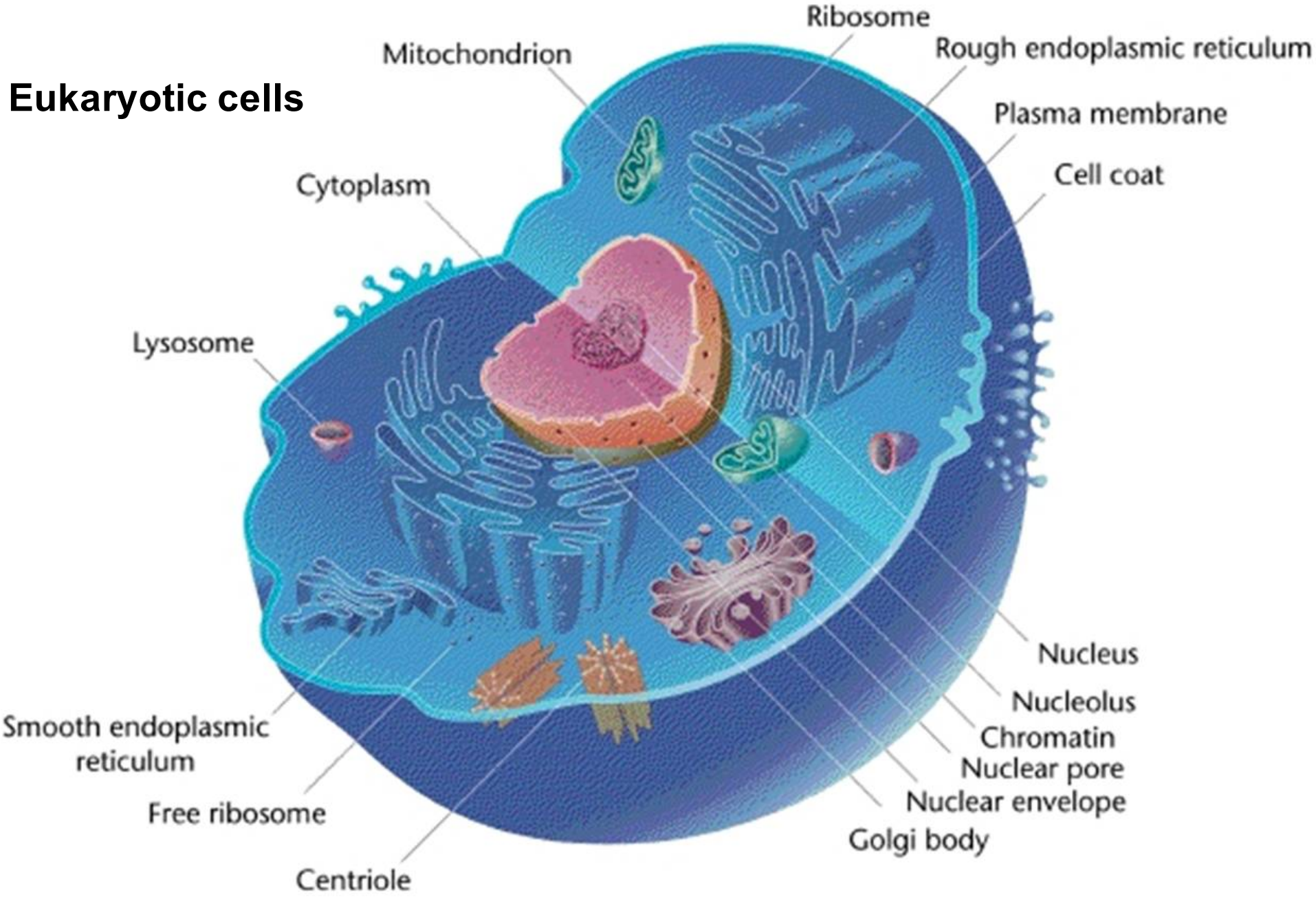
Controllo dell'espressione genica negli eucarioti

1. **Regulation of transcription**
- Introduction
- Transcription factors – Activators of transcription
- Basic mechanisms of transcriptional activation
- Integration of signals
- Signal transduction

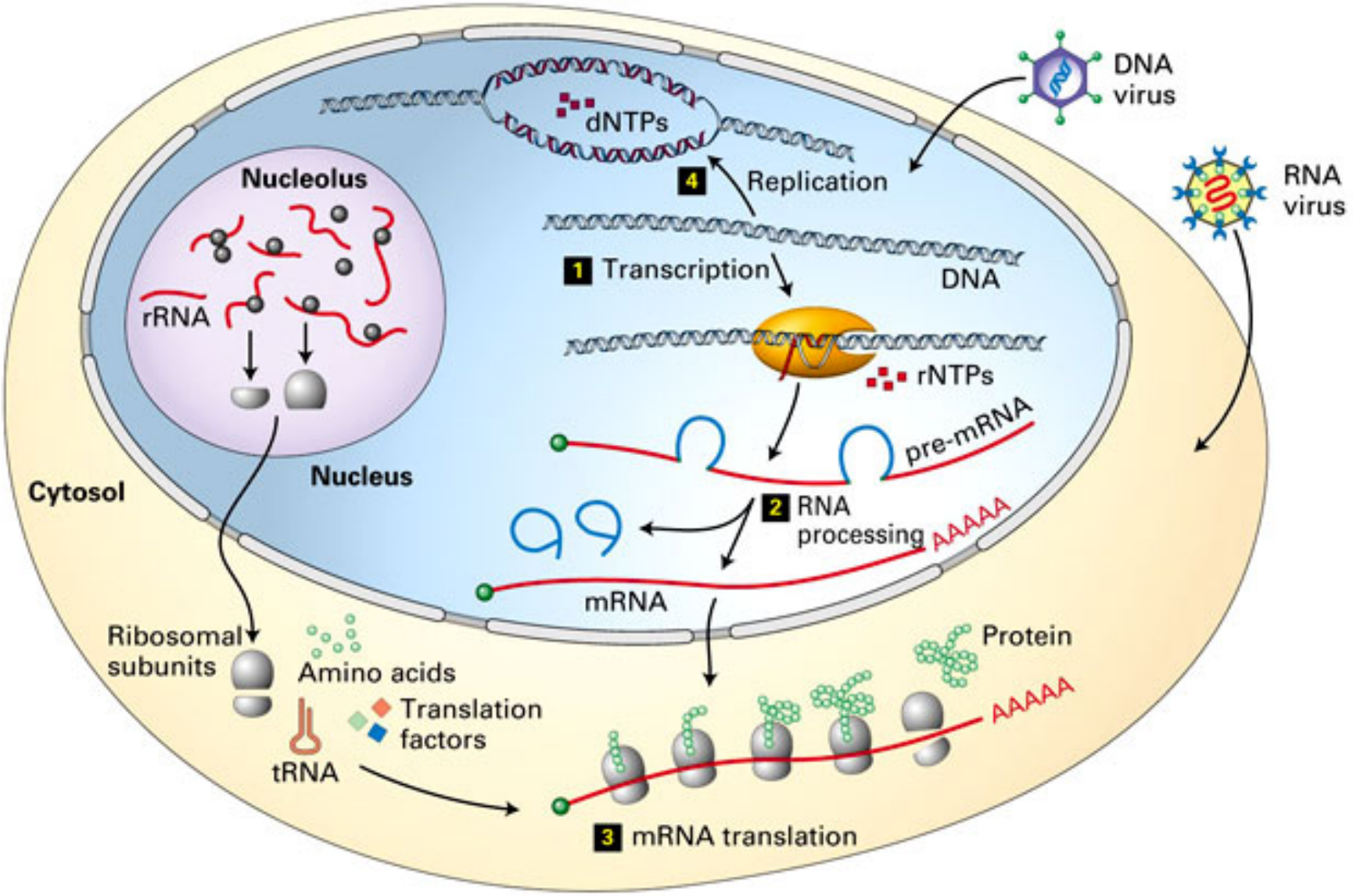
2. **Post-transcriptional gene regulation**
- Chromatin regulation
- ncRNA - miRNAs

INTRODUCTION

Eukaryotic cells



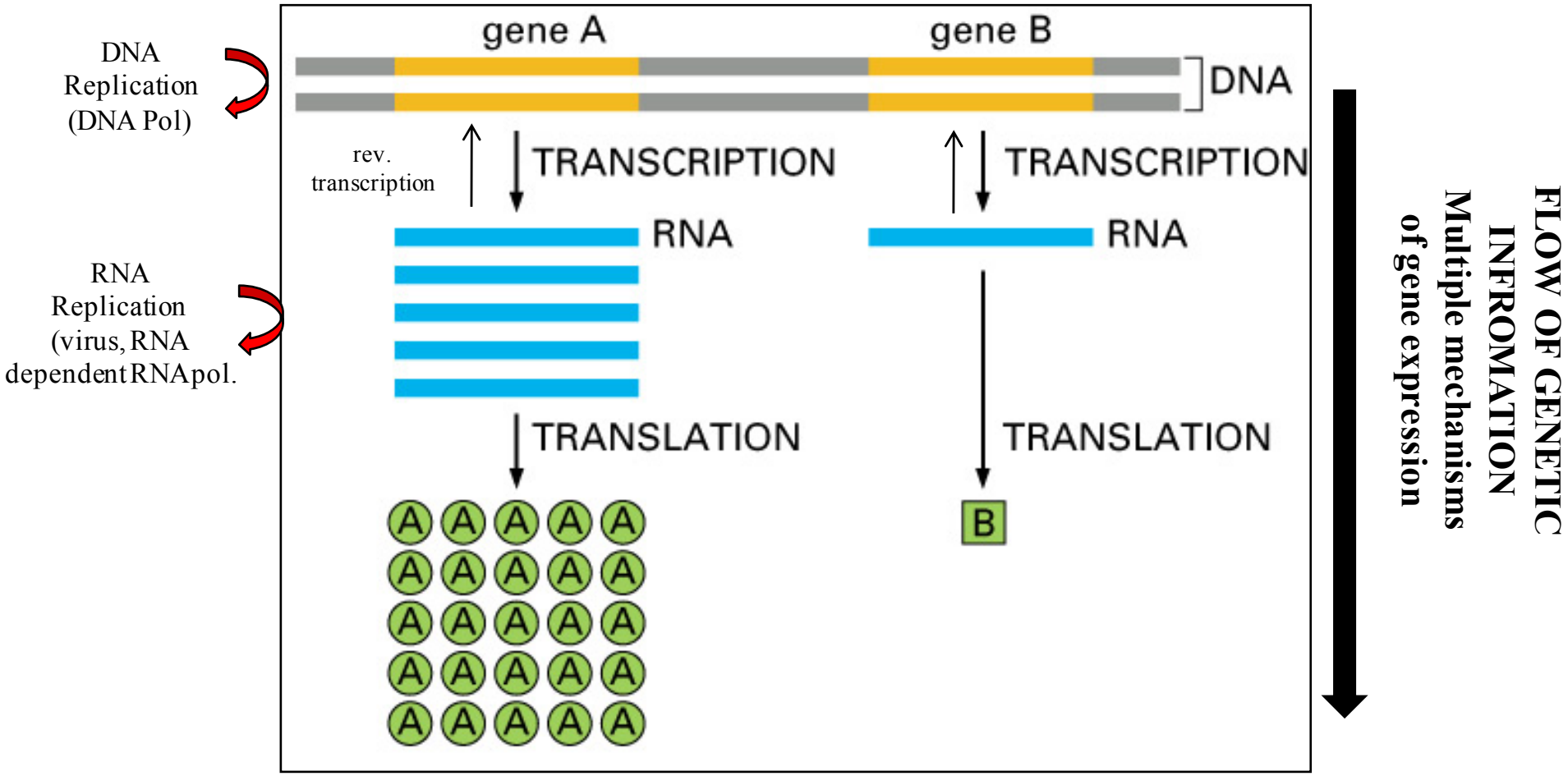
The flow of information in eukaryotic cells



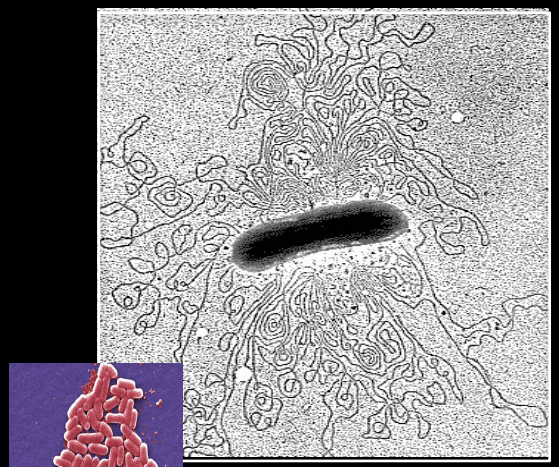
"IL DOGMA CENTRALE"

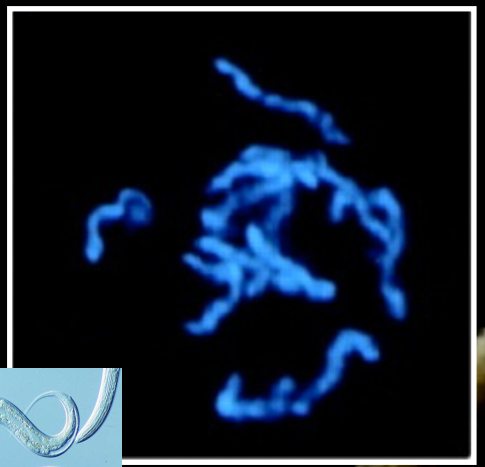
DNA→RNA→PROTEIN

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.



The coding and non-coding genome evolution

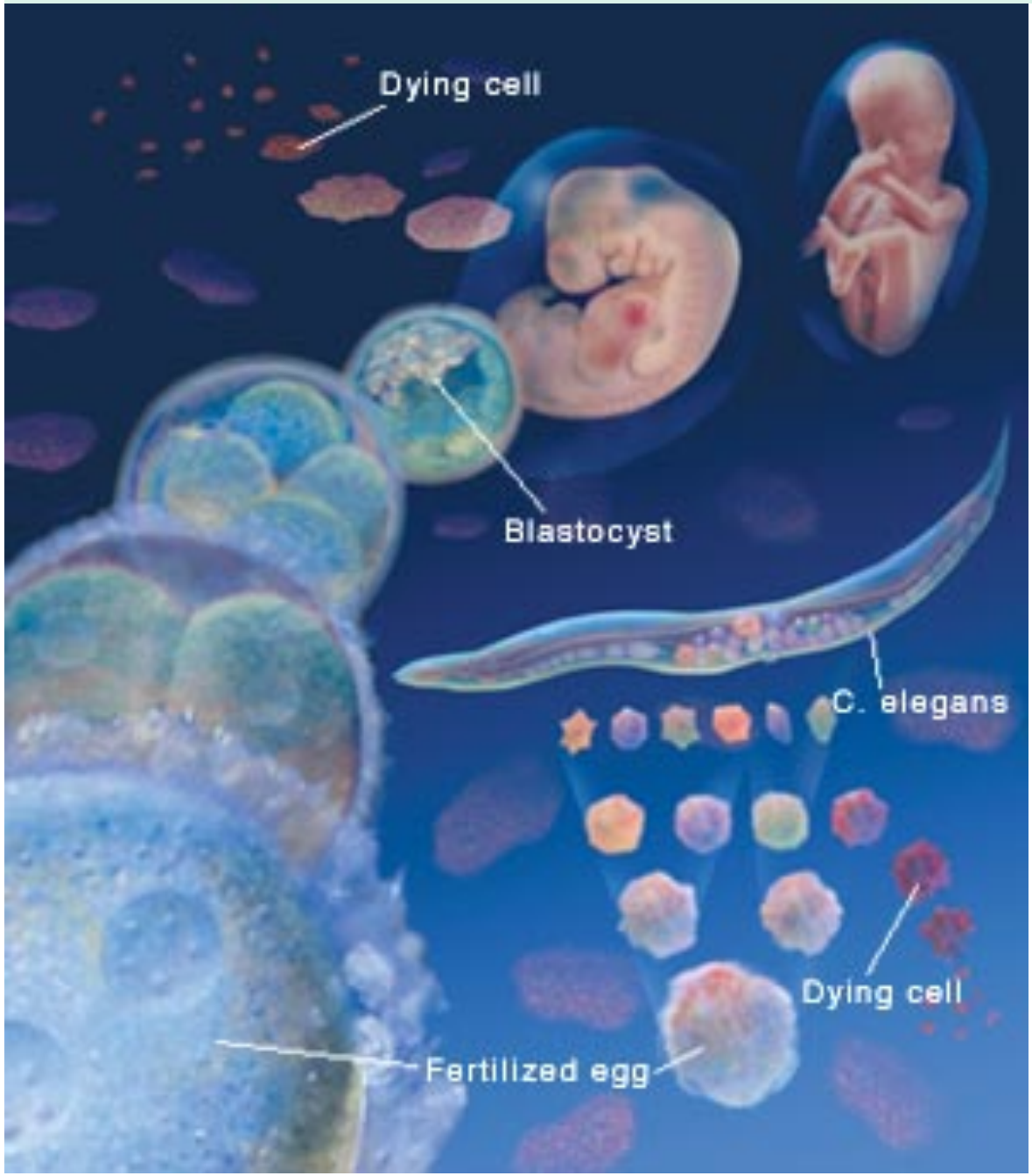




Genome	$5x10^6$ bp	$1x10^8$ bp	$3x10^9$ bp
Chromosomes	1	6	23
Coding genes	6692	20541	21995
ncDNA	5%	60%	**98%**
non-coding RNA genes	15	23136	ca. 40000
miRNAs	0	224	4274
pseudogenes	21	1522	10616

ENSEMBL 11/2014

Differential gene expression in organismal development



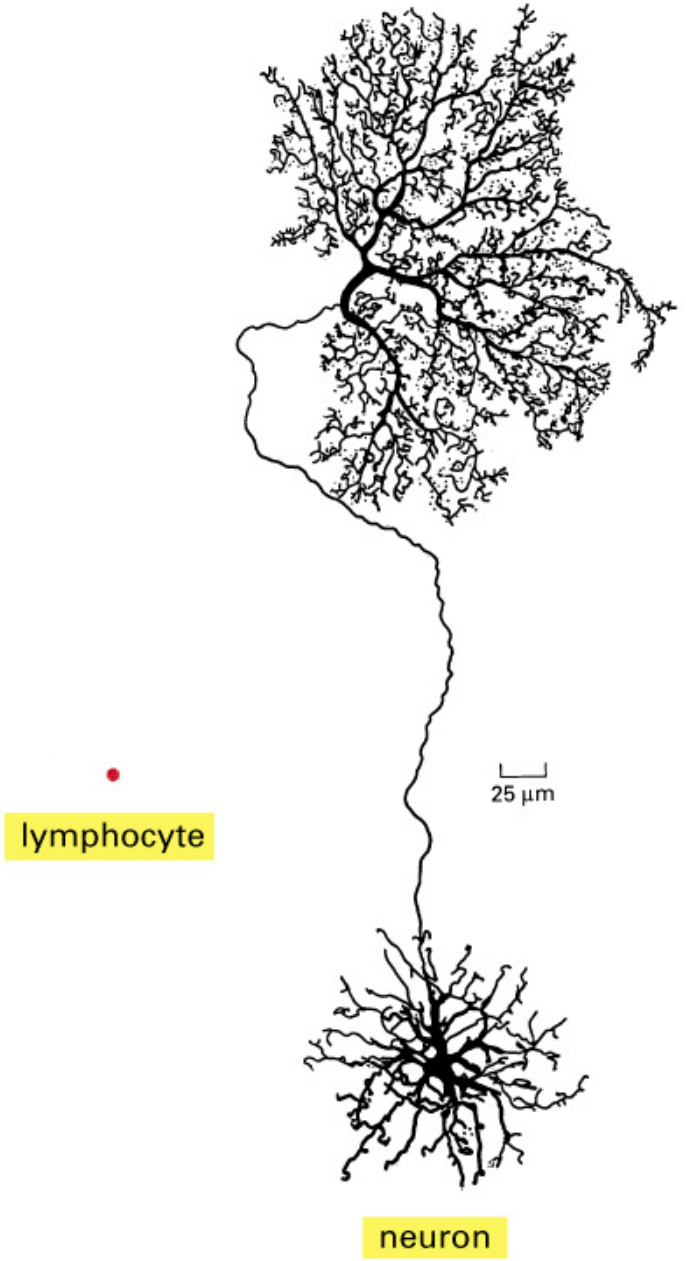
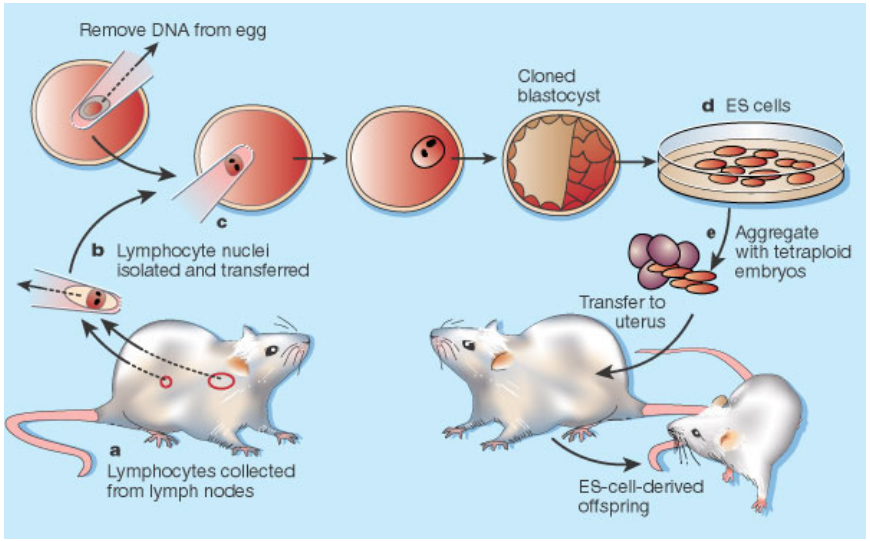


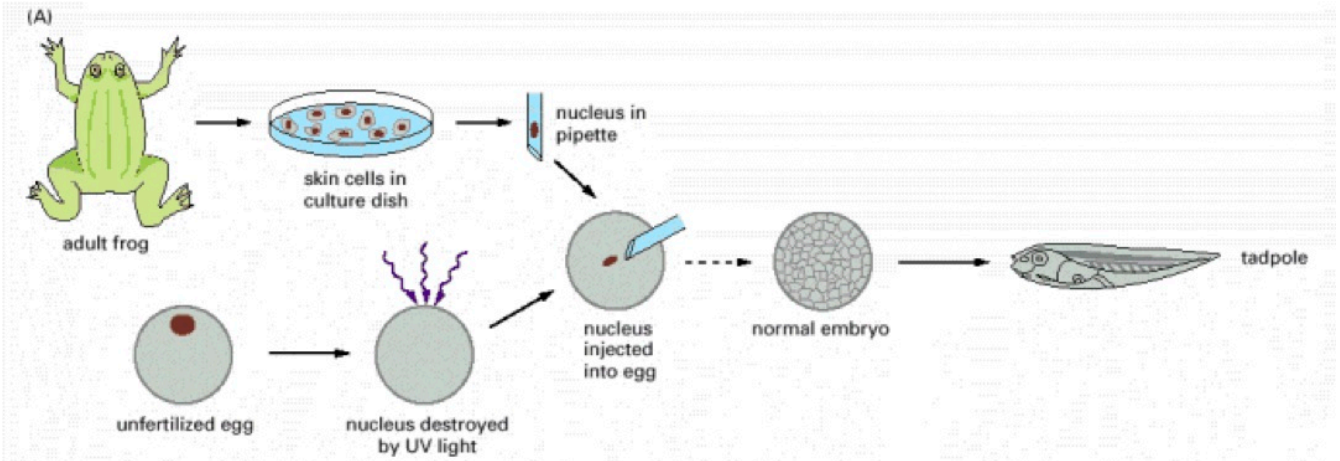
Figure 7–1. Molecular Biology of the Cell, 4th Edition.

