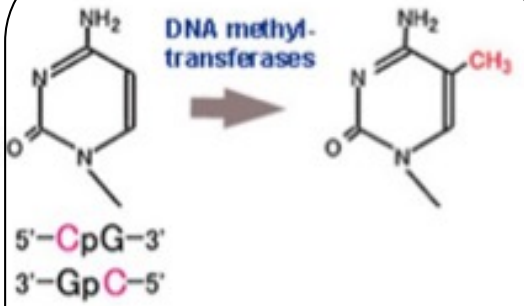
(active or repressive chromatin structure)



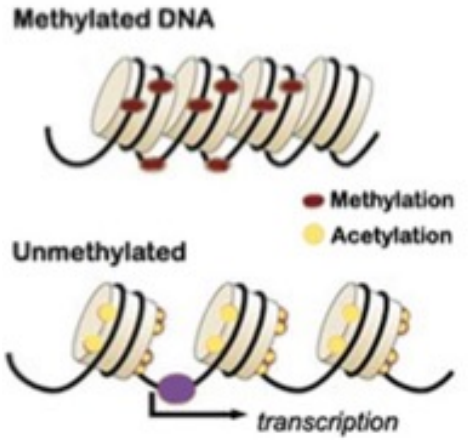
THE "HISTONE CODE"

DNA METHYLATION

(repressive chromatin structure)

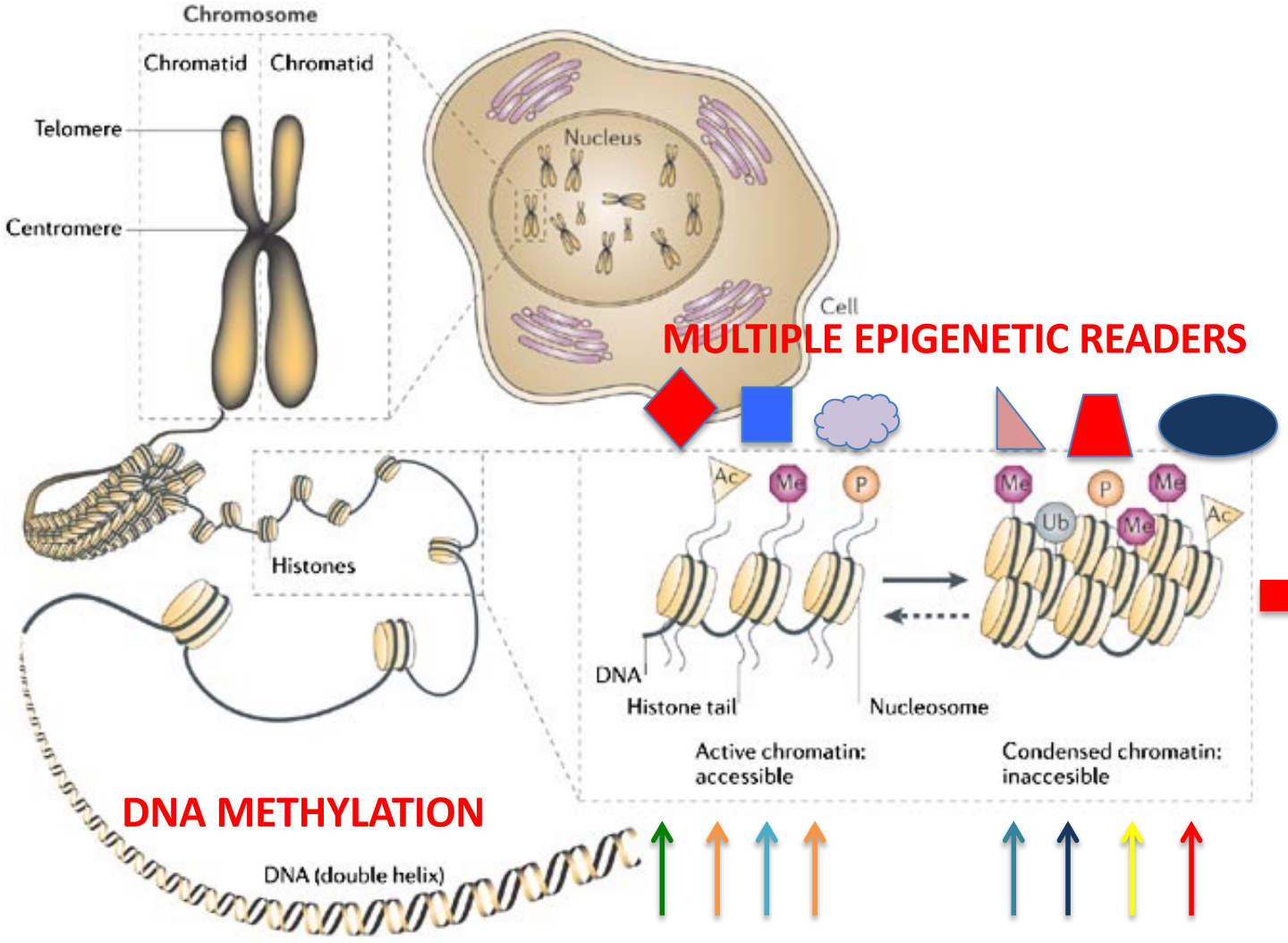


Vertebrate DNA methyltransfererases
DNMT1, DNMT3a, DNMT3b
act only on CpG di-nuceotides



Mammals, Plants, Tetrahymena (6mA)
Less freqeunt in Drosophila, Yeast
Not detectable in C. elegans

CONCEPT OF EPIGENETIC WRITER AND READERS



Epigenetic code (histone code)