Evidence that a differentiated cell contains all the genetic instructions necessary to direct the formation of a complete organism

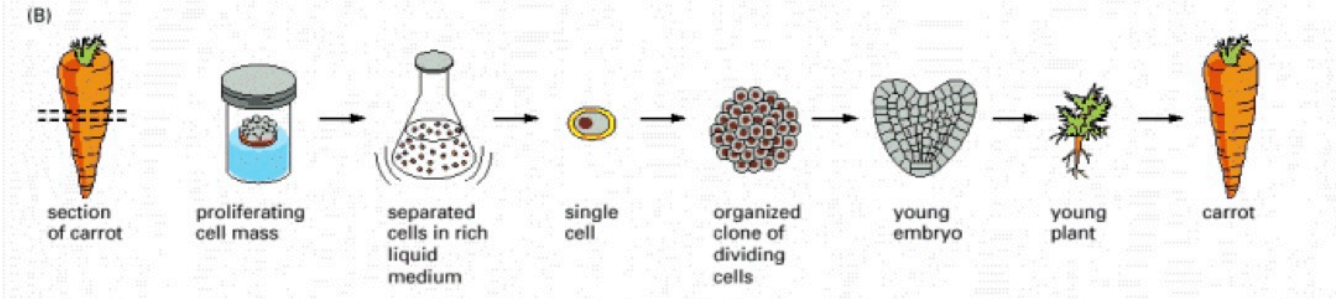
mouse



frog



plants



The nucleus of a skin cell from an adult frog/mouse transplanted into an enucleated egg can give rise to an entire tadpole(mouse)
→ nuclear cloning

In many types of plants, differentiated cells retain the ability to "dedifferentiate," so that a single cell can form a clone of progeny cells that later give rise to an entire plant.

Tissue
spcific gene expression

- 1800 genes
- green = low expression
- red = high expression

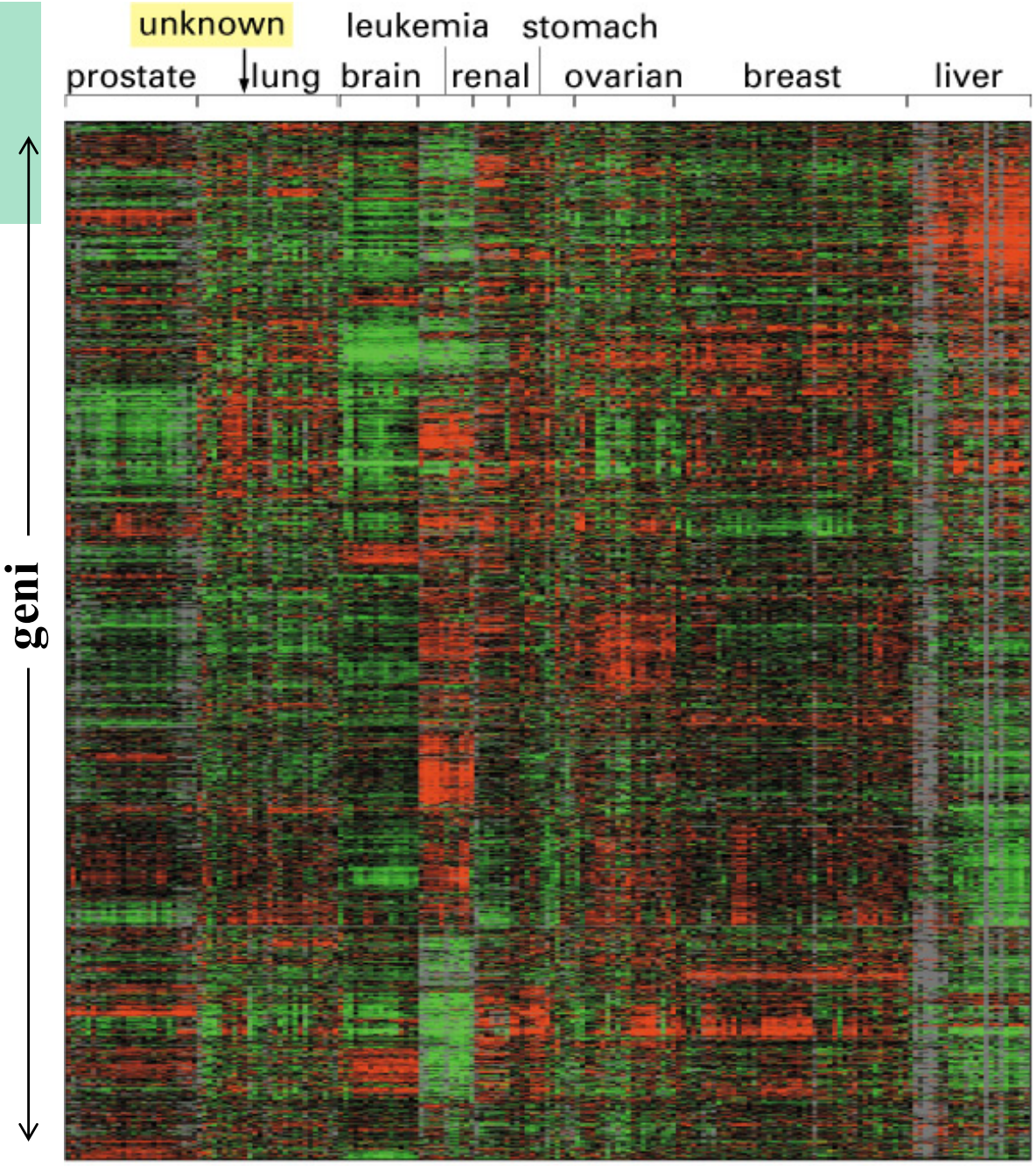
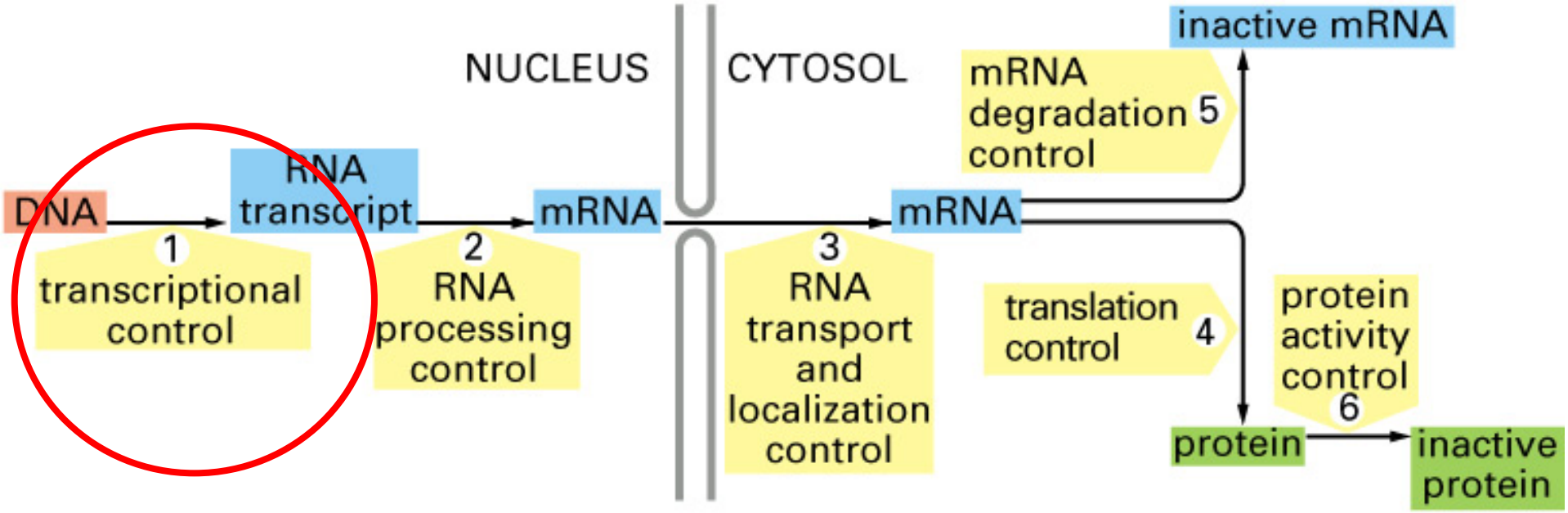


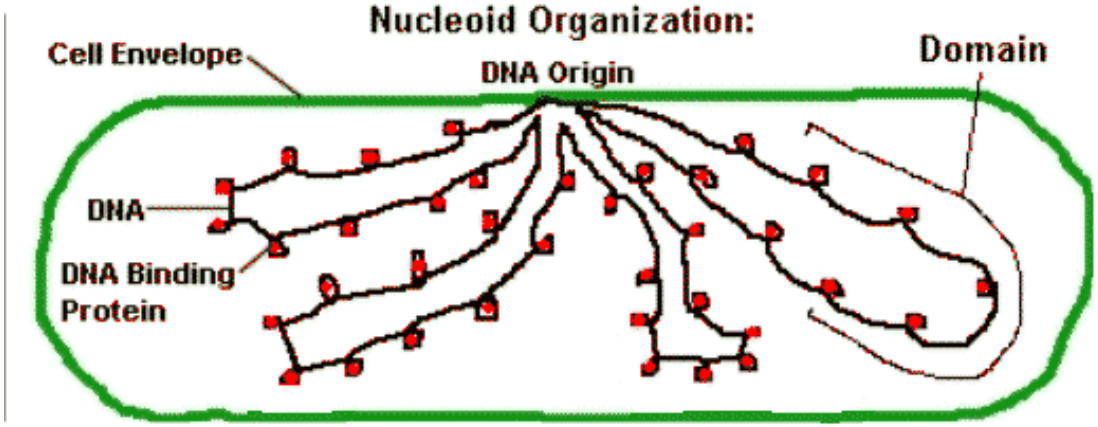
Figure 7–3. Molecular Biology of the Cell, 4th Edition.

Gene expression is regulated at multiple levels

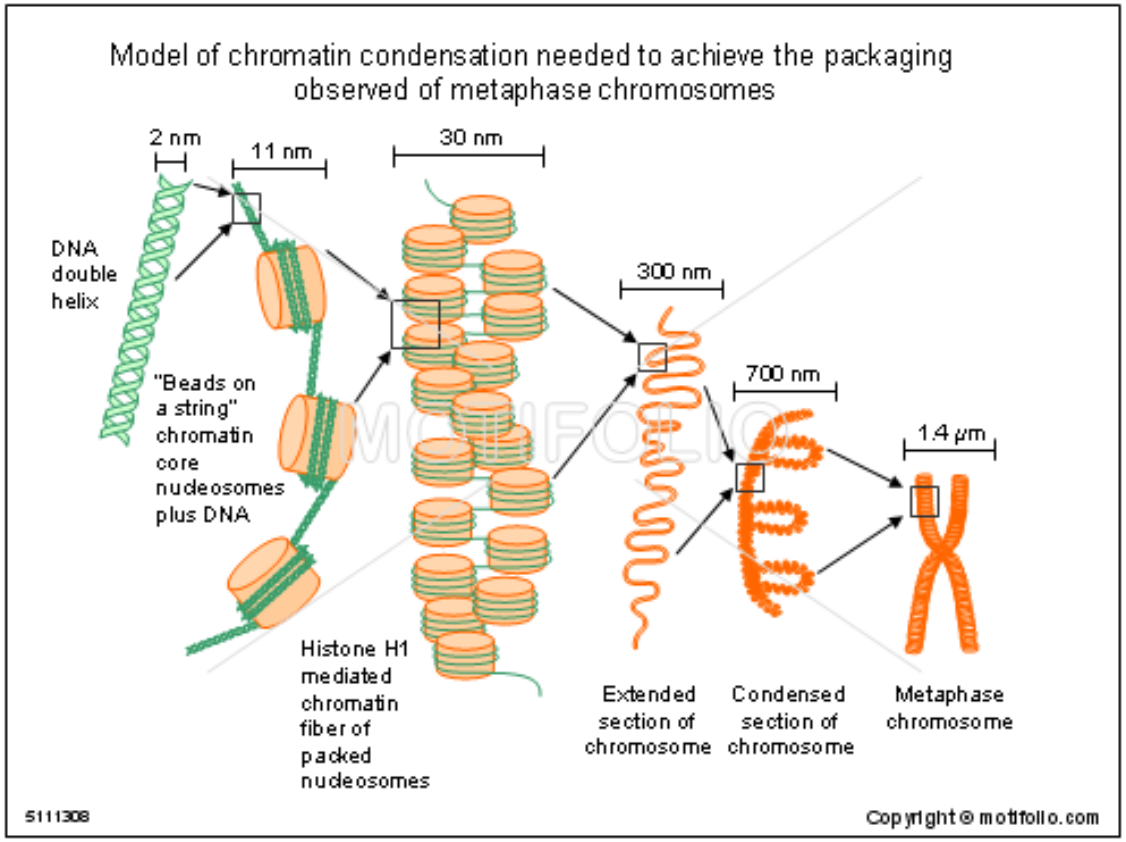


Differences between pro- and eukaryotes that increase the complexity of gene expression

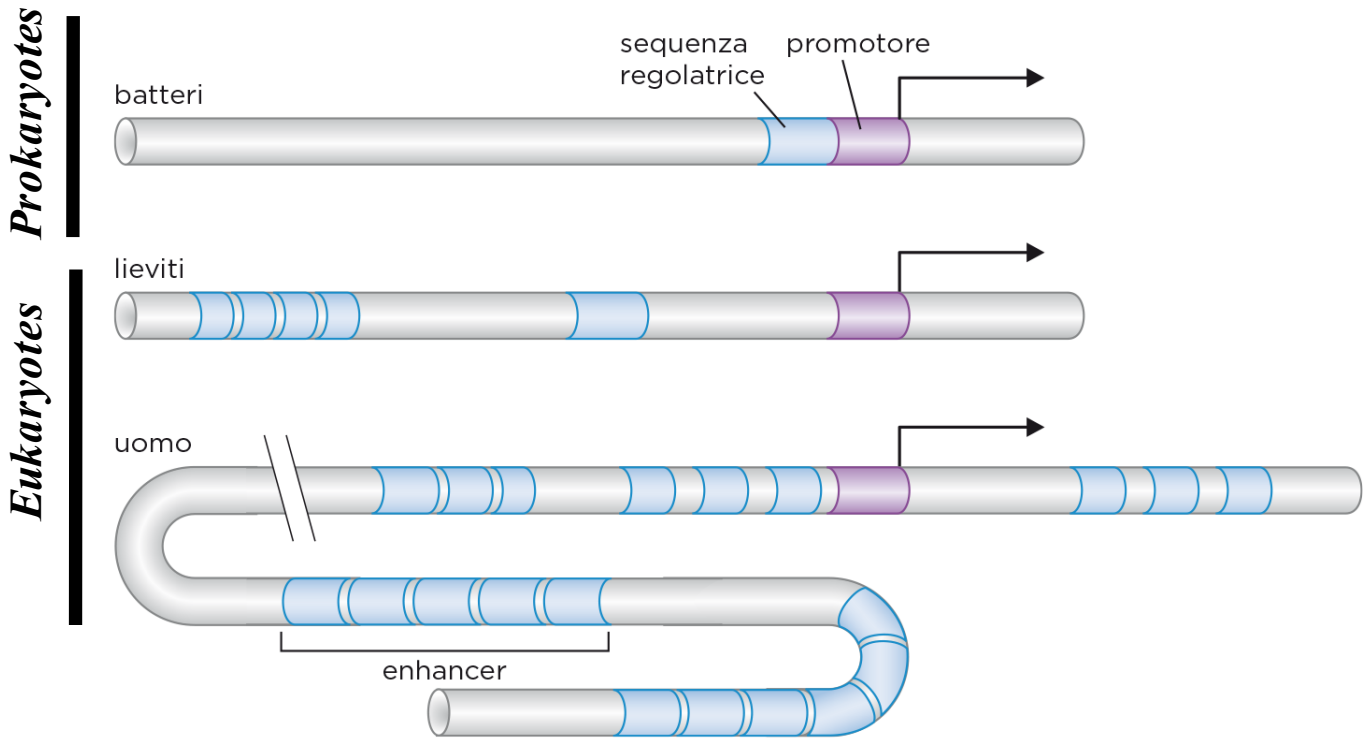
Simple chromatin
→Direct access
of RNA Pol holoenzyme
to promoter
(+/- activator/repressor)



complex chromatin
→Chromatin modifying complexes
chemically modify histones
→change the efficiency of interaction
of transcription factors and RNA Pol II
with target site on DNA



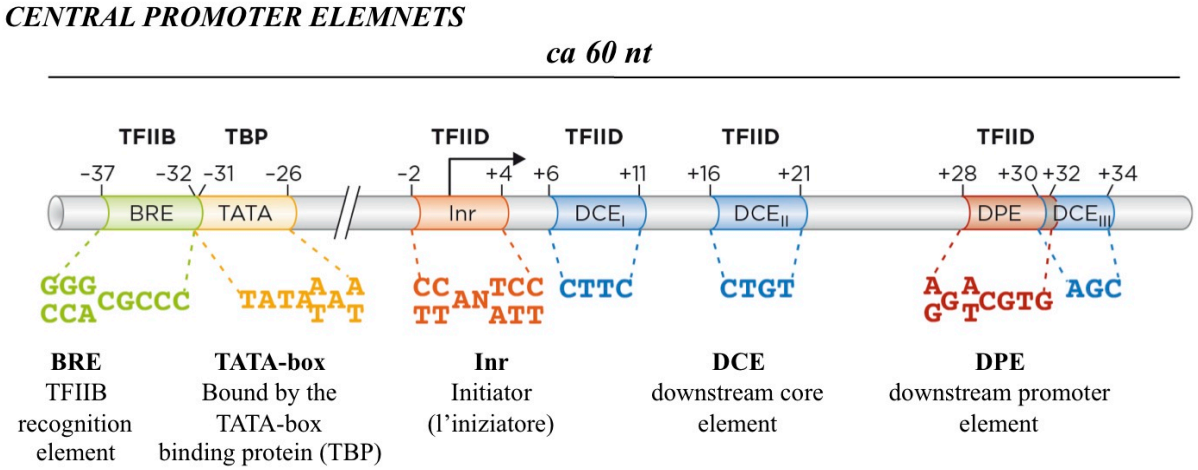
Differences between pro- and eukaryotes that increase the complexity of gene expression