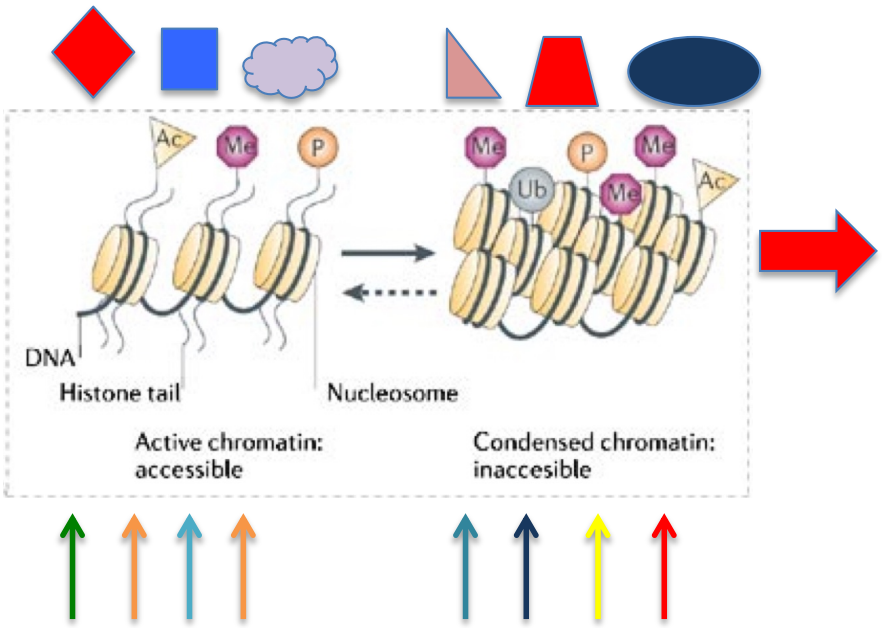
- Functionally redundant
- Reversible
- Heritable to next generation of cells

MULTIPLE EPIGENETIC WRITERS

Impose multiple modifications at histone tails and DNA

CONCEPT OF EPIGENETIC WRITER AND READERS

MULTIPLE EPIGENETIC READERS



MULTIPLE EPIGENETIC WRITERS

DNA METHYLATION

Specific expression of transcription factors epigenetic writers and epigenetic readers create an epigenome along the genome that defines specific gene expression programs

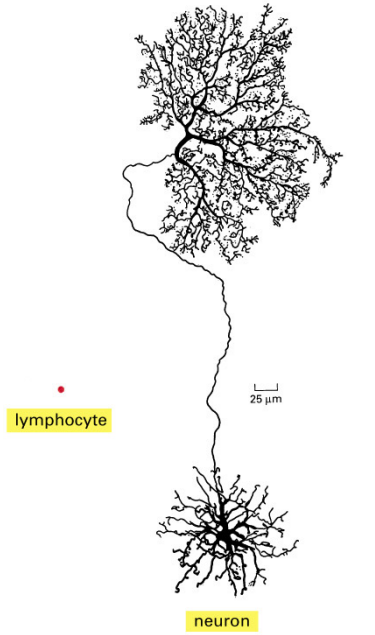


Figure 7–1. Molecular Biology of the Cell, 4th Edition.

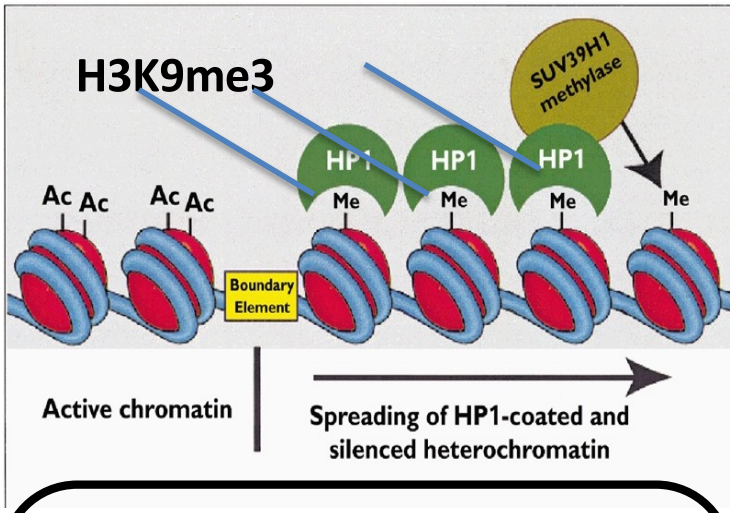
GENE EXPRESSION PROGRAMS: →CELL TYPE SPECIFIC

→NORMAL/ CANCER-DISEASE

Key factors in epigenetics: 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