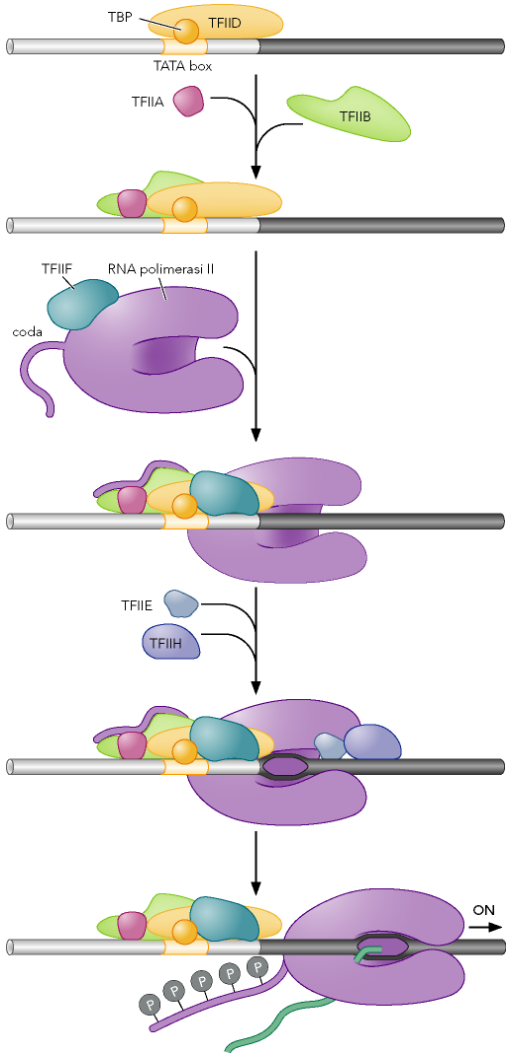


Eukaryotes

- More regulatory proteins
- More regulatory sequences
- Regulatory sequences also far away from the promoter (max 1 Mb)
- Function of regulatory sequences
    - enhancer (1 - x elements): regulate one gene in a particulare moment and/or at different cell types and can respond to signals
    - silencing elements: mediate the repression of a promoter
    - insultors/bounday elements: sequenences that direct enhancer function to a particular gene

Regulatory sequences control the activity of the basal apparatus at the promoter

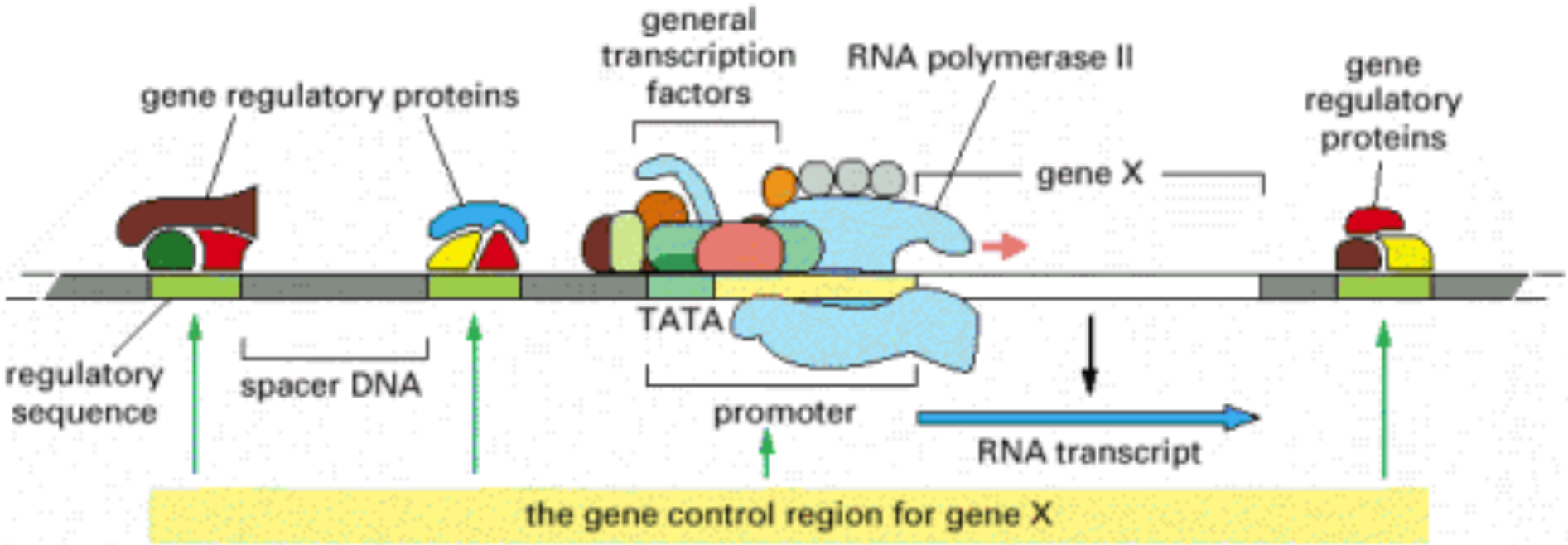




- Binding of **TFIID/TBP** to the TATA box is the first step in initiation.
- Other basal transcription factors bind to the complex in a **defined order**, extending the length of the protected region on DNA.
- When **RNA polymerase II** binds to the complex, it **initiates transcription**.

Regulatory sequences/transcription factors control transcriptional initiation/elongation by the basal apparatus

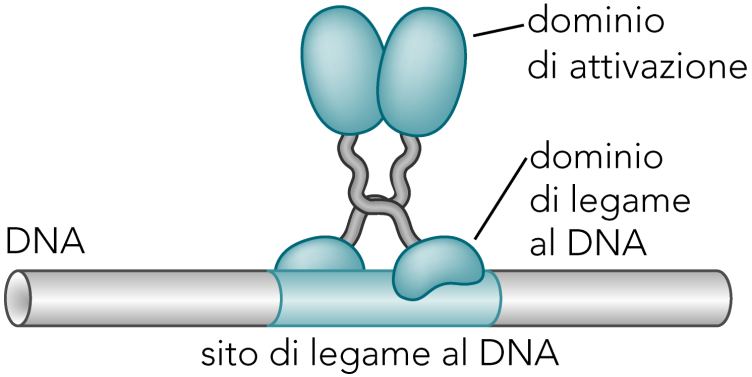
A complex interplay of regualtory sequences and transcription factors control the basal transcription complex



The gene control region of a typical eucaryotic gene. The ***promoter*** is the DNA sequence where the general transcription factors and the polymerase assemble. The ***regulatory sequences*** serve as **binding sites** for **gene regulatory proteins**, whose presence on the DNA affects the rate of transcription initiation. These sequences can be located **adjacent** to the promoter, far **upstream** of it, or even **within introns** or **downstream** of the gene. **DNA looping** is thought to allow gene regulatory proteins bound at any of these positions to interact with the proteins that assemble at the promoter. Whereas the **general transcription factors** that assemble at the promoter are **similar for all** polymerase II transcribed **genes**, the **gene regulatory proteins** and the **locations** of their binding sites relative to the promoter are **different for each gene**.

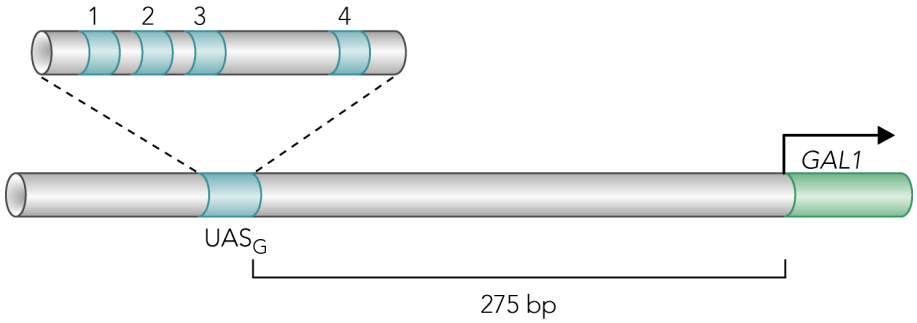
Transcriptional activators

A classic example from S. cerevisiae: Gal4 and the GAL1 gene promoter



Transcriptional activators have a modular composition

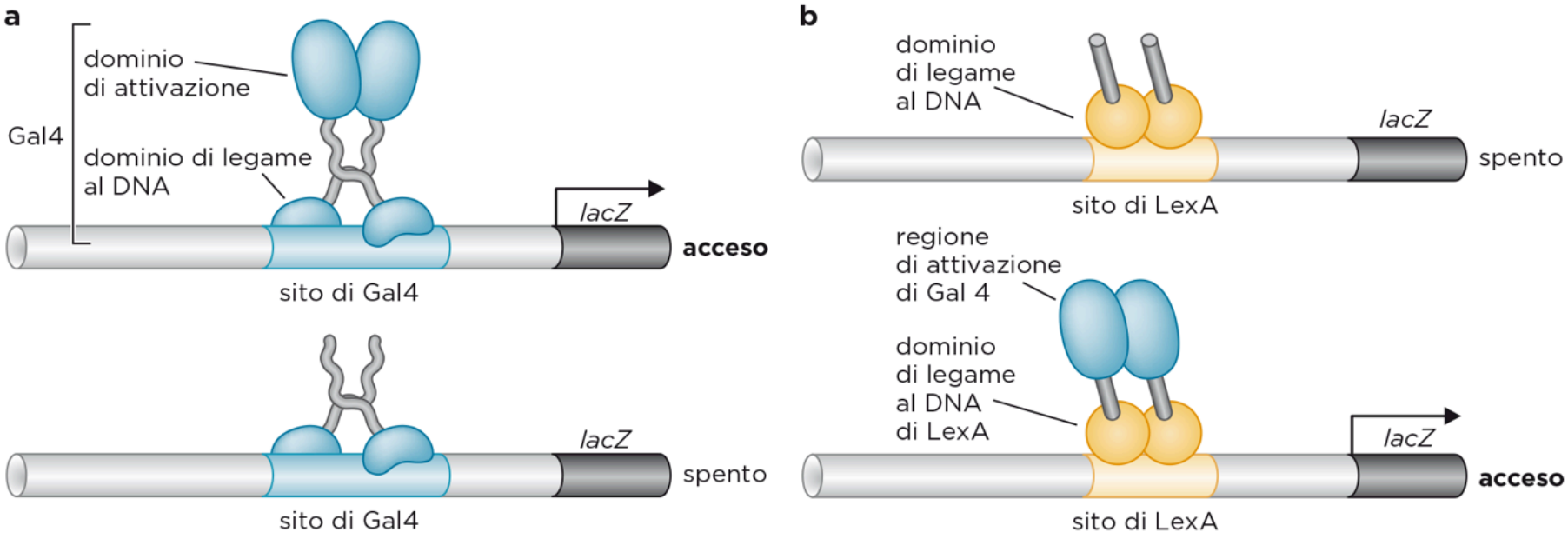
- DNA binding domain → brings TF to regulatory sequence
- Activation domain: Protein-Protein interaction domain → interaction with components of the basal transcription apparatus



- Gal4 dimers (bound to galactose) binds UAS$_G$
  (upstream activating seqeunce of Gal1)
  (4 Gal4 binding sites, each has 17nt)
  Gal1 expression: conversion of Galactose to Glucose

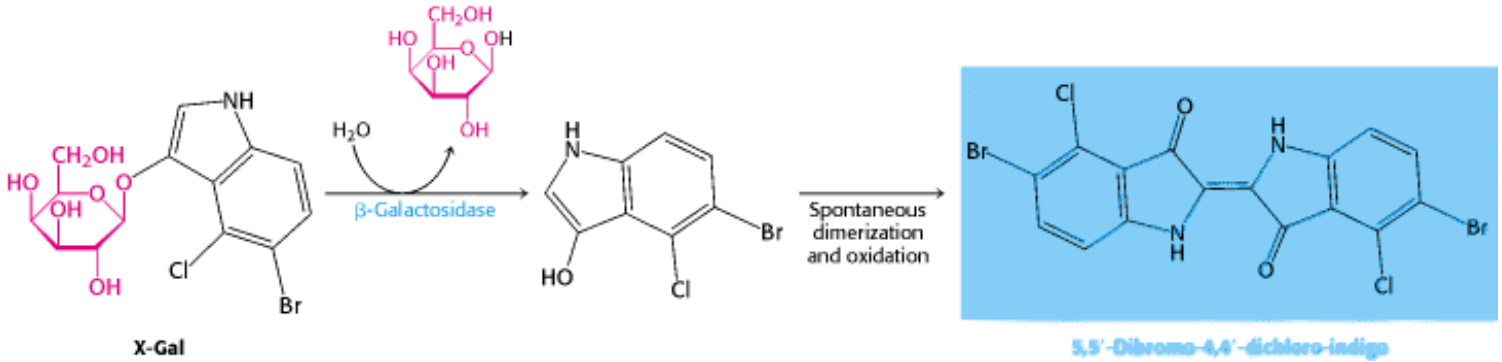
Transcriptional activators have a modular composition

Domains can be exchanged (S. cerevisiae)

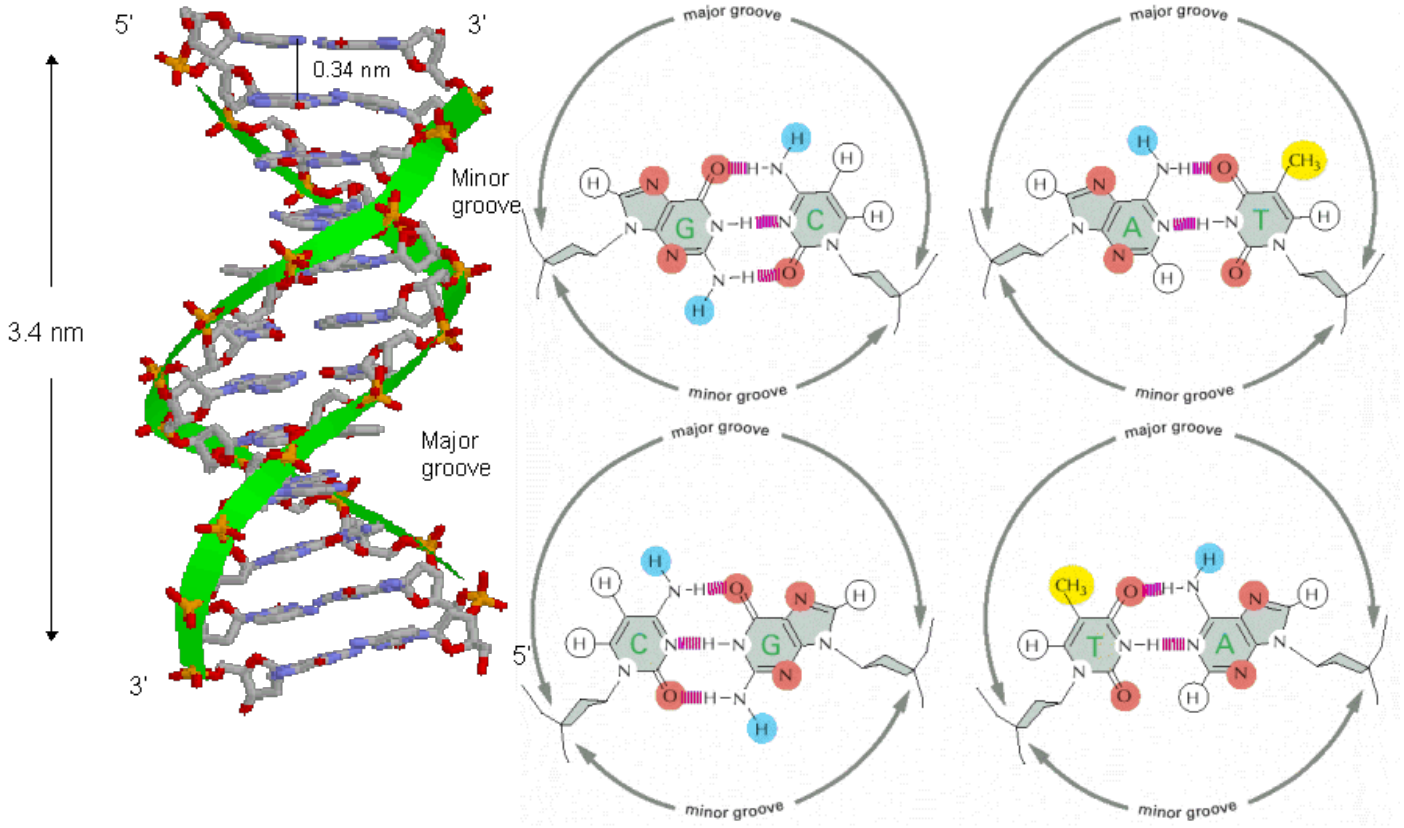


Gal1 regulatory seqeunences upstream of lacZ reporter

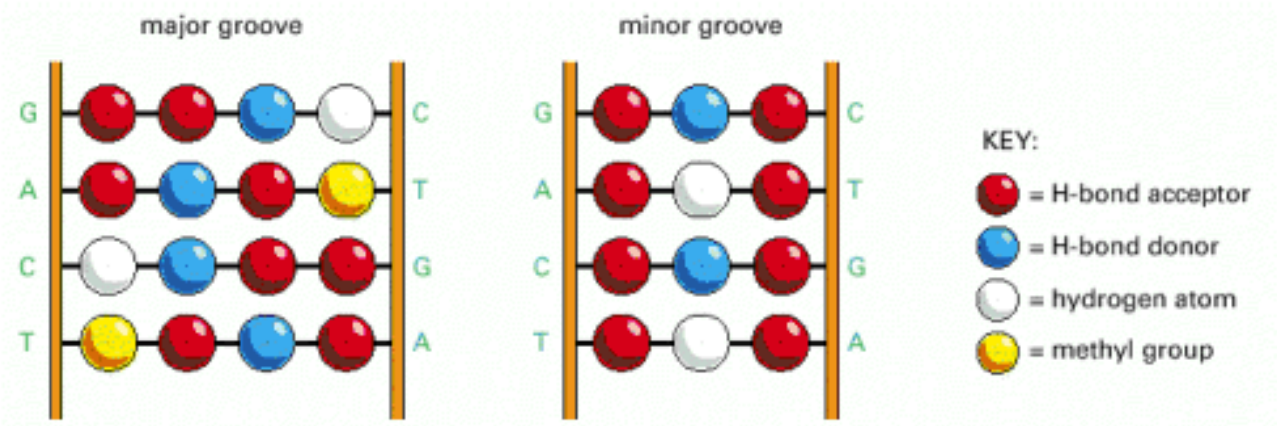
Gal4 transactivation domain fused To the LexA transcriptional activator
→Drives lacZ reporteractivity by binding to
→The LexA binding element



Transcription factors bind to specific positions in the major and minor groove of regulatory sequences



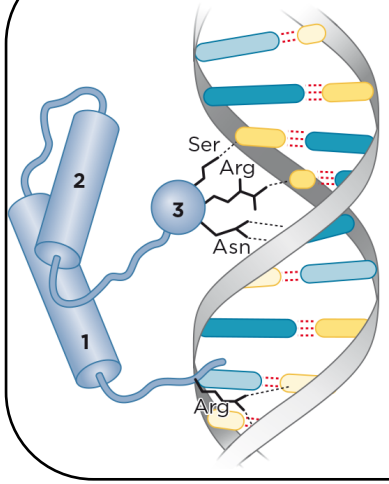
Nucleotides in solco minore e maggiore (minor and major groove) expose H-bond acceptors, H-bond donors or hydrophobic methyl group (thymine) that can be used in DNA-Protein interactions. DNA sequeunce expose a code of interacting residues that determine binding specificity with AA of the DNA binding domains of transcriptional activators.



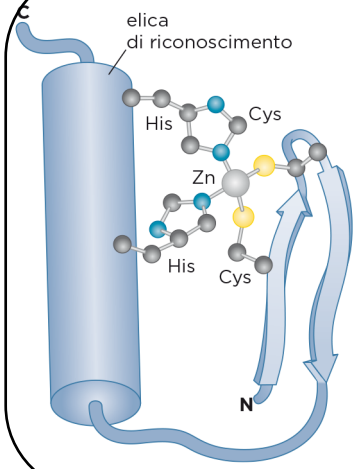
Major and minor groove codes:
Minor groove encodes lower information: A:T → T:A change or G:C→CG change does not change the code.

DNA binding motifs in Eukaryotes

Procaryotes: homodimers
Eukaryotes: homodomer, heterodimer, monomers

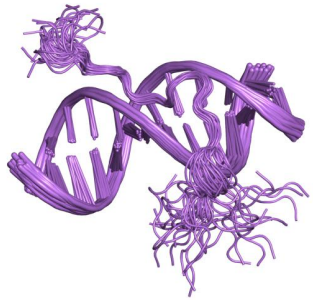


Homeobox DNA binding protein:
3 helices (2 form helix-turn-helix motif)
Helix 3 interacts with major groove
Form very often heterodimers

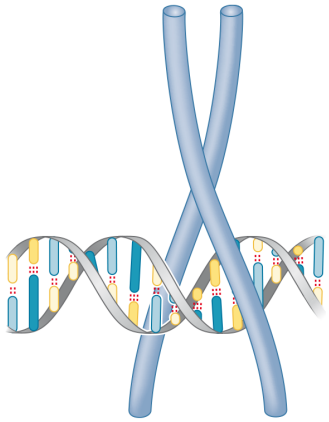


Zink finger DNA binding protein:
Frequent
Zn interacts with Cys to create a structure that allows the alpha helix to interact with the major groove
Proteins can have 1 or more alpha-helices

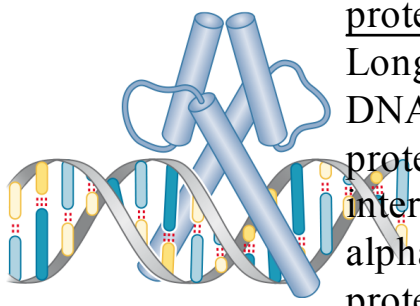
AT-hooks



Interact with the minor groove
of AT-rich DNA
1-30 AT-hooks per protein.
High mobility group protein
Contain AT-hooks (proteins
Involved in transcription, Recombination, replication, etc)



Leucine Zipper
2 apha helices
Dimerisation domain by coiled-coil domains
That interact with the major groove.
Monomers interact by hydrophobic interaction between leucines
Form hetero and homodimers



Helix-turn-helix DNA binding proteins:
(classic DNA binding protein in bacteria)
Long alpha helix for DNA binding and protein-protein interaction; smaller alpha-helix for protein-protein interaction
Form dimers

Activating domains in eukaryotic activators

→ No specific structures known

→Interaction between activating domains and components of the basal transcription apparatus occurs via flexible structures that are often induced by Protein-Protein contact (often via helices)

→ advantage: allows interaction of activator with multiple proteins

→Frequent mechanism: via acidic or hydrophobic interactions

→Frequent mechanism: short peptide repeats that have a cooperative effect in binding the basal transcription apparatus

Basic mechanisms of transcriptional activation

-**Basal transcription factors and RNA polymerase** bind promoter at start point

↕

-**Activators** (type of transcription factors) bind specific target sequences in promoter region or enhance regions =RESPONSE ELEMENTS

-**Co-activators** interconnect activators and the basal transcription apparatus located at promoter

-**Chromatin modifying proteins** change chromatin conformation (hyperacetylation → active transcription)

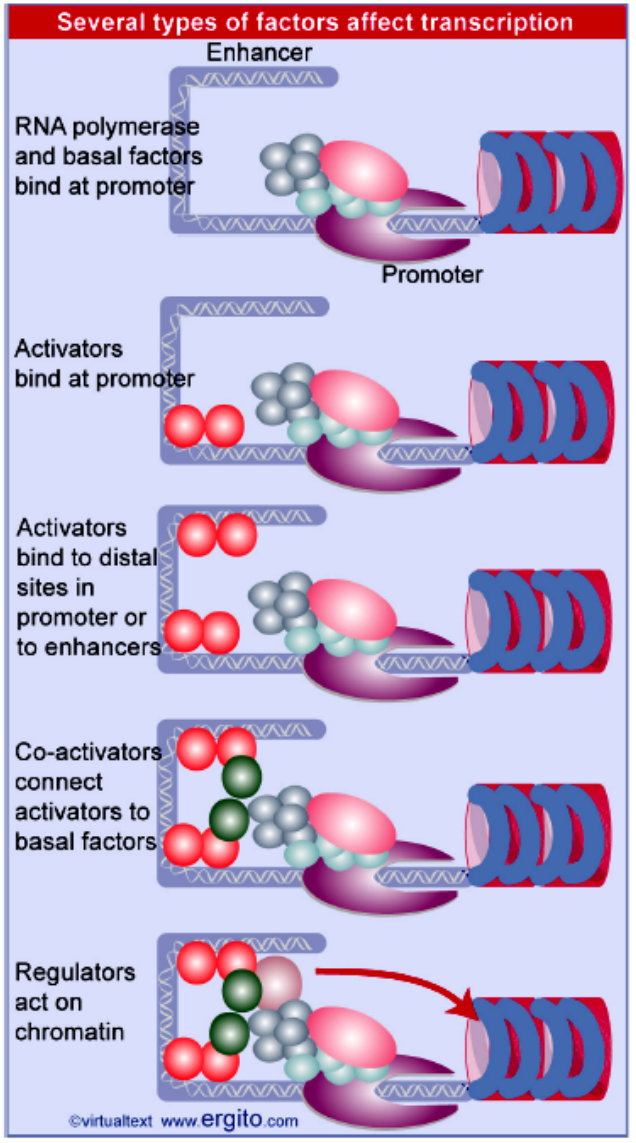


Figure 22.2 Factors involved in gene expression include RNA polymerase and the basal apparatus, activators that bind directly to DNA at the promoter or at enhancers, co-activators that bind to both activators and the basal apparatus, and regulators that act on chromatin structure.

Basic mechanisms of transcriptional activation

Prokaryotes: recruitment of RNA-Polymerase by activator/without activator
Eukaryotes: recruitment of chromatin modifying activites
recruitment of general transcription factors (see TAFs of the TFIID complex, Mediator complex
recruitment of specific transcription factors (see Gal4, etc)
recruitment of proteins that stimulate the initiation/elongation of transcription

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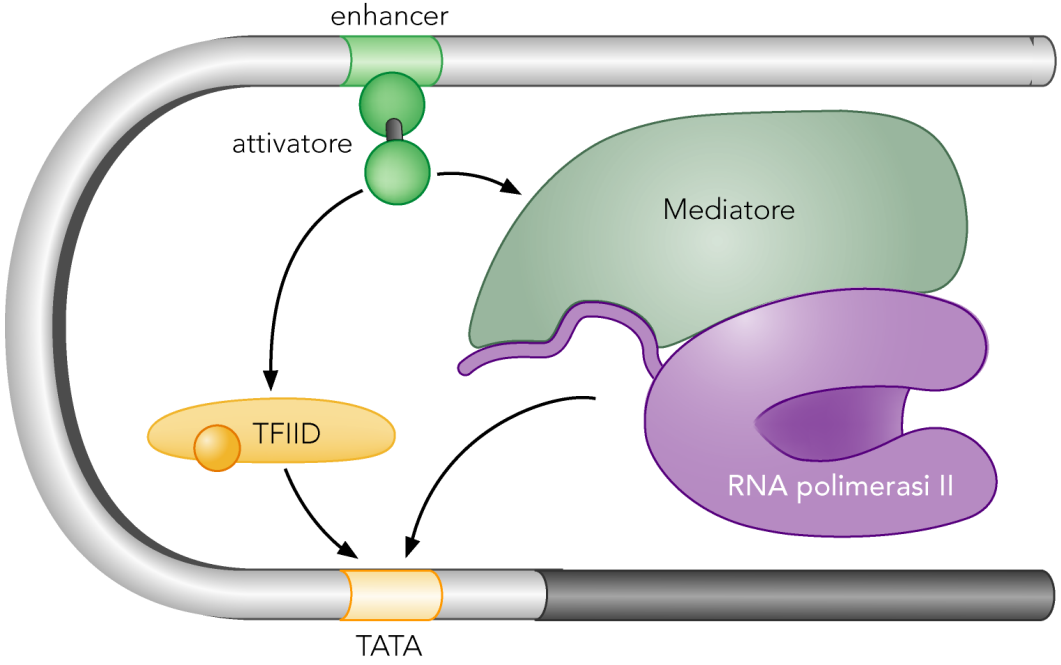
1. Activators and/or co-activators recruit the transcription complex to promoter
2. Chromatin modifying complexes open chromatin
3. Induction of changes in the transcription complex
allow a more efficient initiation/elongation of transcription
4. Isolators control enhancer function

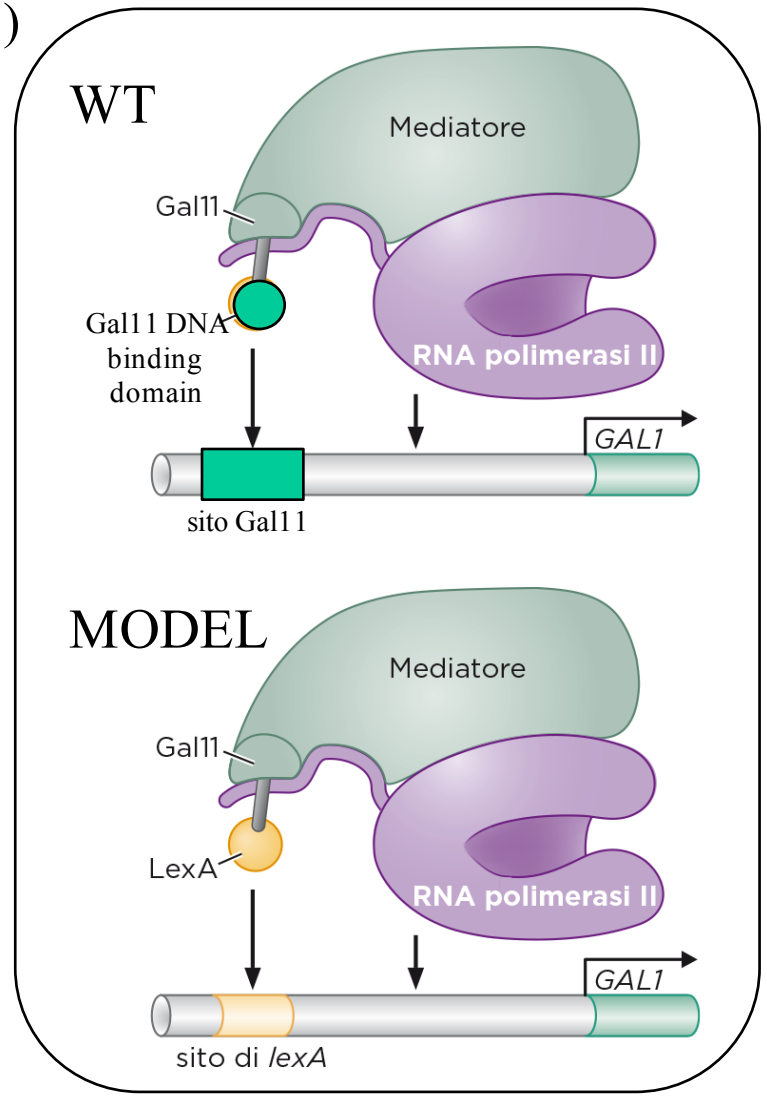
Due modelli generali :

- Il modello di **reclutamento** implica che il solo effetto è di aumentare il legame della RNA polimerasi al promotore.
- Un modello alternativo è che esso induca dei **cambiamenti conformazionali** nel complesso trascrizionale, che ne aumenta l'efficienza.

1. Activators and/or co-activators recruit the transcription complex to promoter

- The activator (bound to the enhance can bind the mediator compex or the basal transcription factors (TFIID components to the promoter
- Note: multiple components of the mediator complex/TFIID can interact with activating proteins (→ not very high specific – but cooperative!)





1. Activators and/or co-activators recruit the transcription complex to promoter

- Tutti i componenti necessari per una trascrizione efficiente– basal factors, RNA polymerase, activators, coactivators – formano un apparato molto grande, di **>40 proteine.**
- Alcuni attivatori, coattivatori, e fattori basali possono assemblarsi uno dopo l'altro al promotore, ma poi possono unirsi ad un complesso molto grande fatto dalla RNA polimerasi preassemblata con altri attivatori and coattivatori.

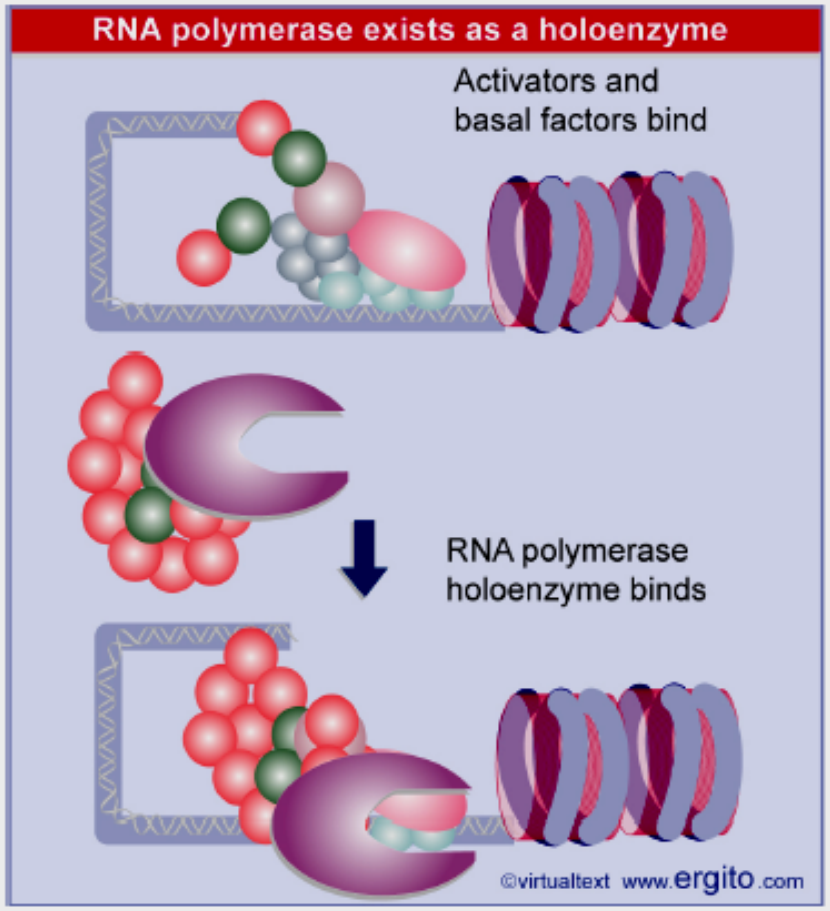
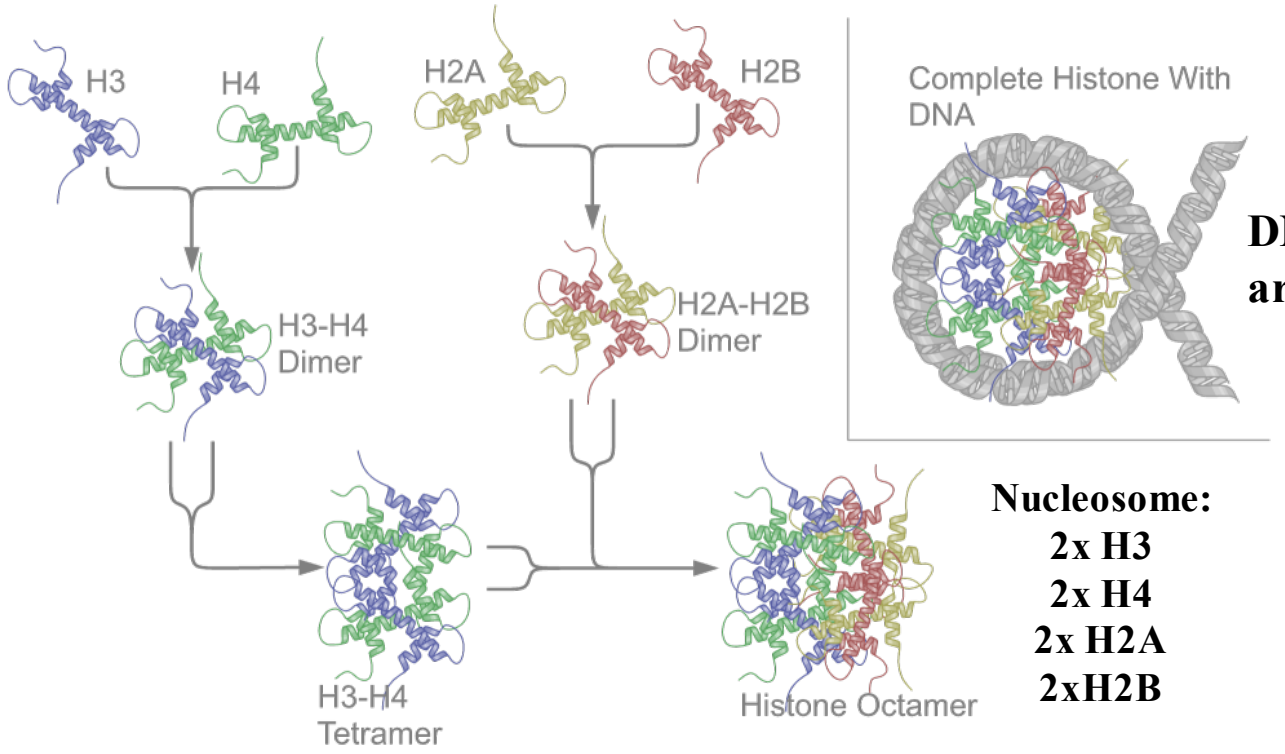


Figure 22.9 RNA polymerase exists as a holoenzyme containing many activators.

2. Chromatin modifying complexes open chromatin

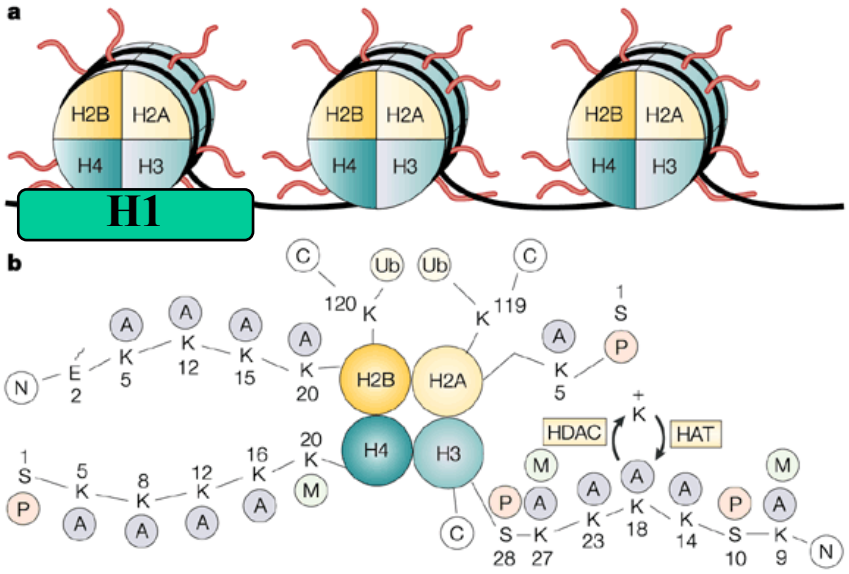
histones



DNA wrapped around: 147nt

Nucleosome:
2x H3
2x H4
2x H2A
2xH2B

Histone H1 compacts nucleosomes



chemical modification of histone tails

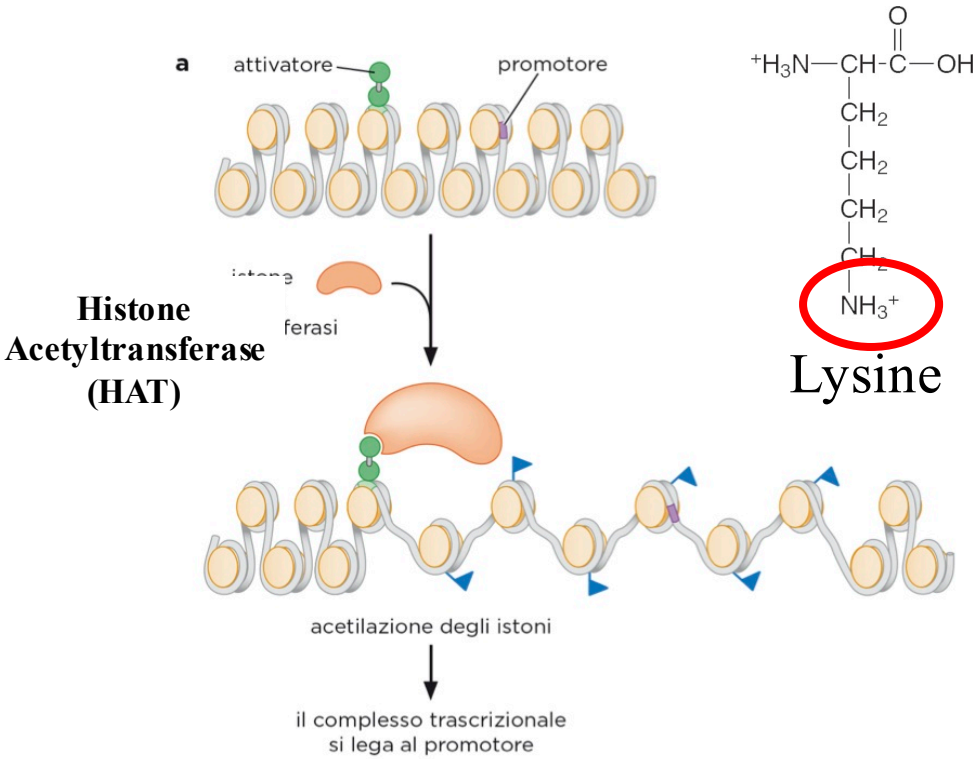
2. Chromatin modifying complexes open chromatin

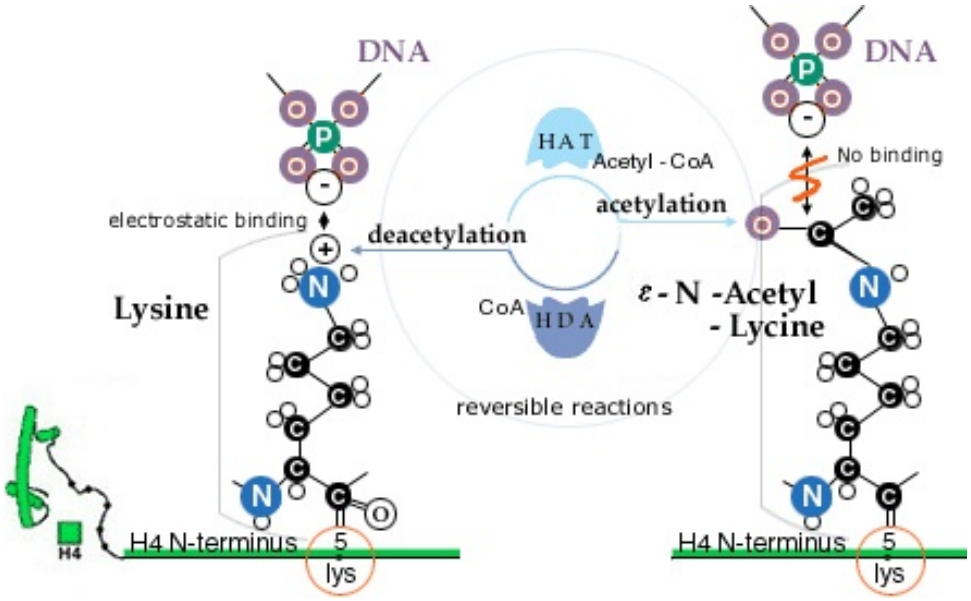
-Activator can recruit a **chromatin remodelling complex** → SWI/SNF complex, moves nucleosomes to make promoter/response elements accessible