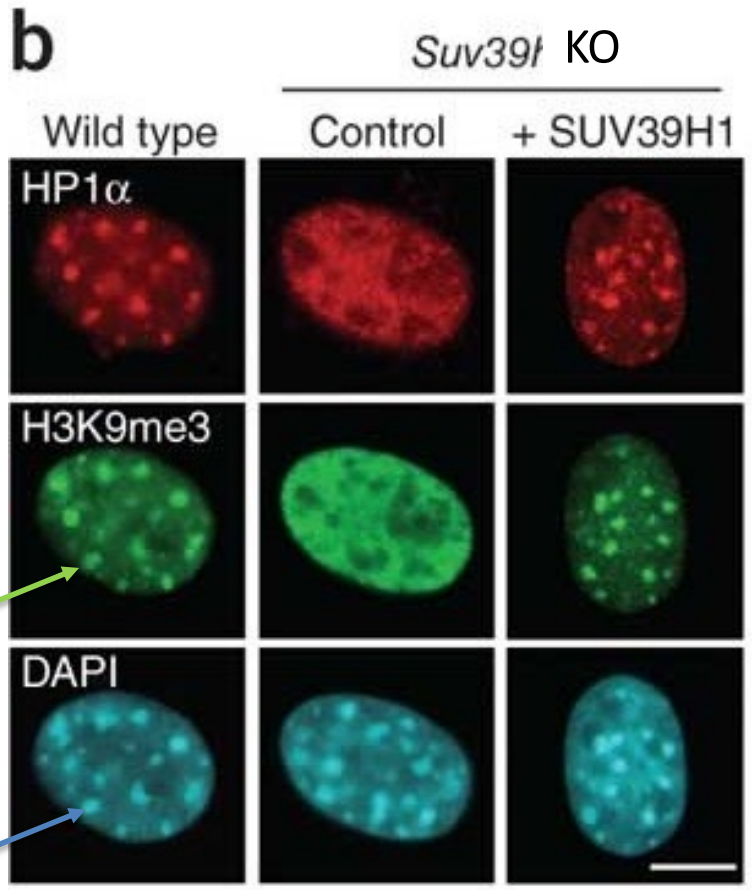
Suv39h1/2 tri-methylate Lysine 9 on histone H3

↓

tri-methylated Lysine 9 on histone H3 recruits HP1

↓

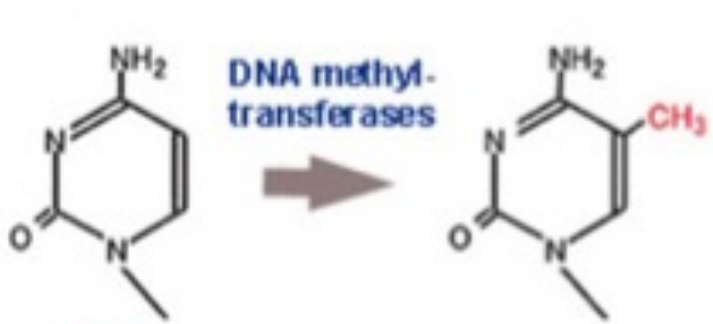
heterochromatin



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

DNA METHYLATION IS "READ" BY SPECIALIZED PROTEINS

Keyfactors in epigenetics: DNA METHYLTRANSFERASES AND METHYL CpG BINDING PROTEINS:



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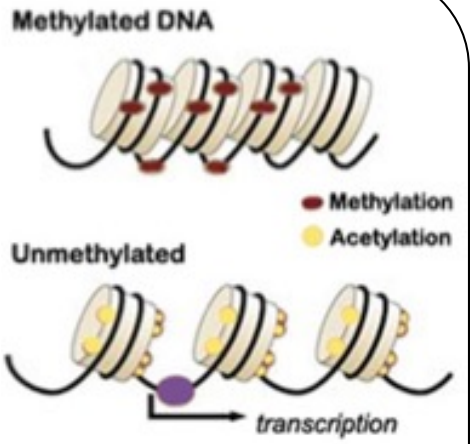
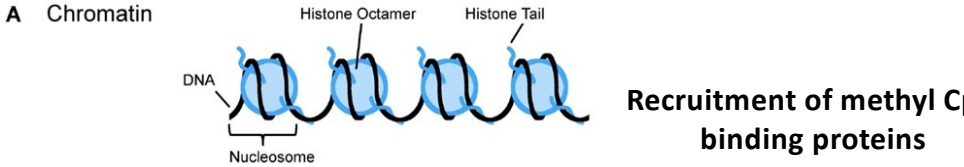
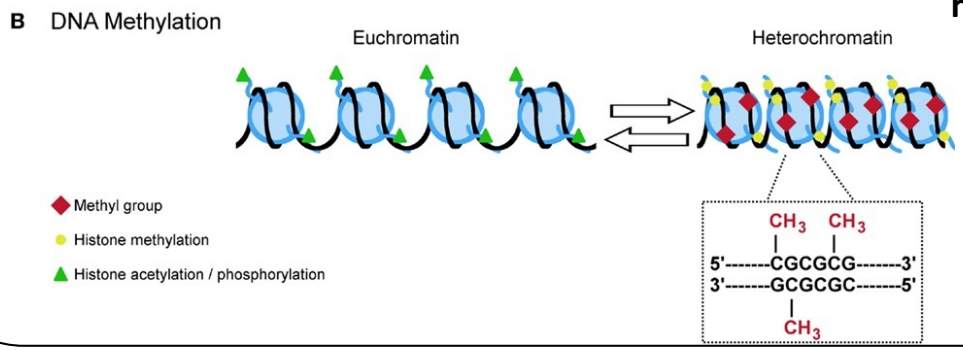