-Activator can recruit a **histone acetyl transferase** → add acetyl groups to lysine histone tails (p300,, GCN5, MOF, etc)

→arrangement of nucleosomes change at response elements

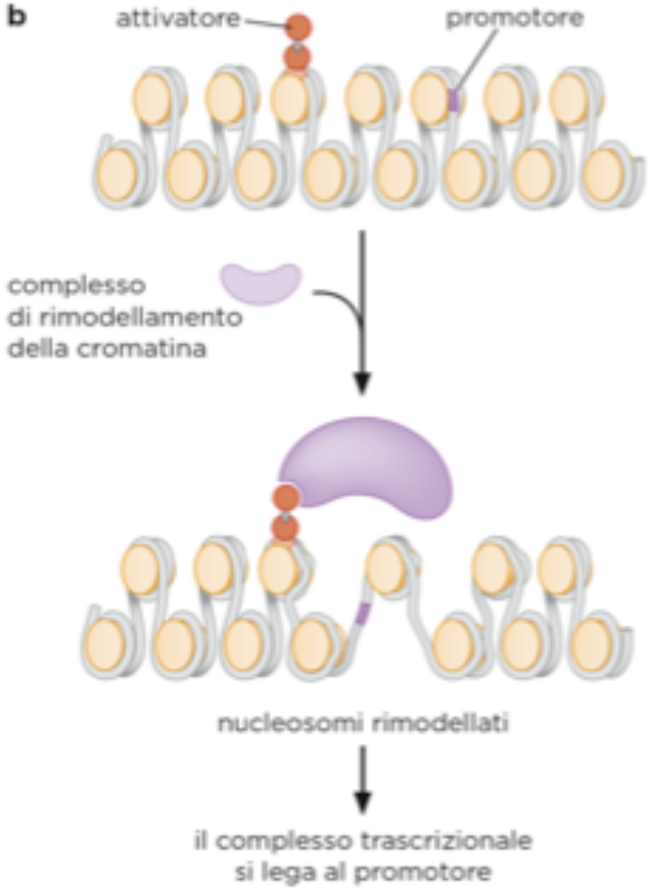
→acetylated tails serve as a binding site for bromo-domain proteins (TFIIH contains such protein)

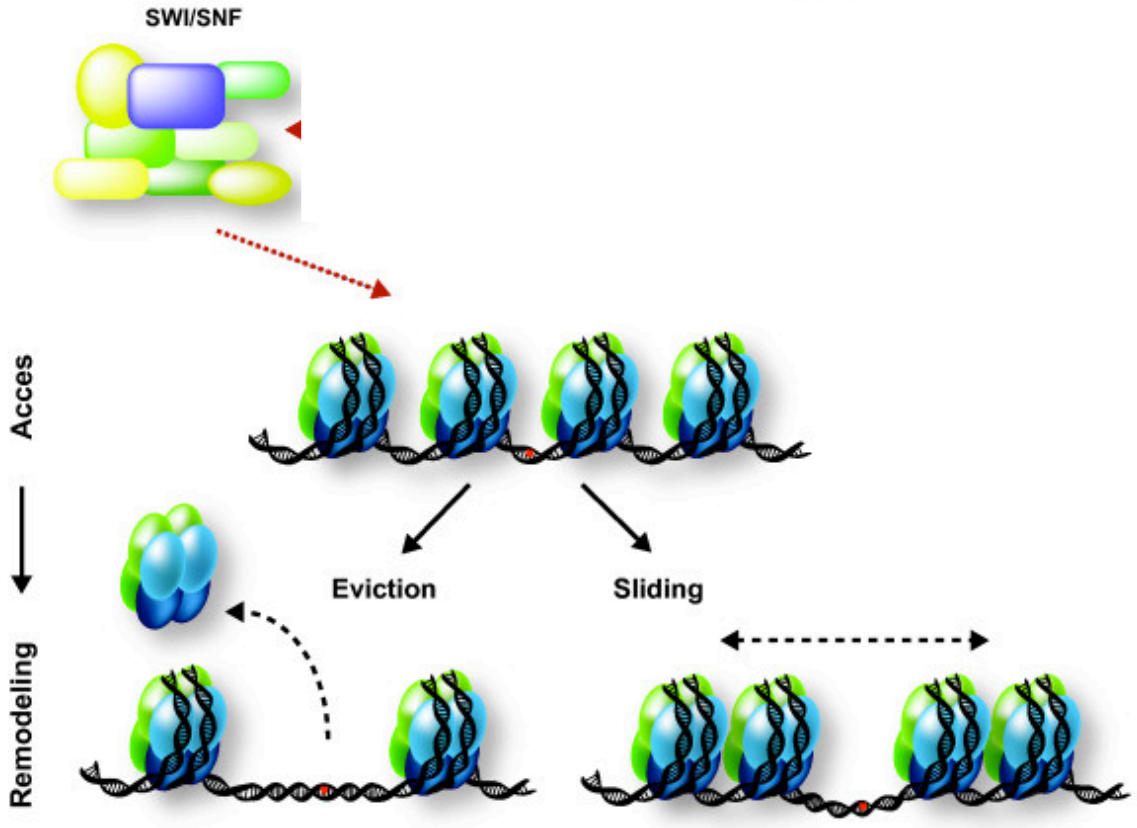




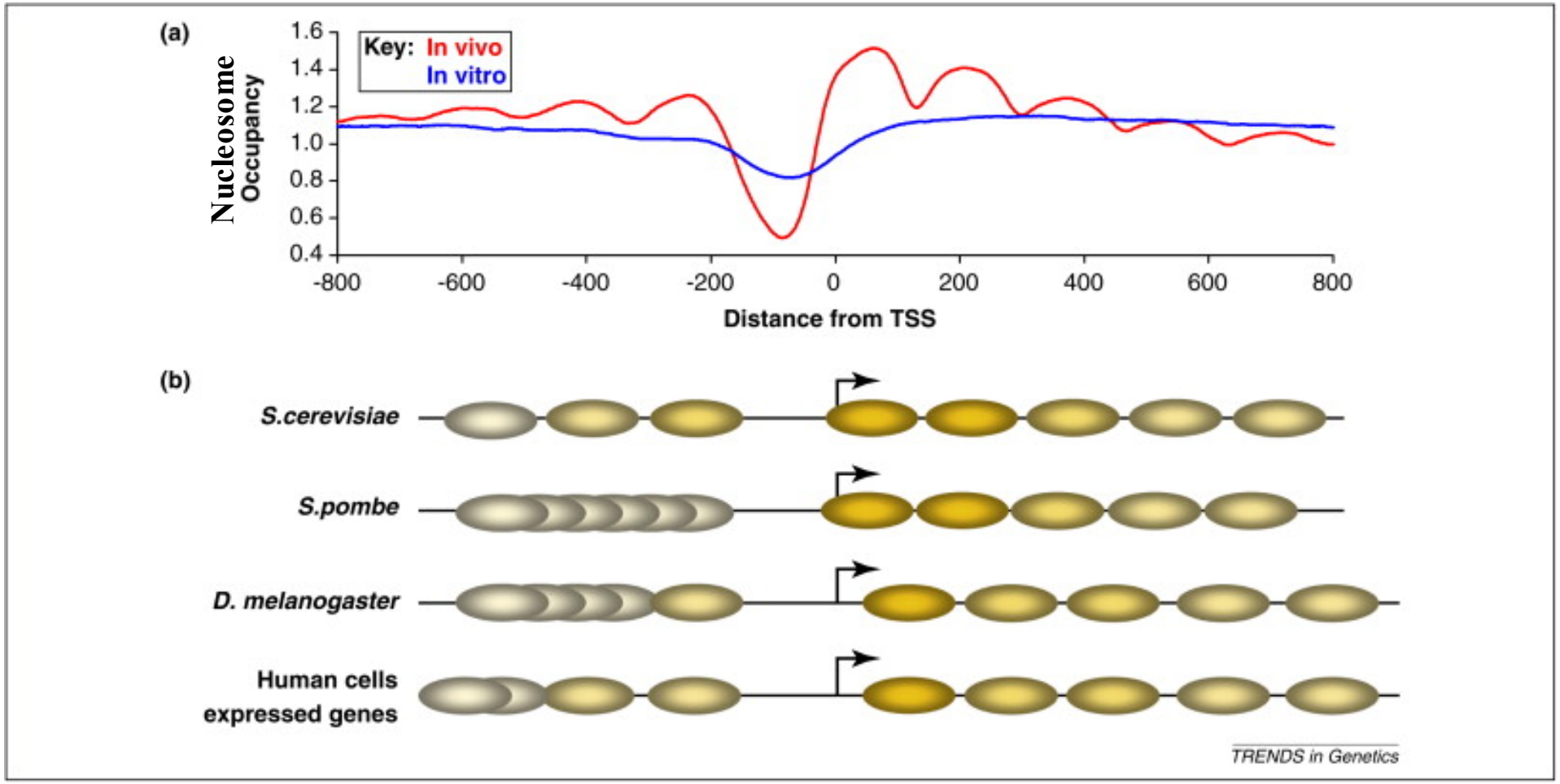
2. Chromatin modifying complexes open chromatin

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



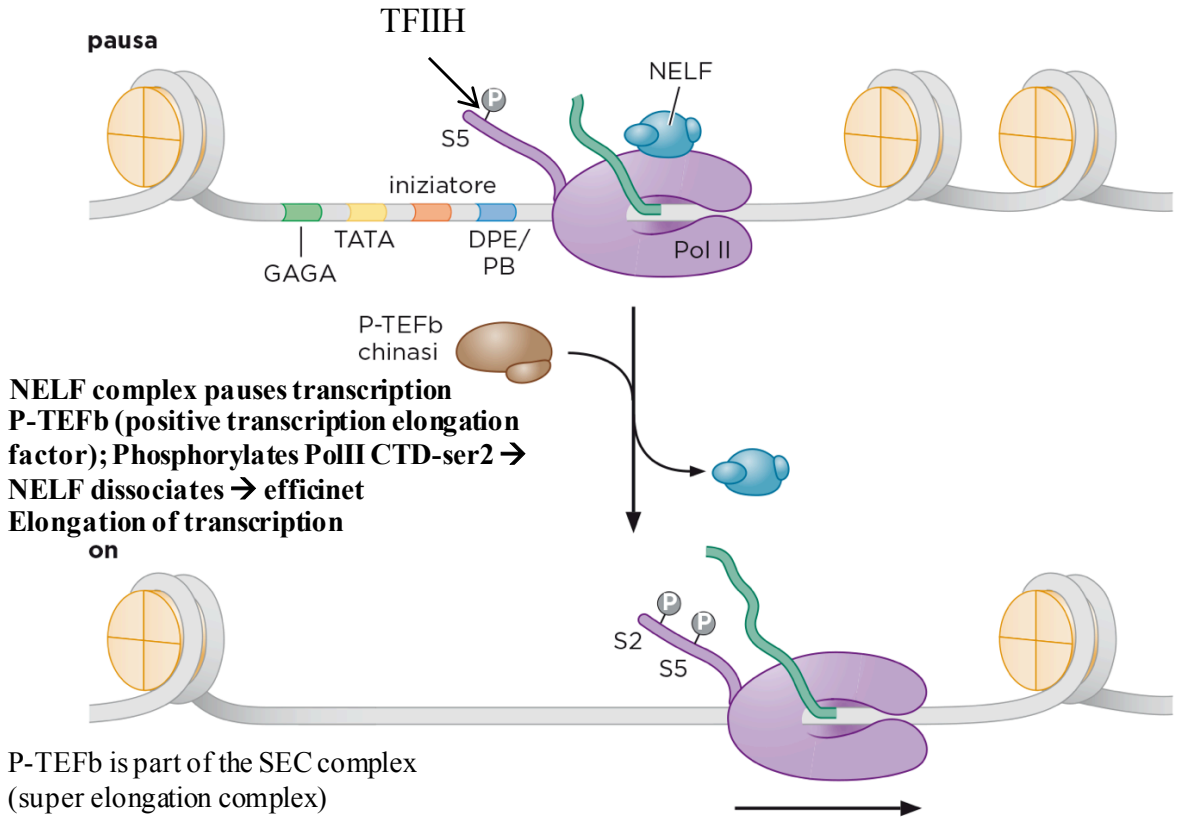


2. Chromatin modifying complexes open chromatin
Transcriptional start sites lack nucleosome (nucleosome eviction)



3. Induction of conformational changes in the transcription complex allow a more efficient initiation/elongation of transcription

1. CTD ser5-P: evasione from promoter (TFIIH)
2. 30% of genes still blocked at the promoter (during drosophila development)
3. P-TEFb kinase required to establish CTD-Ser2-phospholylation



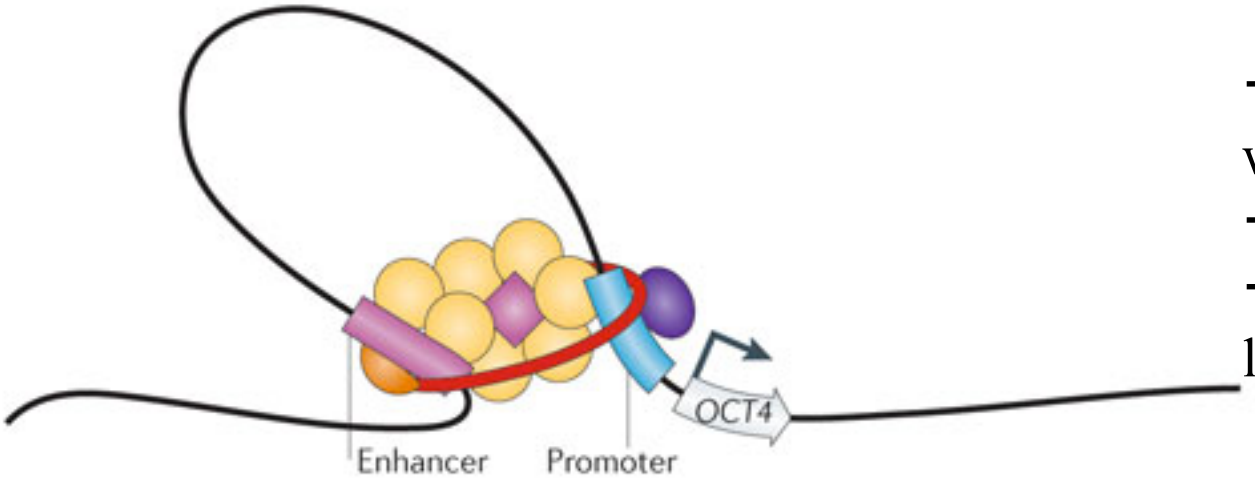
P-TEFb is part of the SEC complex (super elongation complex)

S.cerevisiae: GAL4 recruits P-TEFb
Drosophila: under thermic shock the Heat shock factor (HSF) binds to response elements in the HSP70 Gene and recruits P-TEFb → HSP70 expression → activation of heat shock response.
Human: Acute lymphoblast leukemia: MLL is fused to SEC components → transcription elongation factors+disease

4. Gene regulation by loop formation - enhancers and insulators

Interaction between Enhancer and Promoter

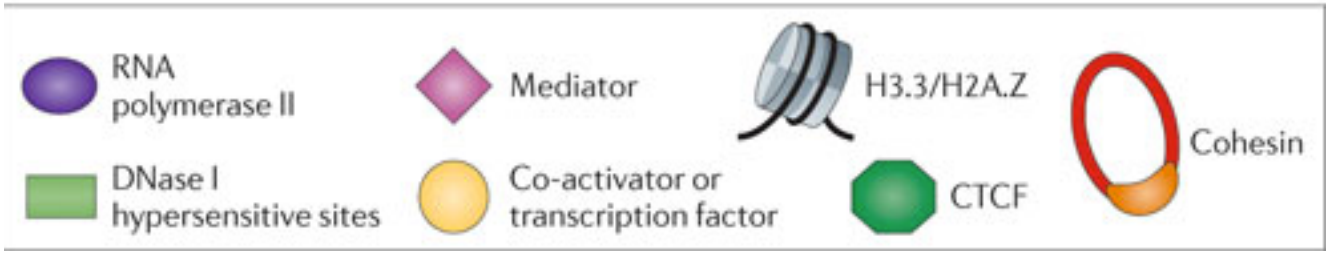
→ Loop formation



→cohesin can interact with the mediator complex

→Stable loop formation

→ activator proteins are loclaized at promoter/enhancer



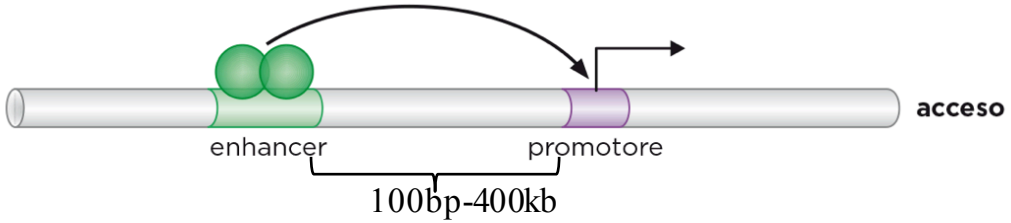
Association of enhancers and promoters at several genes in embryonic stem cells (ESCs) — for example, octamer-binding protein 4 (OCT4; also known as POU domain, class 5, transcription factor 1) is mediated by physical interactions between Mediator and cohesin complexes.

4. Gene regulation by loop formation - enhancers and insulators

Block enhancer function

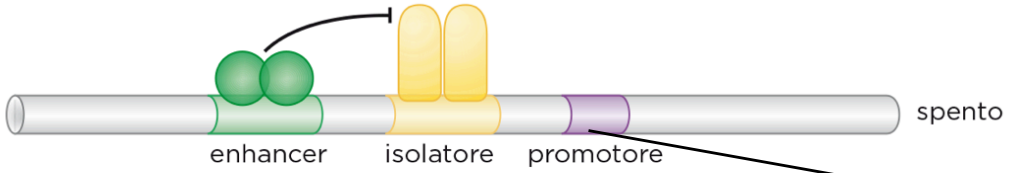
a

enhancers are often far away from promoter



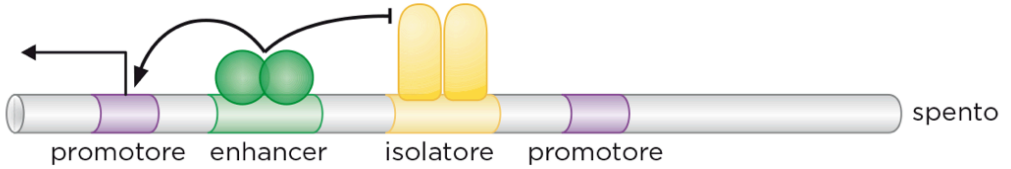
b

Isolator block action of enhancers on gene promoter



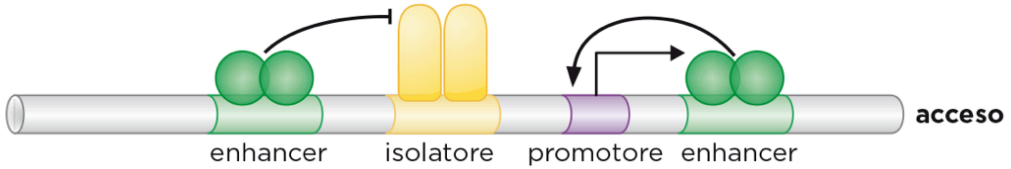
c

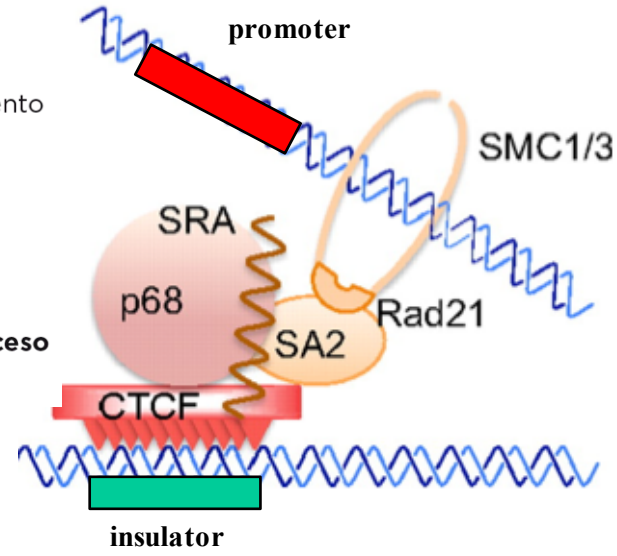
Isolator exclude promoter from interaction with enhancer



d

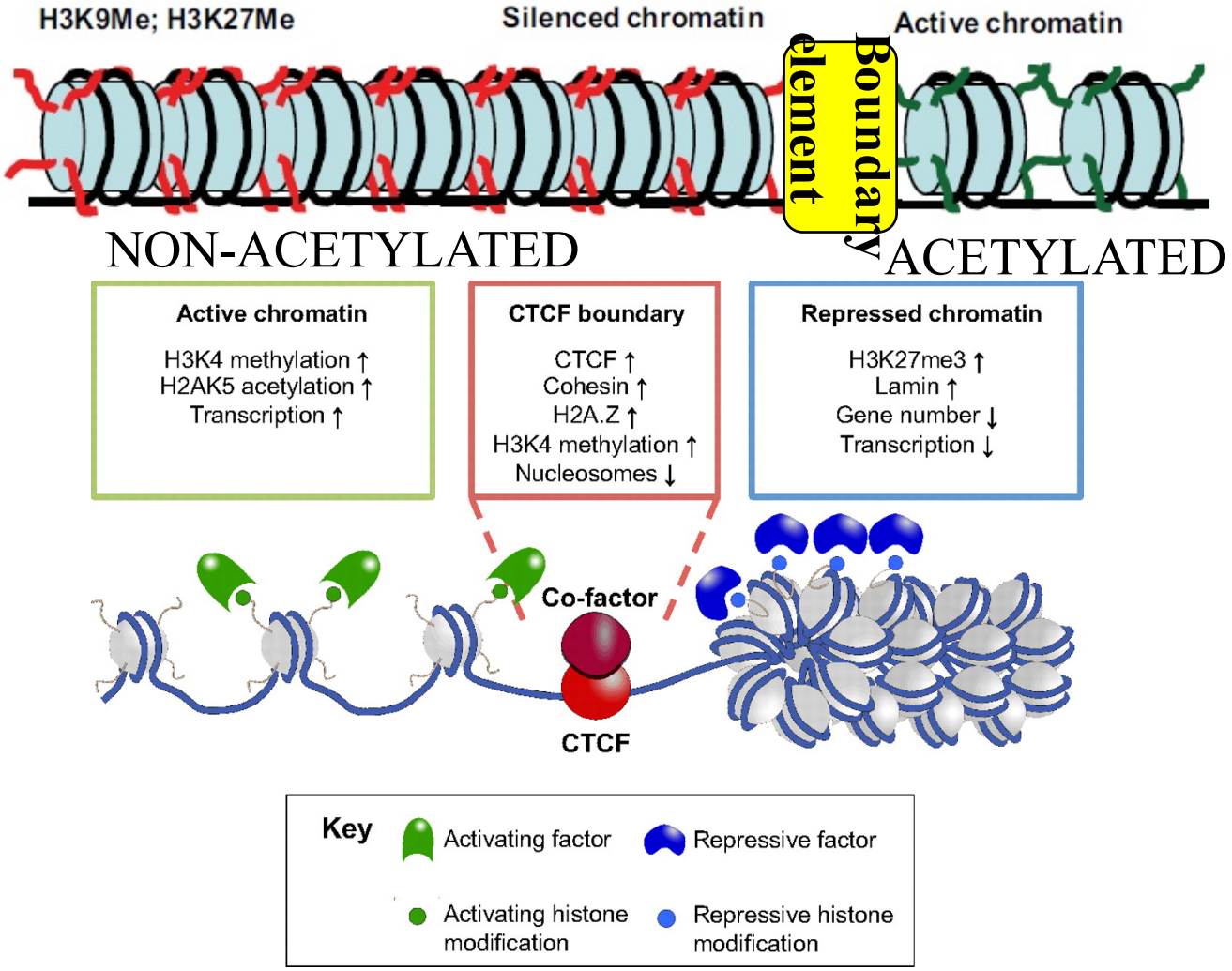
Isolator coordinates enhancer function





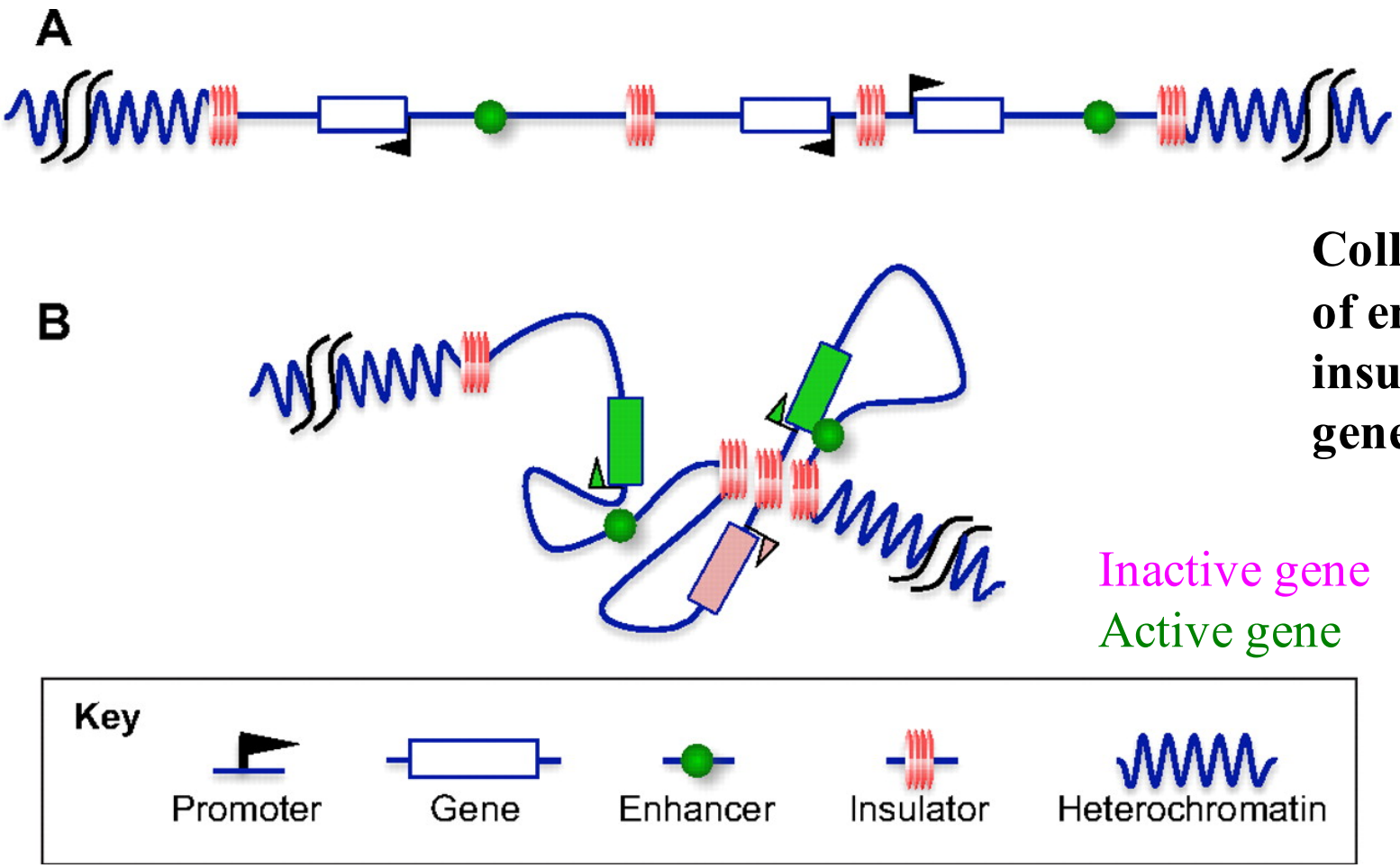
Something more: Gene regulation by loop formation - Isulator/boundary elements

Blocking the spreading of repressive chromatin



CTCF boundary elements are associated with specific chromatin features. CTCF-binding sites are characterised by nucleosome depletion. This may be regulated by specific interaction with remodelling factors. Nucleosomes directly associated with CTCF binding have been reported to be marked by generally active marks (like H3K4me3) or exchange histones (like H2A.Z). Furthermore, CTCF boundaries are located in between domains with contrary modification patterns corresponding to contrary activity states. Active chromatin domains are marked by increased acetylation and methylation correlated with increased transcriptional processes. By contrast, repressed chromatin domains are marked by increased H3K27me3 and are low in gene number.

4. Gene regulation by loop formation - enhancers and insulators



Schematic overview of the domain model of a linear genome, highlighting insulator locations. (A) An active chromatin domain is flanked by heterochromatic regions. Insulator positions are indicated at the domain boundaries (where they can mediate border or barrier function of insulators) as well as within the active domain (where they can mediate enhancer-blocking function). It is not known whether both functions are established by similar or different mechanisms. (B) One aspect of insulator function is to organise chromatin looping by promoting contacts between insulators or with other genomic structures. Depending on the linear and three-dimensional arrangement, looping may interfere with enhancer-promoter interactions (thus mediating the enhancer-blocking function of insulators), resulting in an inactive gene (pink gene and promoter), or it may assist in increasing enhancer-promoter contacts, resulting in an active gene (green gene and promoter on right). Gene activation can also be achieved by direct enhancer-promoter interactions (green gene on left) that can occur independently of the presence of an insulator. Insulators are also found between tandem promoters positioned in a head-to-head orientation ensuring that both promoters can be regulated individually.

Controllo dell'espressione genica negli eucarioti

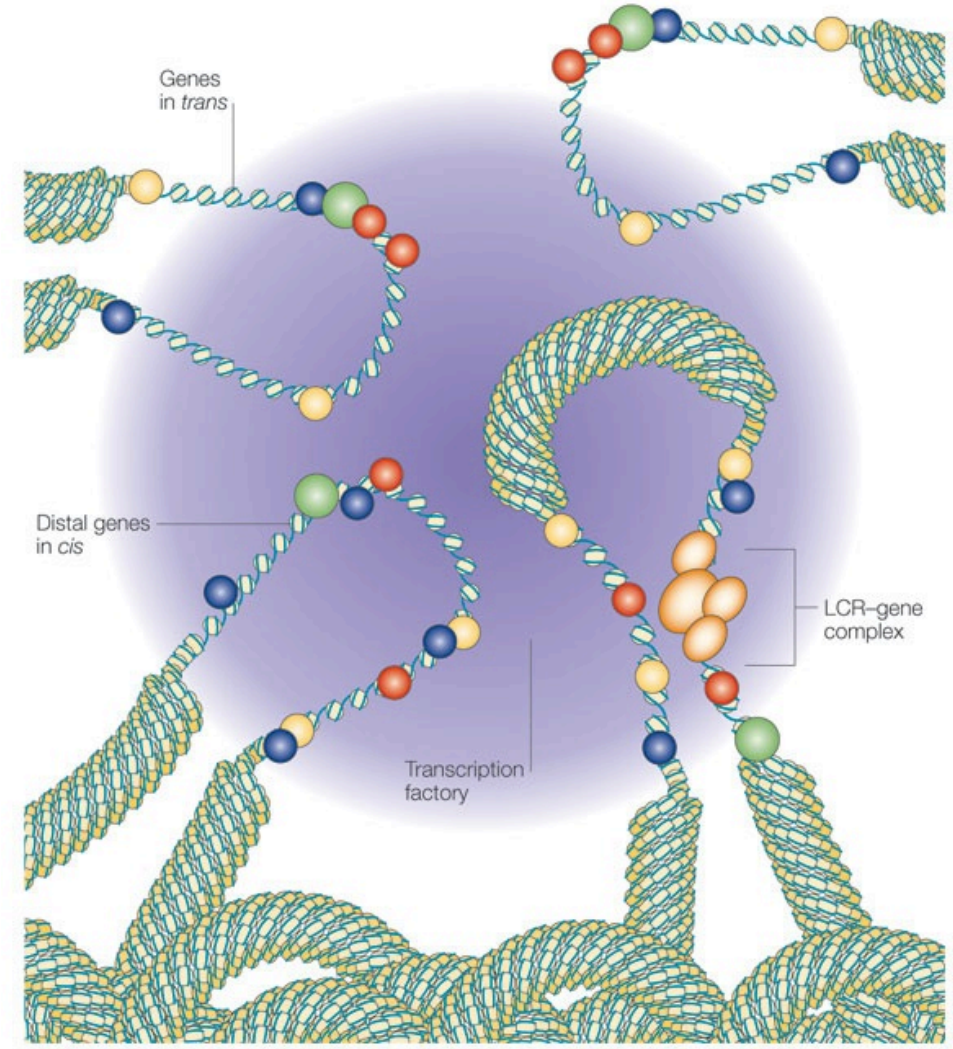
1. **Regulation of transcription**
- Introduction
- Transcription factors – activators of transcription
- Basic mechanisms of transcriptional activation
- Integration of signals
- Signal transduction

2. **Post-transcriptional gene regulation**
- Chromatin regulation
- ncRNA - miRNAs

INTEGRATION OF SIGNALS TO CONTROL GENE EXPRESSION

--

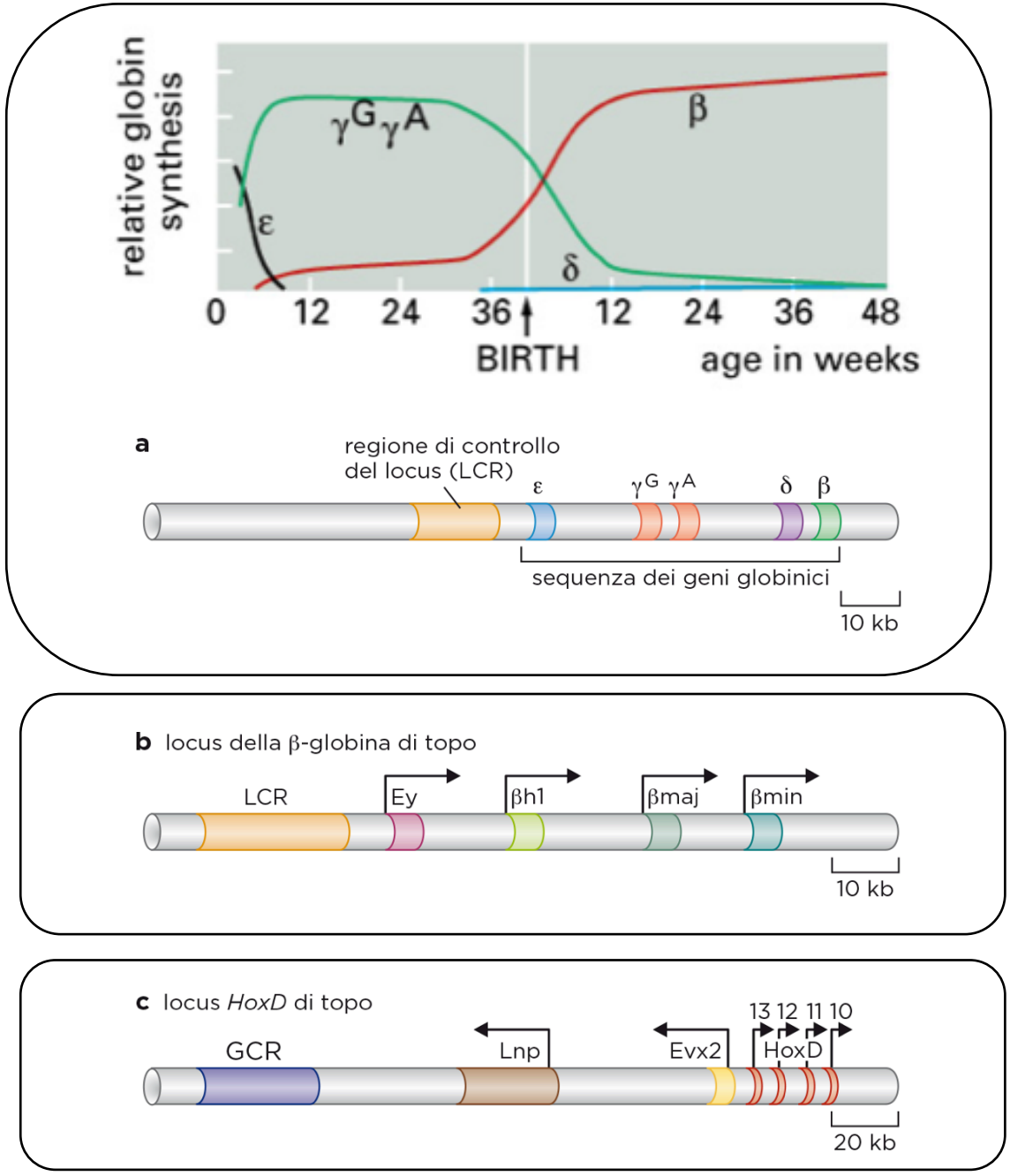
LOCUS CONTROL REGIONS



The locus control region (LCR) is a long-range cis-regulatory element that enhances expression of linked genes at ectopic chromatin sites. It functions in a copy number-dependent manner and is tissue-specific, as seen in the selective expression of β-globin genes in erythroid cells. Expression levels of genes can be modified by the LCR and gene-proximal elements, such as promoters, enhancers, and silencers. The LCR functions by recruiting chromatin-modifying, coactivator, and transcription complexes. Its sequence is conserved in many vertebrates, and conservation of specific sites may suggest importance in function

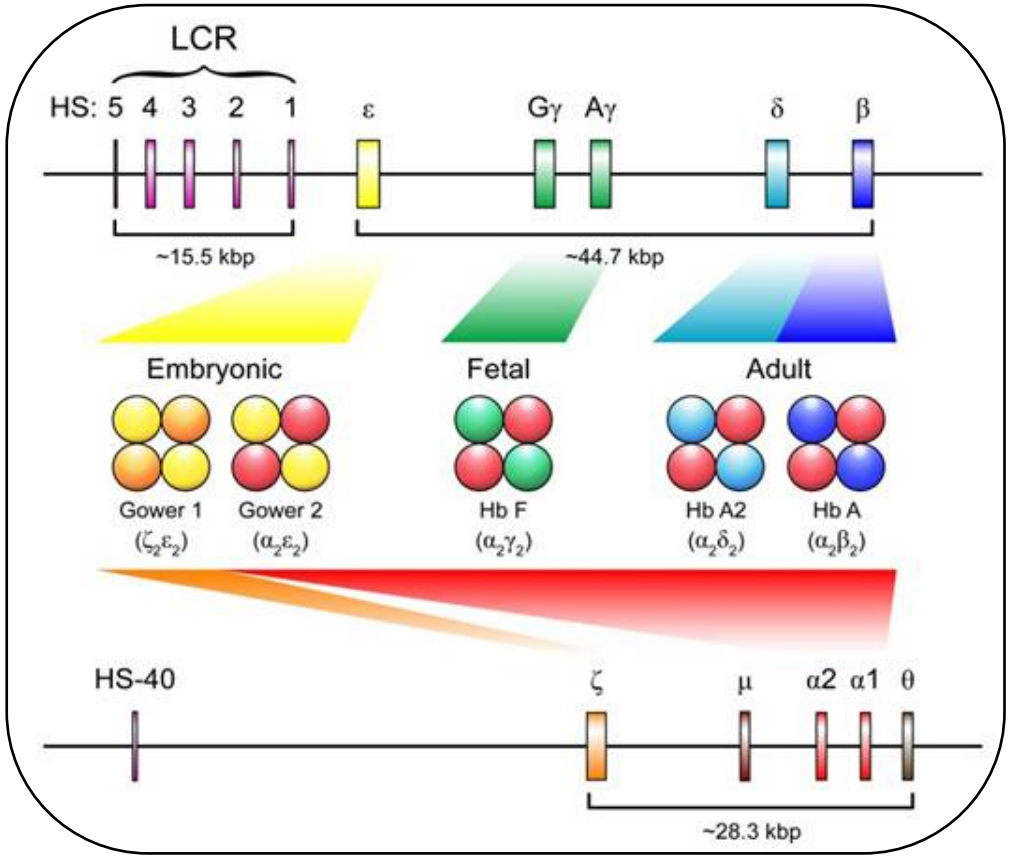
LOCUS CONTROL REGIONS (LCRs) control groups of genes

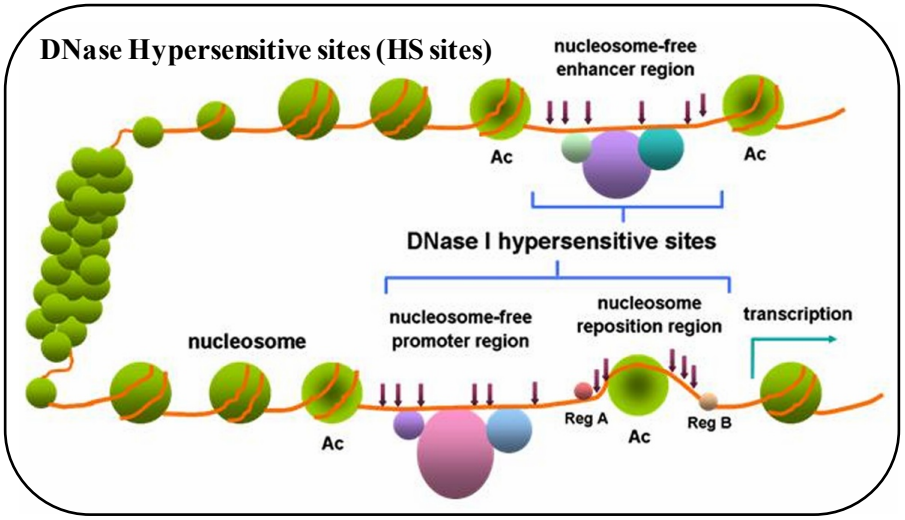
LCRs contain multiple binding sites for regulatory proteins that allow the controlled expression of genes encoded by a locus