Recruitment of methyl CpG binding proteins MDB, MeCP,…



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

= FUNCTIONAL REDUNDANCY TO ENSURE REPRESSION

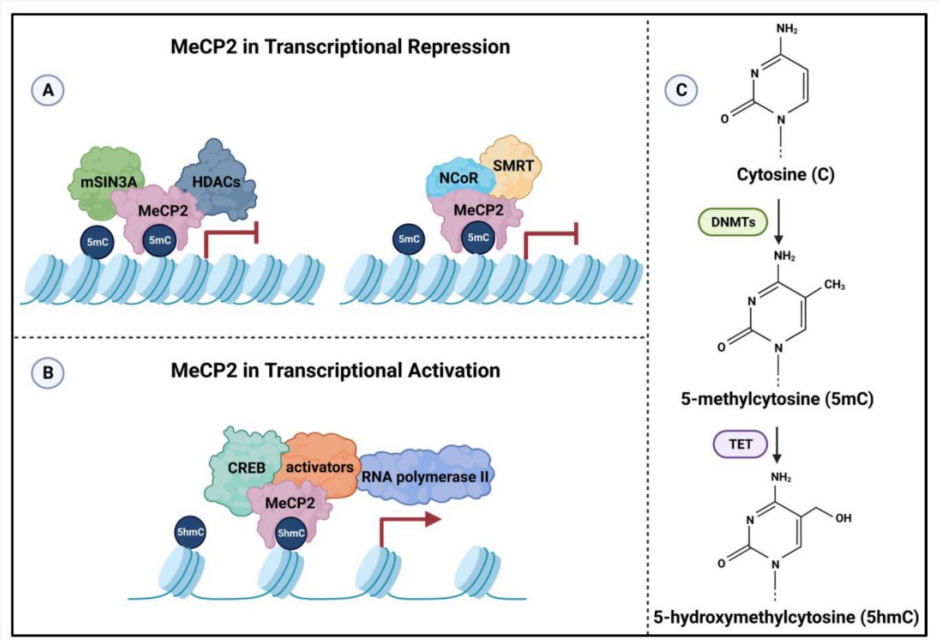
DNA methylation:
Yeast:
NO
C. elegans:
NO
D. melanogaster:
YES, but very low levels
Vertebrates: HIGH, Very important

DNA METHYLATION IS "READ" BY SPECIALIZED PROTEINS

Keyfactors in epigenetics: DNA METHYLTRANSFERASES AND METHYL CpG BINDING PROTEINS:

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 methylation (5mC but also 5hmC at lower affinity

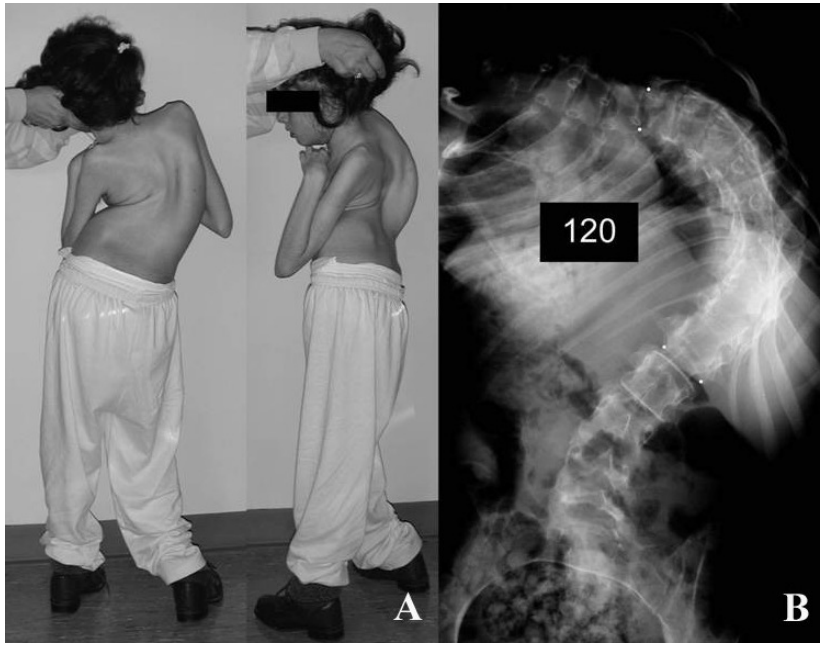


MeCP2 interacts with other factors mediates:

- Gene repression
- Gene activation

MeCP2 mutants show aberrant gene expression → phenotype

Rett syndrome is caused by mutations in MeCP2



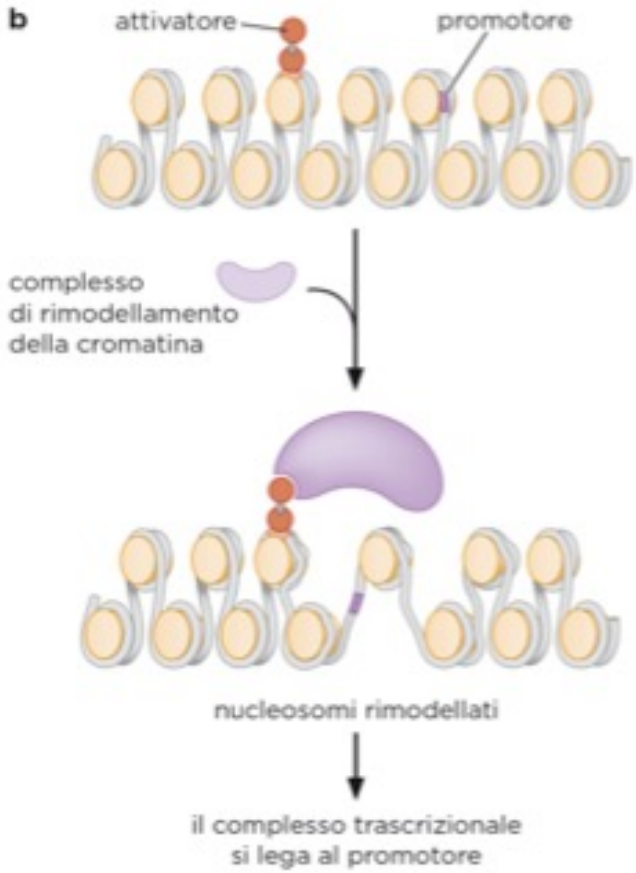
Rett syndrome (RTT), originally termed cerebroatrophic hyperammonemia is a rare genetic postnatal neurological disorder of the grey matter of the brain that almost exclusively affects females but has also been found in male patients. X-linked dominant. The clinical features include small hands and feet 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. Life expectation: 40-50 yrs