LOCUS CONTROL REGIONS (LCRs) control groups of genes

Hemoglobin is a tetramer of globin proteins; different composition during development





LCR control elements are localized Close to the promoter of beta globin gene in adults

LCR elements are subjected to complex chromatin modifications

Looping model: Transcription factors bind to hypersensitive site cores and cause the LCR to form a loop that can interact with the promoter of the gene it regulates

Tracking model: Transcription factors bind to the LCR to form a complex. The complex moves along the DNA helix until it can bind to the promoter of the gene it regulates. Once bound, the transcriptional apparatus increases gene expression

Facilitated tracking model: This hypothesis combines the looping and tracking models, suggesting that the transcription factors bind to the LCR to form a loop, which then seeks and binds to the promoter of the gene it regulates

Linking model: Transcription factors bind DNA regions ranging from the LCR to the promoter in an orderly fashion using non-DNA-binding proteins and chromatin modifiers. This changes chromatin conformation to expose the transcriptional domain

Combination of activator binding sites act in a cooperative manner

→ many activating proteins collaborate To control the activation of genes

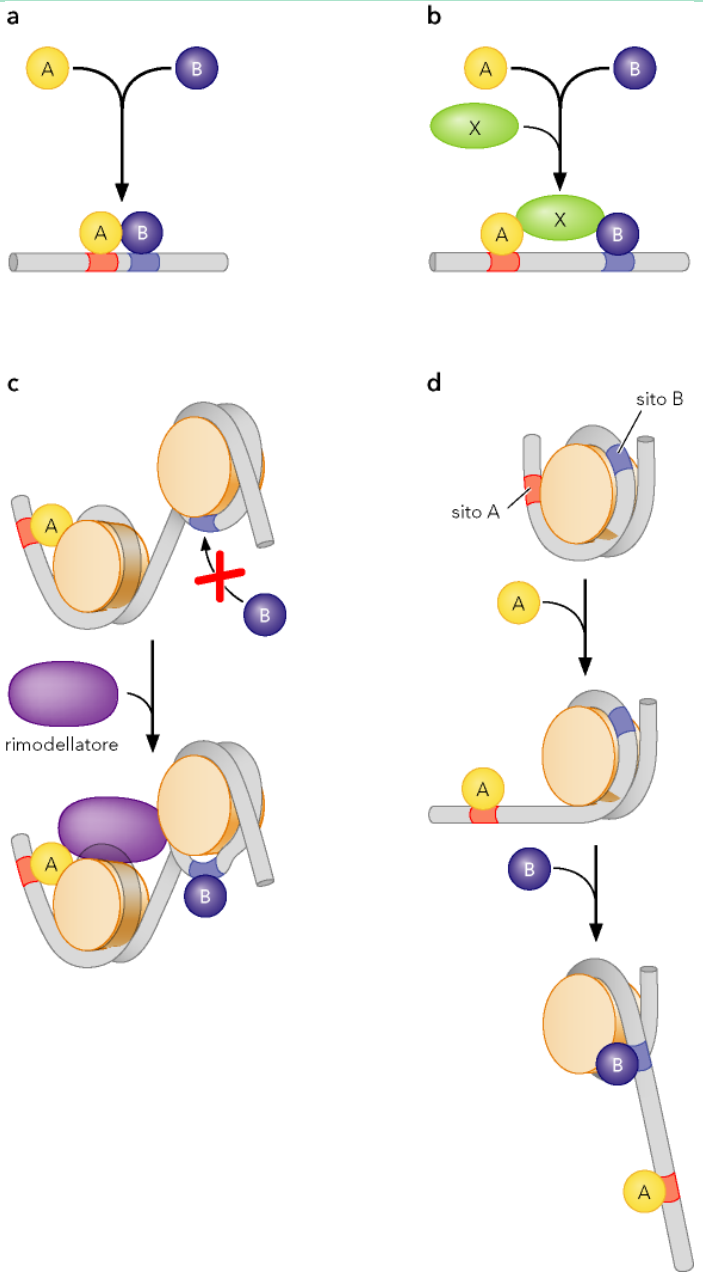
→ combined binding energy increases exponentially when 2 factors bind to in a cooperative manner to DNA

A. Protein a+b form complex and bind 2 DNA sites

B. Protein a+b bind DNA and recruit another activating factor

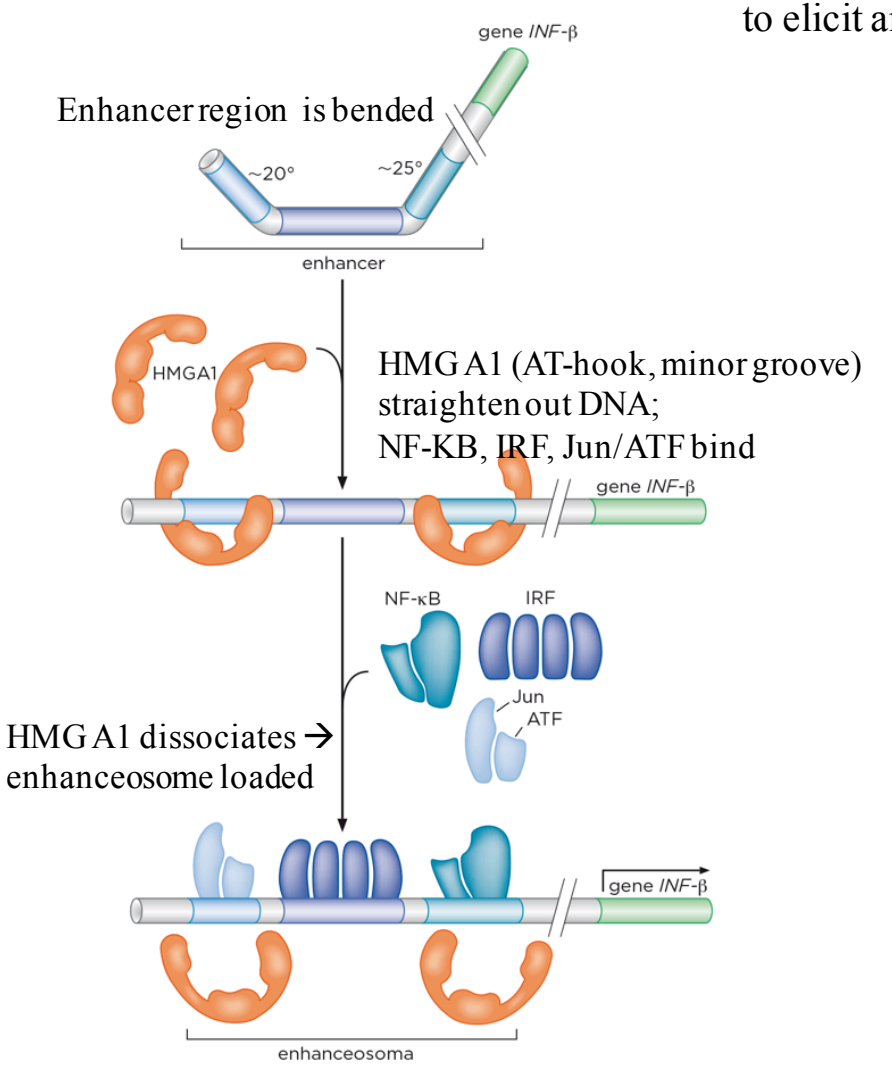
C. Protein a binds → chromatin regulatory factor
→ new site for protein b accessible

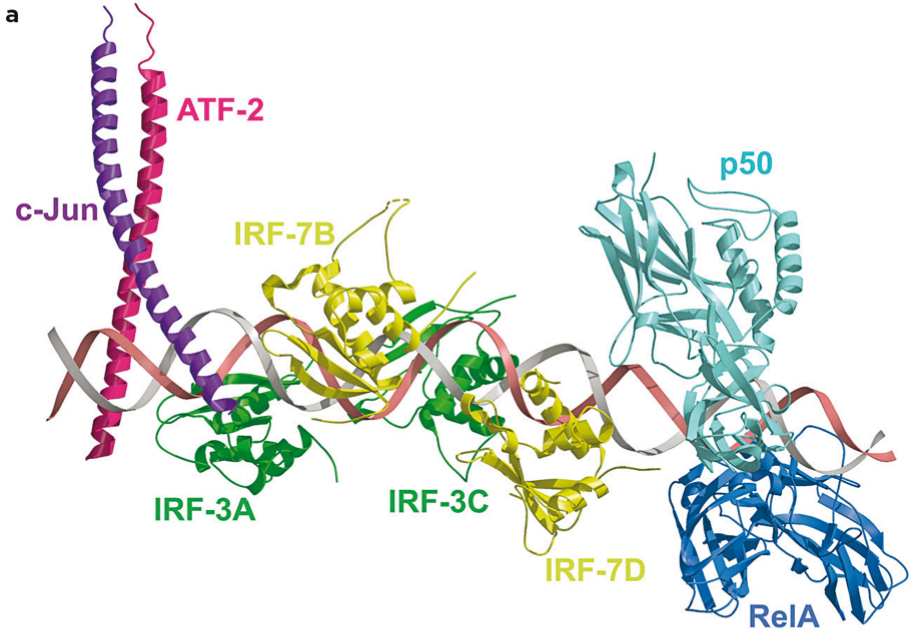
D. Protein a binds site → chromatin remodeling factor → site for protein b accessible



Combination of activator binding sites act in a ccoperative manner --signal integration at the IFN beta gene--

Viral infection → NF-kB, IRF, Jun /ATF gene activation → cooperatively bind IFN beta promoter → form enhancer structure (ENHANCEOSOME) → recruitment of CBP/p300 coactivators (have HAT activity) → SWI/SNF recruitment → IFN beta gene activation → IFN secretion → stimulate macrophages and NK cells to elicit an anti-viral response

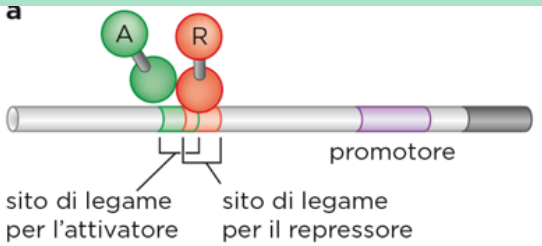




TF binding sites are highly conserved

Repression of transcription

repressor site overlaps with activator site



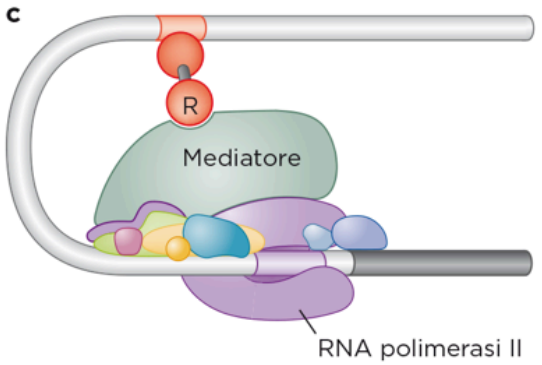
meccanismo:

competizione

Repressor binds activator site and covers the activation domain of the transcriptional activator

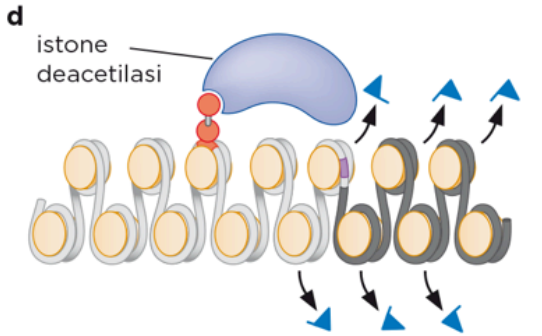
inibizione

A repressor binds to repressor siteand blocks the activation of transcription



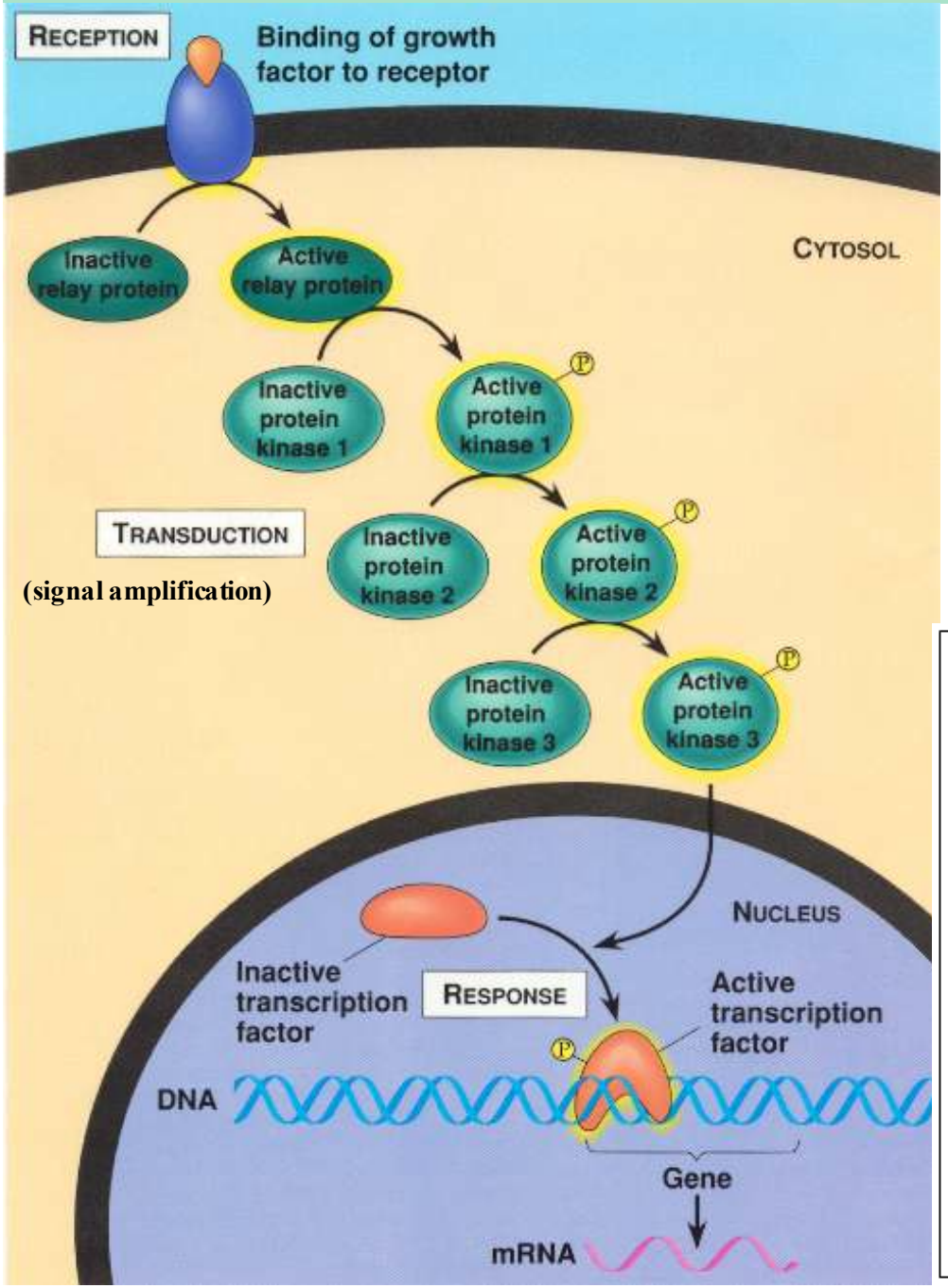
repressione diretta

A repressor proteins recruits histone modifying complexes that silence gene expression my mediating chromatin compaction or impact on nucleosome remodeling



repressione indiretta

SIGNAL TRANSDUCTION: signal and impact on gene expression



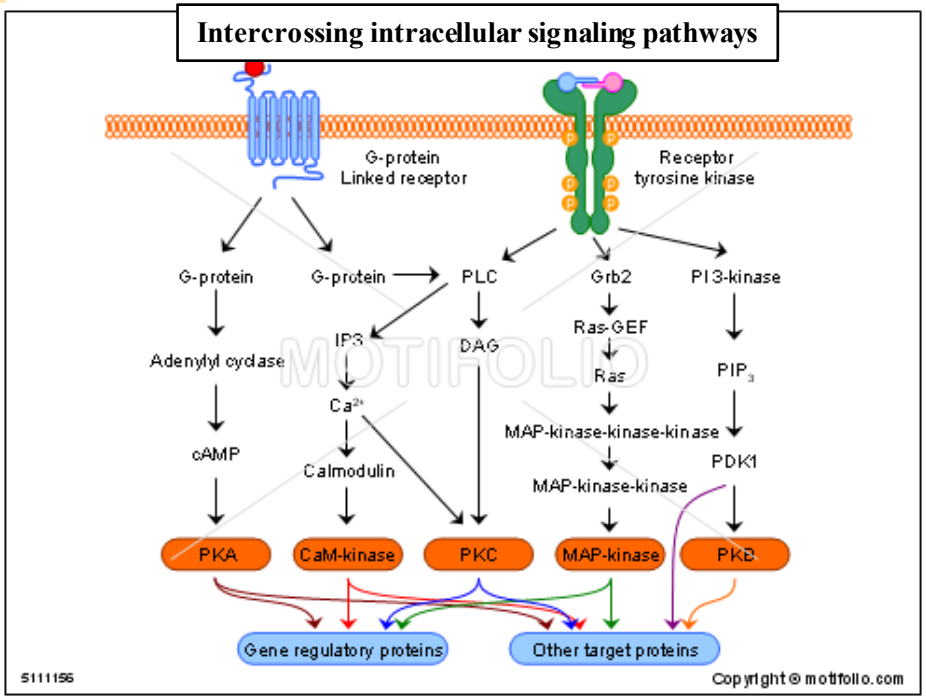
Signal:

1. small molecule (glucose, Hg, galatosio; see bacteria) → diffuse into cells

2. peptides (IFN beta, cytokines) release from a cell → ligands stimulate a receptor in membrane of another cell (paracrine) or the same cells (autocrine) (→ CELL COMMUNICATION)

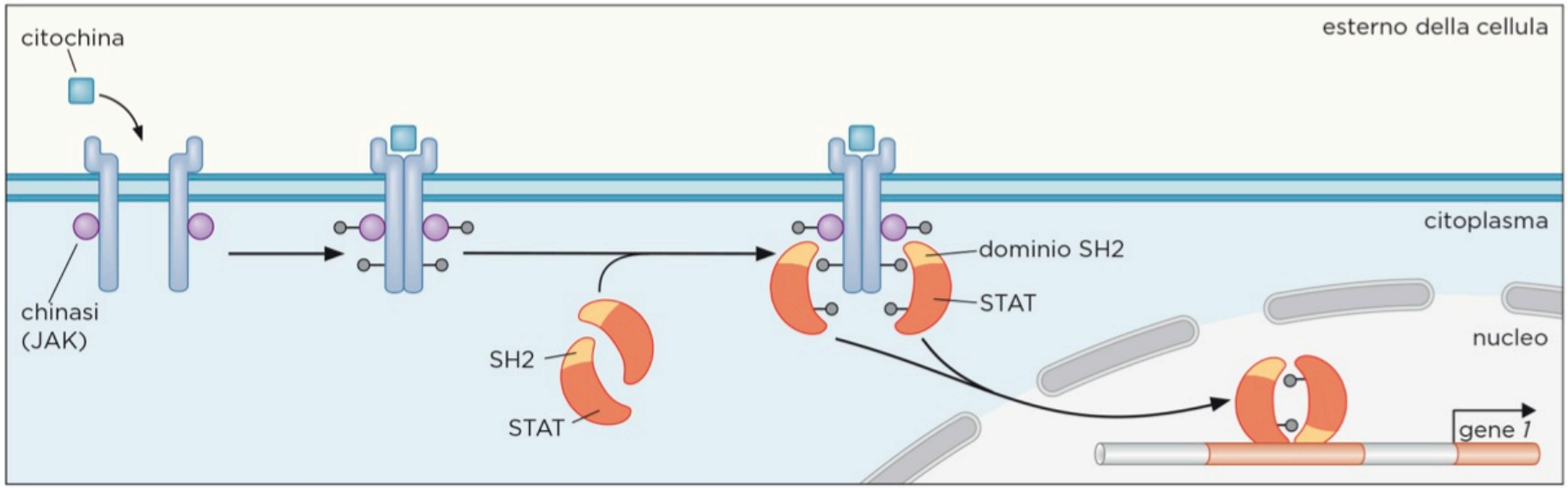
Ligand binding to receptor activates allosteric structural change of receptor → change of activity → a cascade of signals transmitted by signaling kinases transmits signal to nucleus nucleus → alterations of gene expression

Cascade is complex – several pathways can intercross along signal transduction pathways/each step is subjected to regulatory mechanisms.