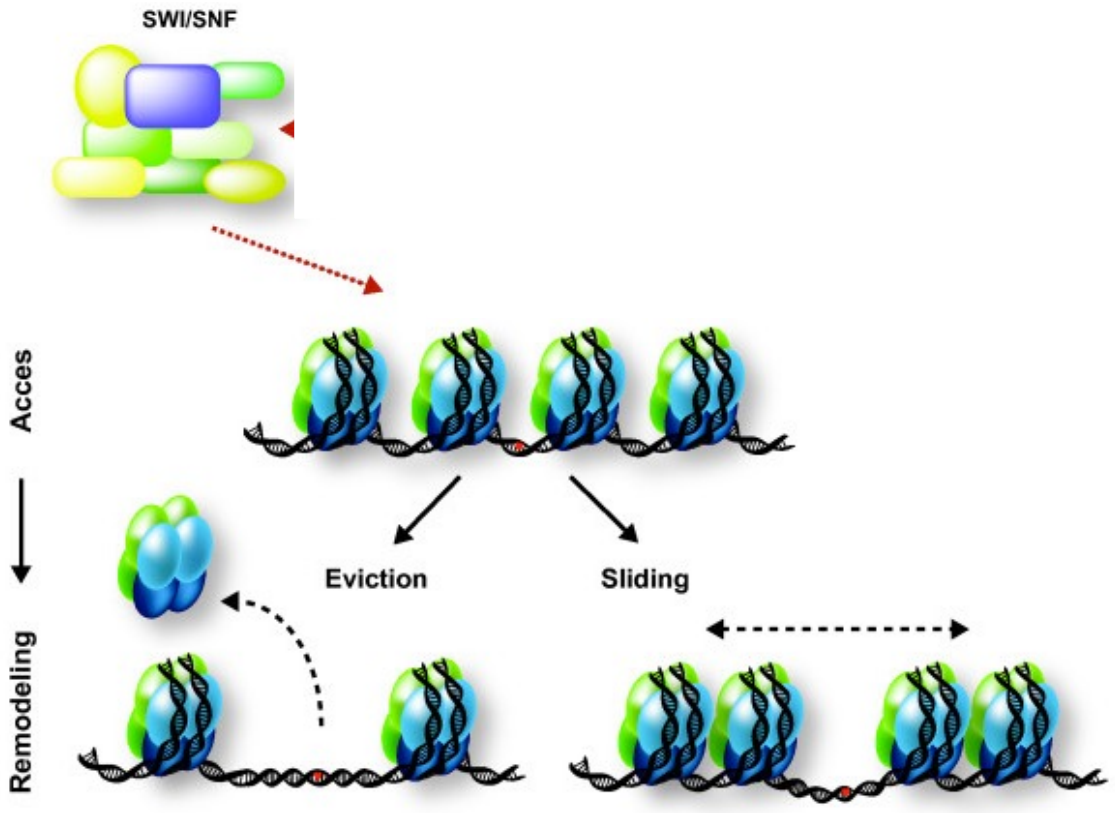
CHROMATIN IS NOT STATIC: CHROMATIN REMODELLING COMPLEXES CAN MOVED OR ELIMINATE OCTAMERES

- Transcriptional 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 (promoters, enhancers,…)
→acetylated tails serve as a binding site for bromo-domain proteins (TFIIH contains such protein)



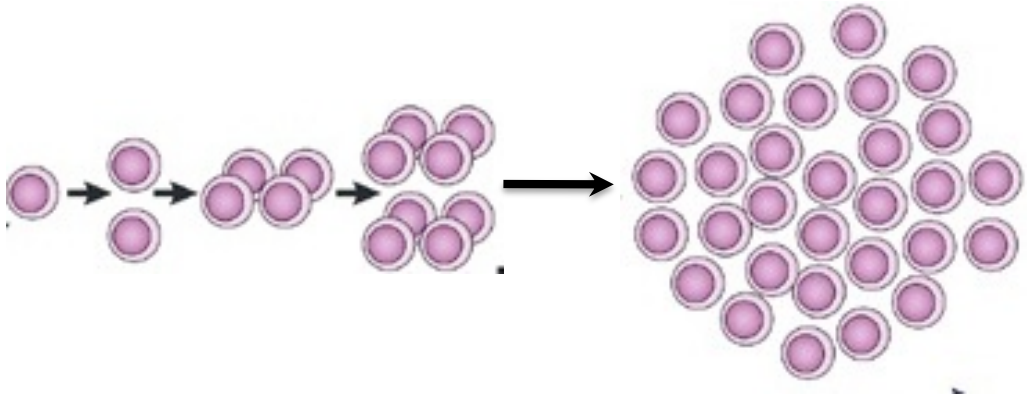


Chromatin has memoy:

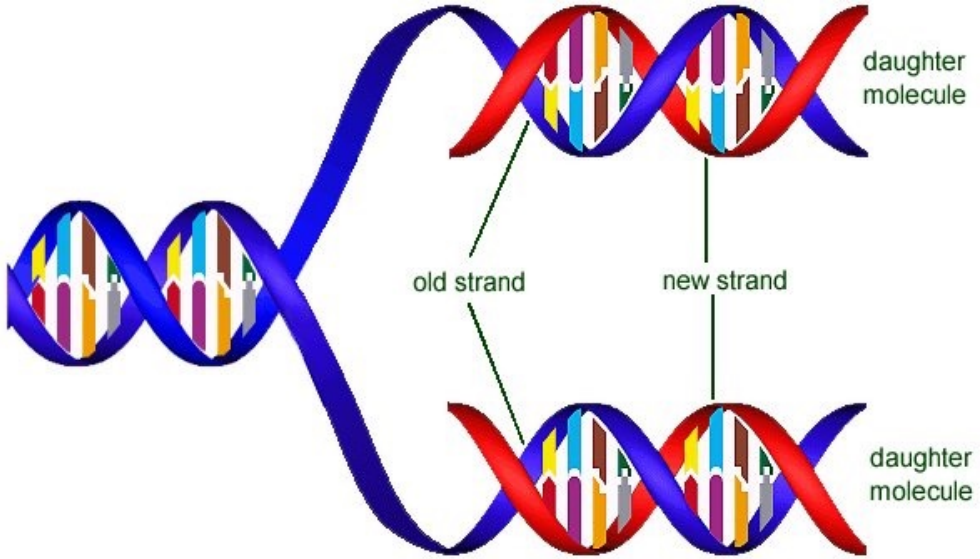
- Maintenance of epigentic information

MANTENAINACE OF EPIGENTIC INFORMATION: how can epigentic information be conserved after dna replication??

Cell proliferation



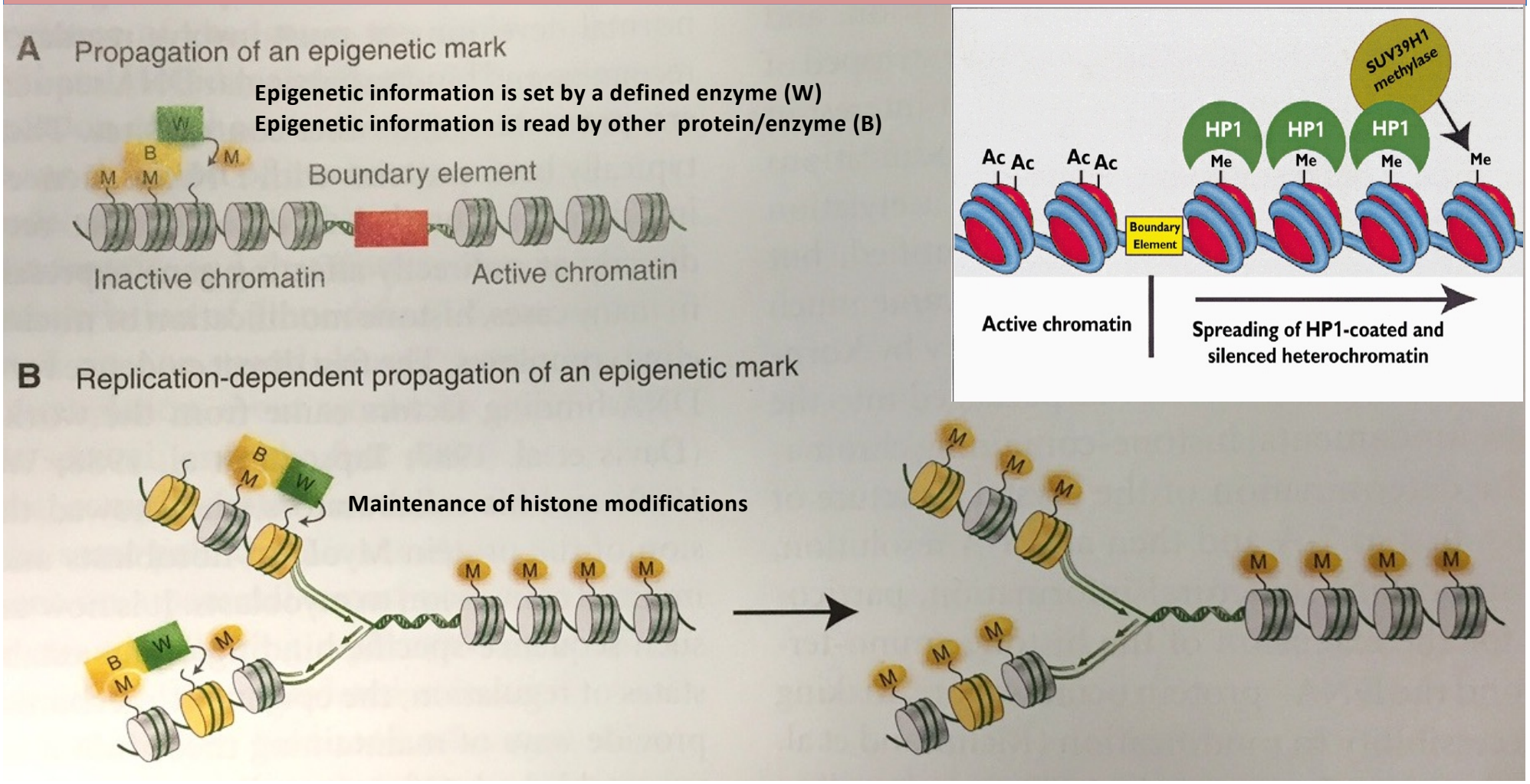
SEMICONSERVATIVE DNA REPLICATION



WHAT IS HAPPENING WITH EPIGENTIC INFORMATION DURING DNA REPLICATION

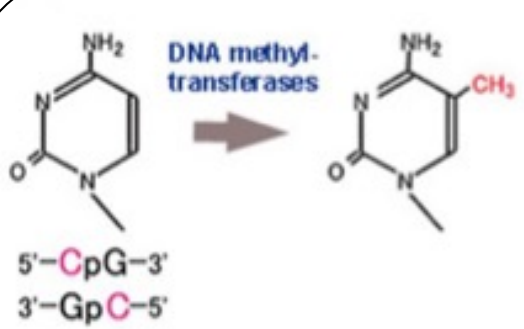
HOW CAN IT BE MAINTANED DURING SEMICONSERVATIVE REPLICATION??

The propagation of epigenetic marks on histones

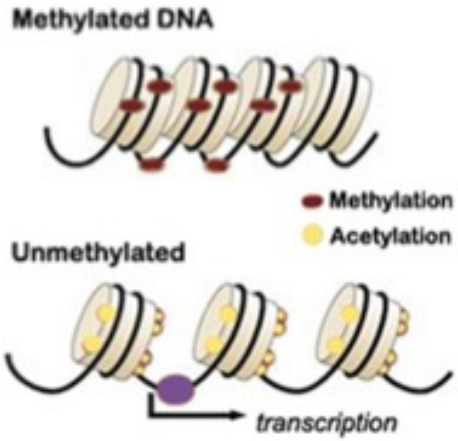


S-Phase: New, randomly deposited histones octameres without histone modifications are inserted during DNA replication. Epigenetic writers associated with the parental DNA now Impose the parental histone code to newly incorporated histones. Epigenetic code is maintained in both daughter cells

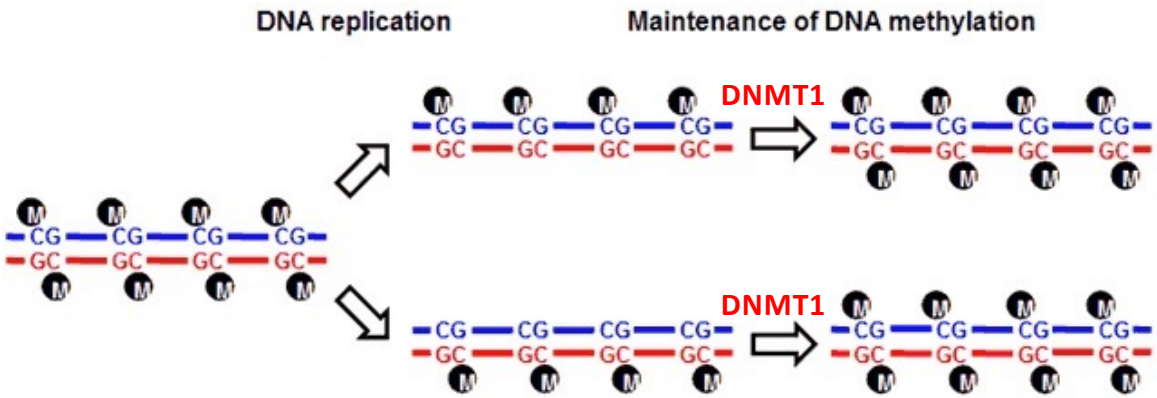
The propagation of epigenetic marks on DNA



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 RECRUITMENT OF ENZYMATIC ACTIVITIES (EPIGENTIC WRITERS)

→ RERUITMENT OF EPIGENTIC READERS TO TRANSLATE EPIGENTIC MODIFICATIONS INTO FUNCTION

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

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

→ CONTROL OF 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

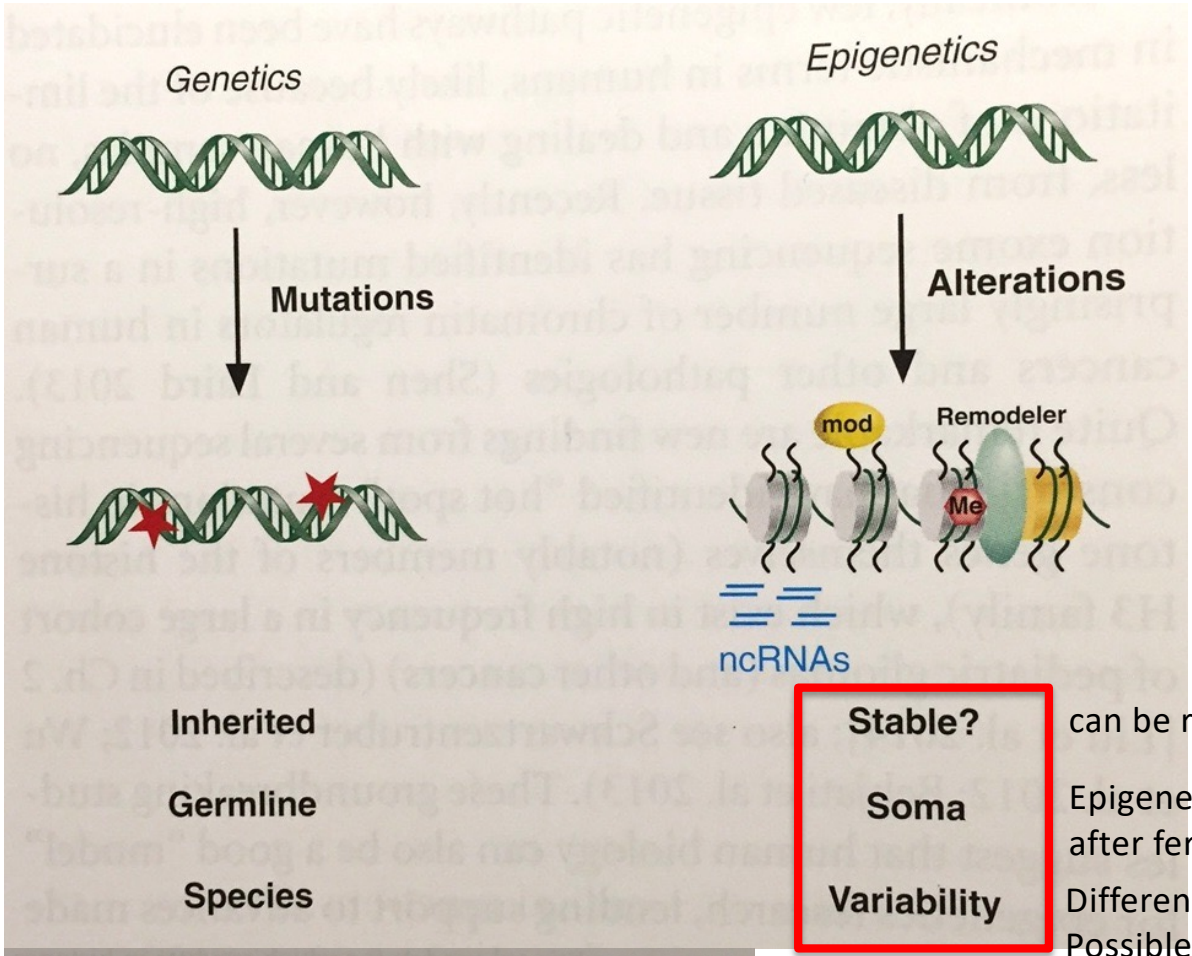


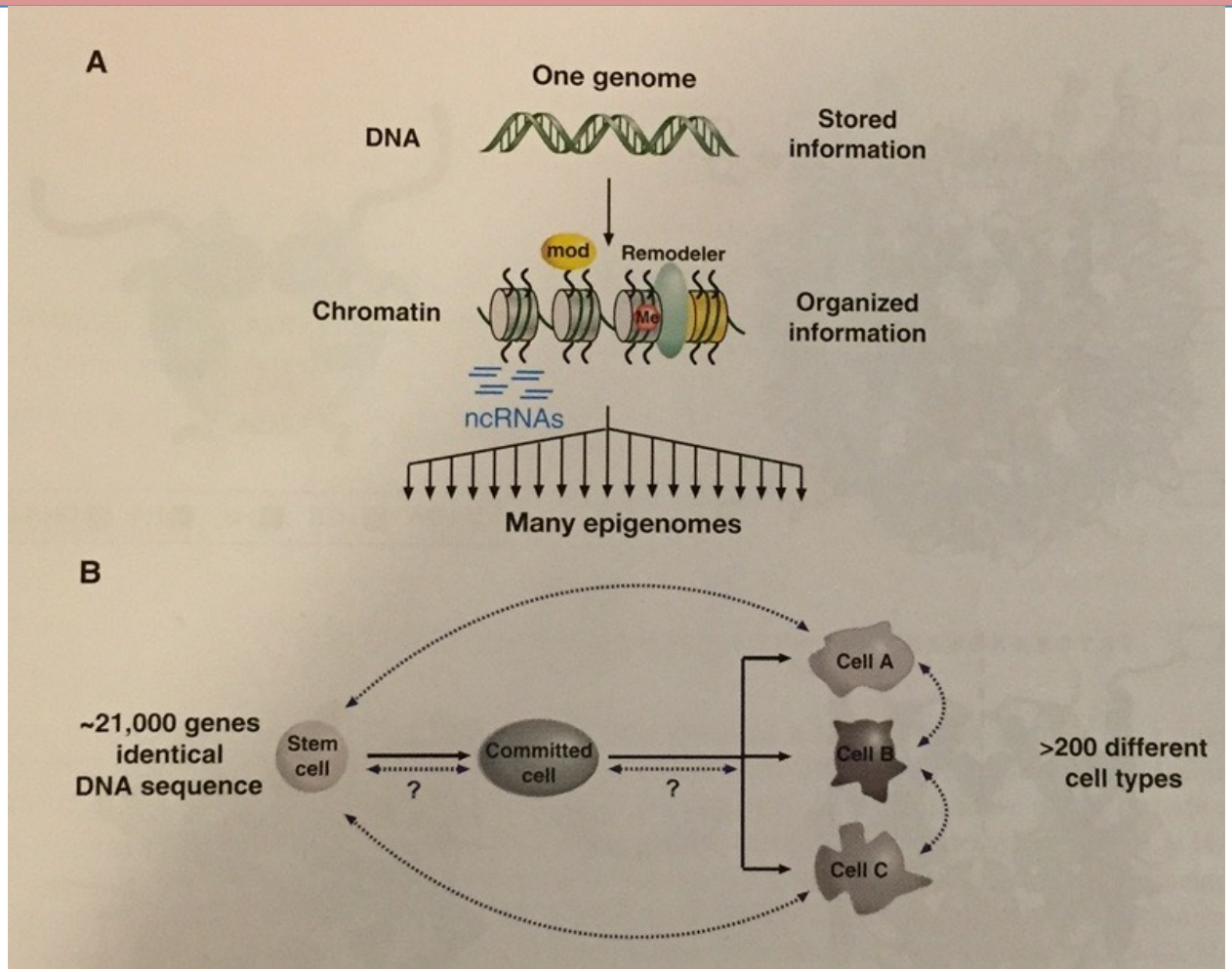
Figure 3. Genetics versus epigenetics. Genetics: Mutations (red stars) of the DNA template (green helix) are heritable somatically and through the germline. Epigenetics: Variations in chromatin structure modulate the use of the genome by (1) histone modifications (mod), (2) chromatin remodeling (remodeler), (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

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

Understanding of molecular principles

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