Signal transduction: JAK-STAT SIGNALLING

STAT: signal transducer and activator of transcription

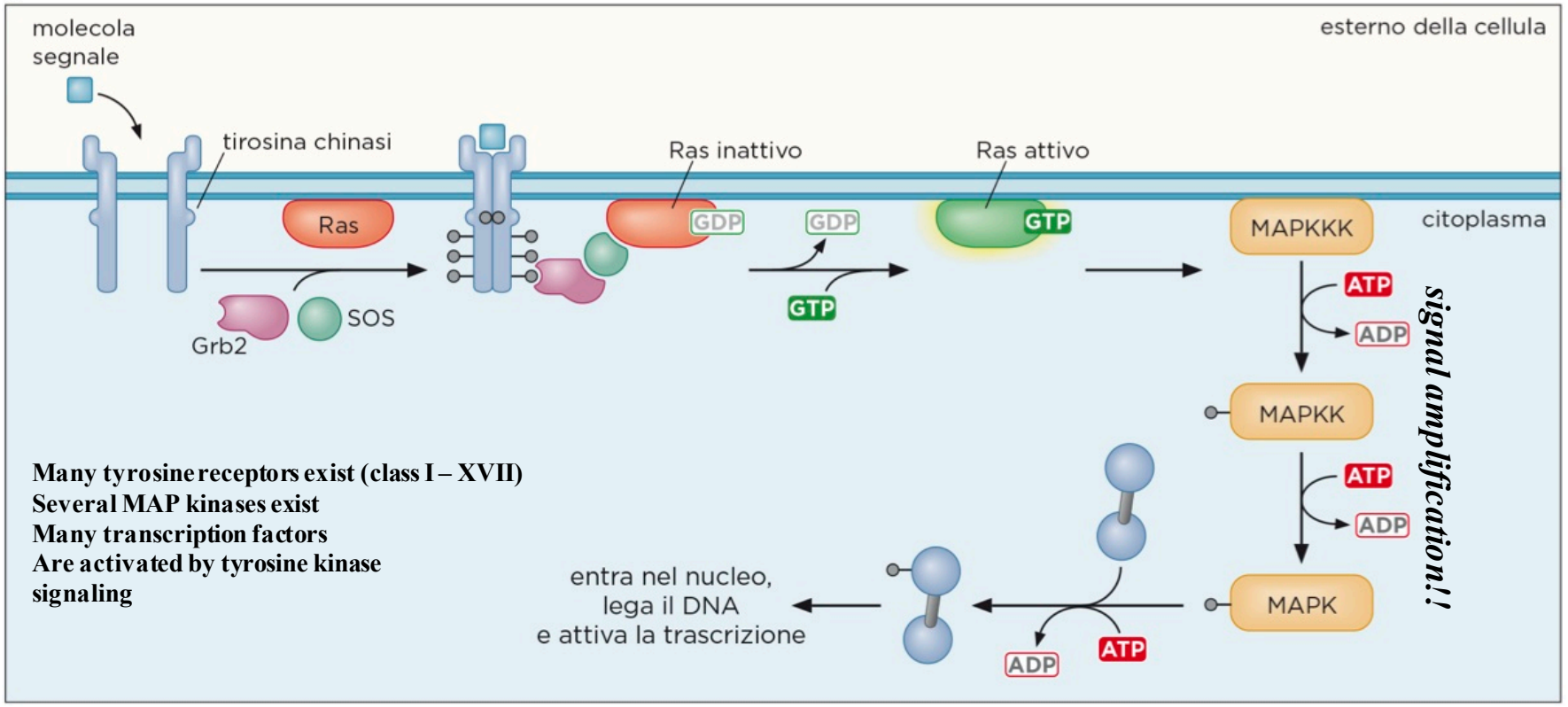


1. Cytokine binds receptor
2. Receptor dimerizes and allosteric change activates adjunct Janus kinase (JAK)
3. JAK autophosphorylates and phosphorylates intracellular receptor chain
4. STAT protein binds receptor chains and performs autophosphorylation
5. P-STAT forms dimer and translocates to nucleus and binds gene promoters

Background STAT transcription factors:
STAT1α/β, STAT2, STAT3α/β, STAT4, STAT5A, STAT5B and STAT6, that transduce signals from a variety of extracellular stimuli initiated by different cytokine families that aside from interferons (interferon α, β and γ) include gp130 cytokines, i.e., IL-6, IL-12, IL-23 and γC cytokines that include IL-2, IL-15 and IL-21.

Although structurally similar, the seven STAT family members possess diverse biological roles and are engaged in numerous processes from embryonic development, organogenesis, cell differentiation to regulation of immune processes. Awareness of their important role in regulation of cell proliferation, differentiation and survival has spurred interest in investigation of their activity in malignant transformation. STAT proteins play an important role in pathogenesis of leukemias and numerous solid tumors

Signal transduction: MAPK/ERK SIGNALING PATHWAY



MEK = mitogen activated kinases

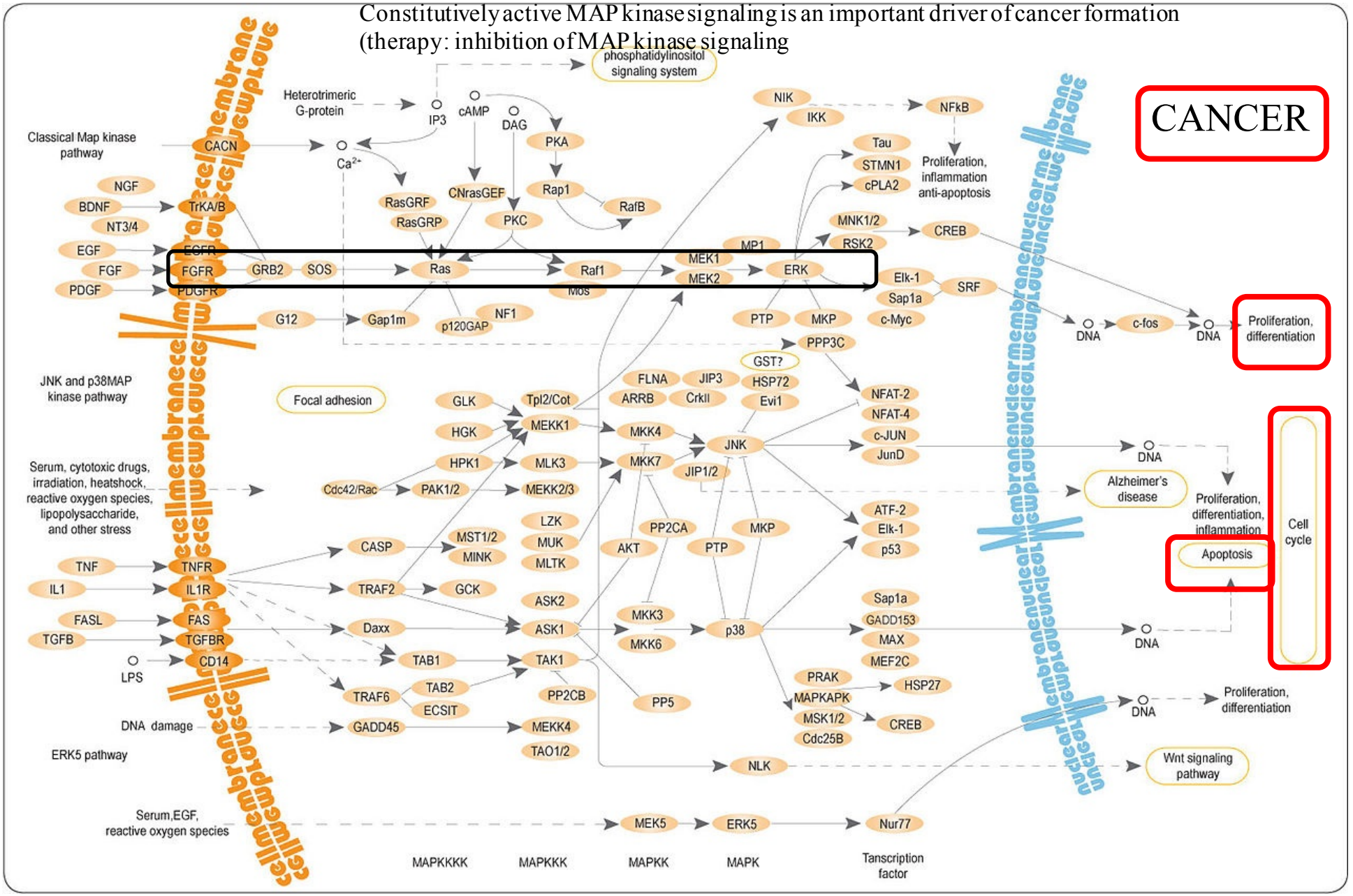
1. Growth factor (for example EGF) binds receptor
2. Receptor dimerizes and cytoplasmatic kinase domain phosphorylates the intercellular receptor domain of the partner receptor
3. Phosphorylated Tyrosin receptor dimer recruits Grb2 via its SH2 domain (binds P I receptor)
4. Grb2 brings SOS that exchanges GDP for GTP in the Ras protein (small GTPase protein)
5. RAS-GTP changes conformation and activtes the kinase of the MAP kinases (MAPKKK for Raf)
6. MAPKKK phosphorylates MAPKK (for example Mek); MAPKK phosphorylates MAP kinase (MAPK for examplr Erk)

→ signal amplification: each kinase can activate mutiple downstream kinases!!

7. MAPK phosphorylates transcription factors (for example fos)

Signal transduction: MAPK/ERK SIGNALING PATHWAY

Constitutively active MAP kinase signaling is an important driver of cancer formation
(therapy: inhibition of MAP kinase signaling



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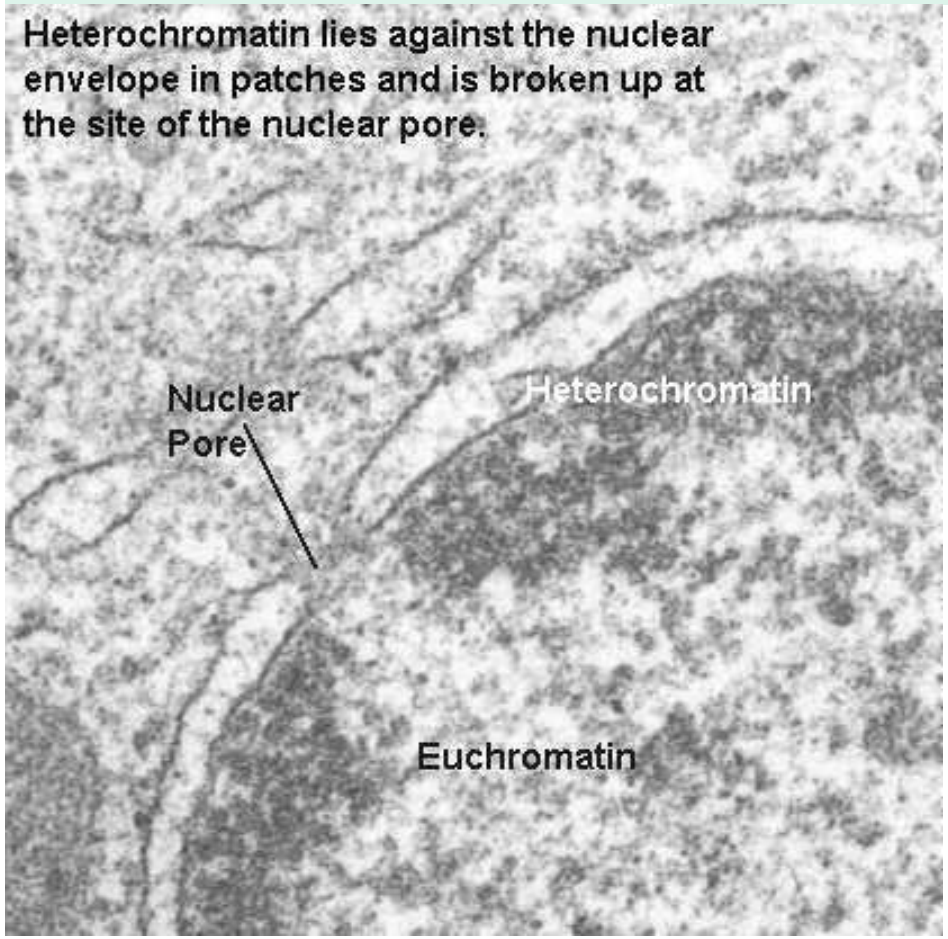
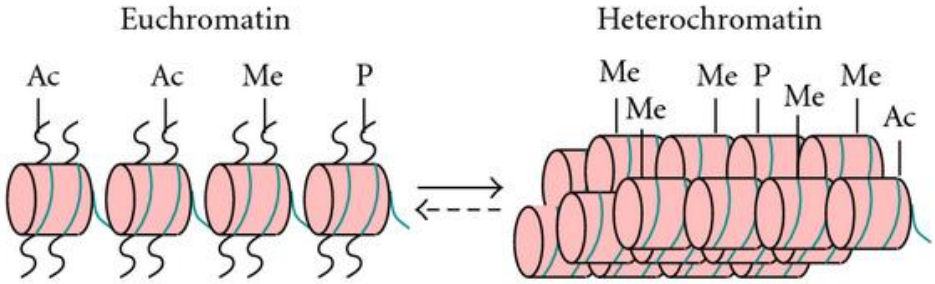
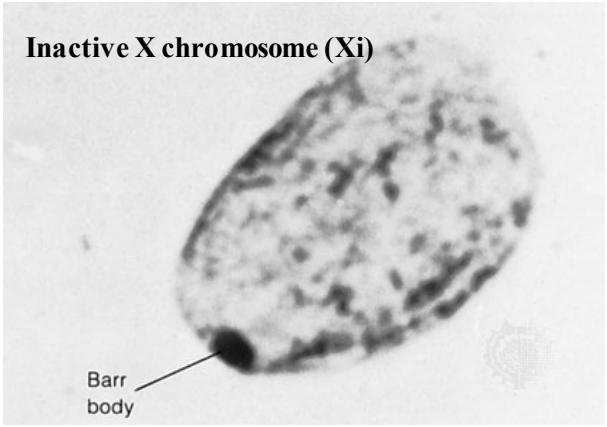
POST-TRANSCRIPTIONAL GENE REGUALTION

-

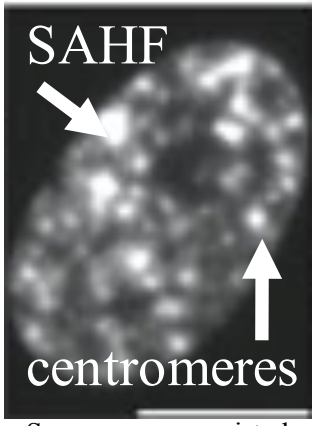
CHROMATIN REGULATION







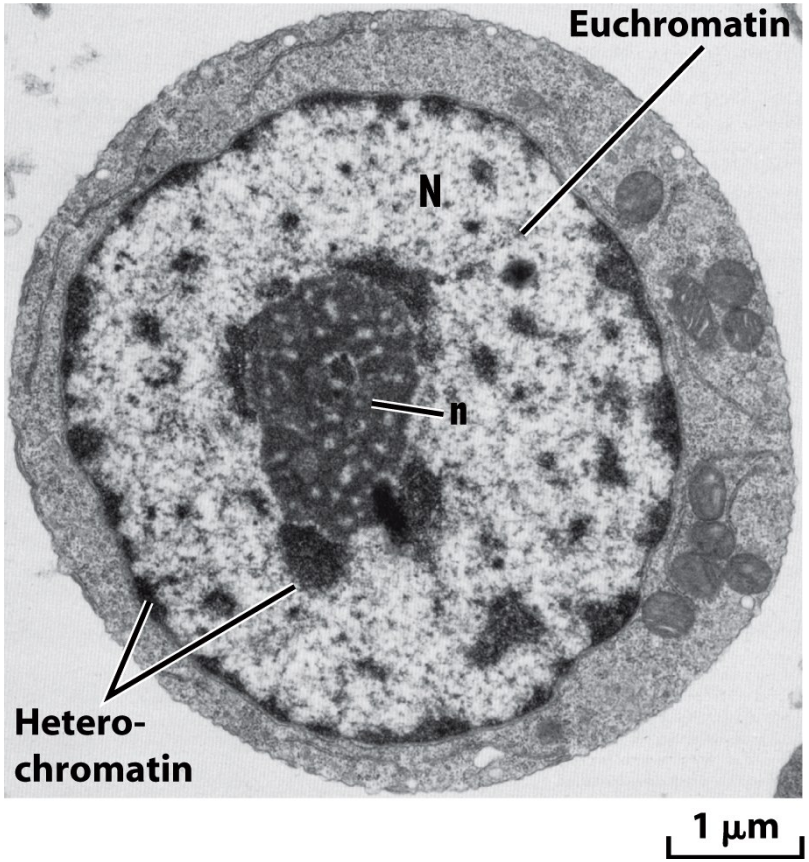
Telomeric heterochromatin



Senescence associated heterochromatic foci

Heterochromatin is frequently localized in nuclear periphery

FACTS ON HETEROCHROMATIN



Darkly stained and condensed

Present at centromeres and telomeres and repeated DNA sequences

Repressive structure are propagated during cell division. This ensures that Daughter cells maintain a defined gene expression program (same is also true for active chromatin structure (acetylation)

Euchromatic gene placed in heterochromatin is repressed

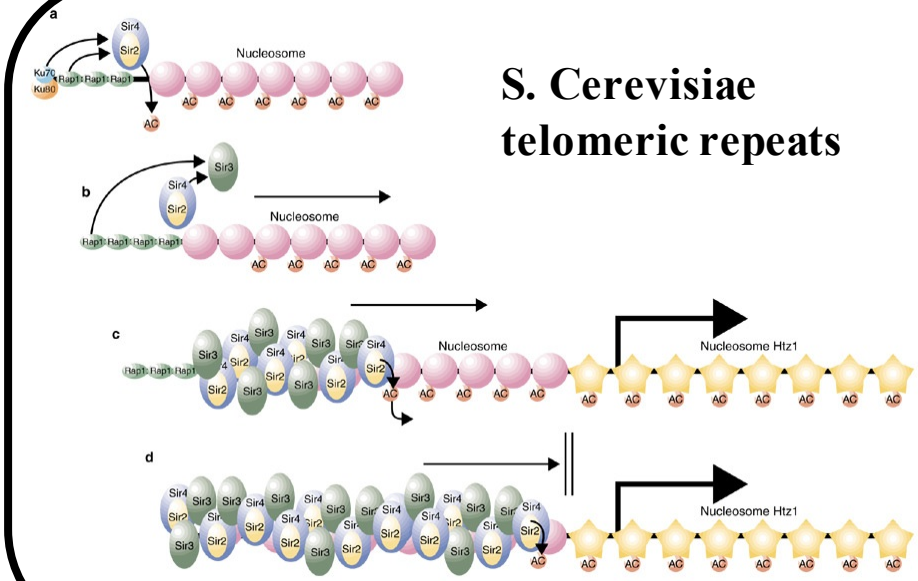
CONSTITUTIVE HETEROCHROMATIN: Always condensed: centromere, telomere DNA repeats

FACULTATIVE HETEROCHROMATIN: can switch from condensed to open structure

→ example: at genes

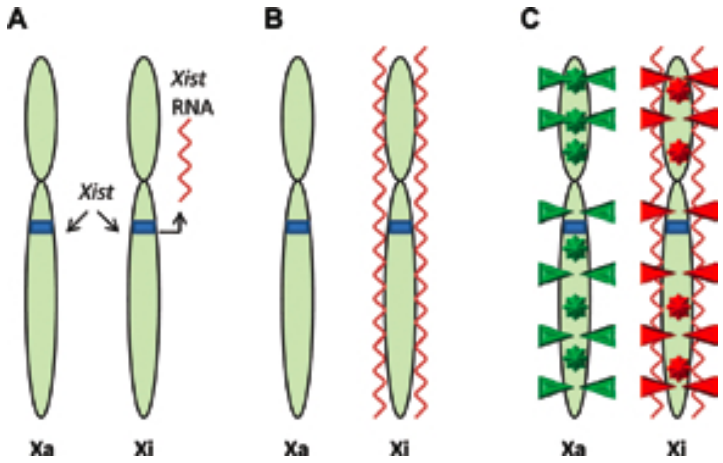
Heterochromatin can spread along DNA sequencens but also acts at limited regions (promoters, enhancers)

spreading

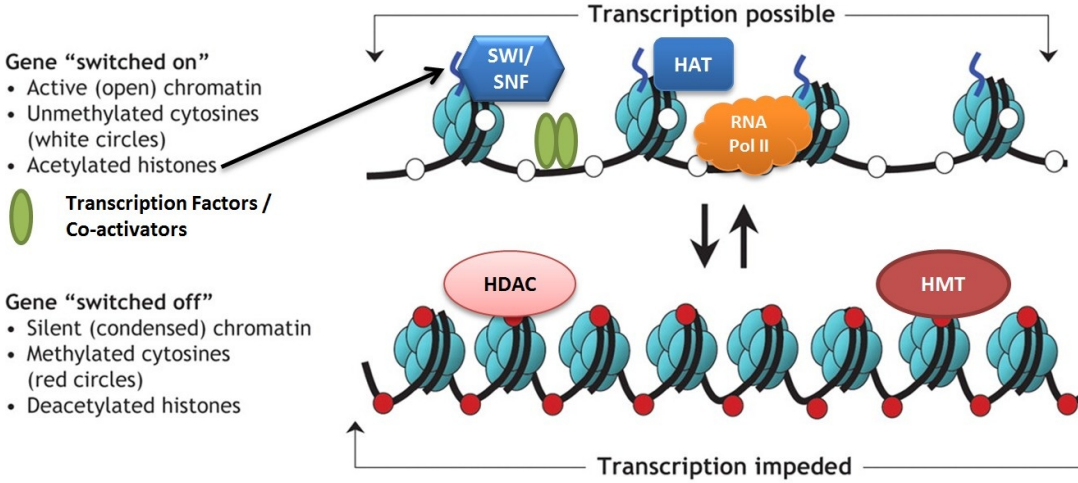


S. Cerevisiae telomeric repeats

Mammalian X-inactivation



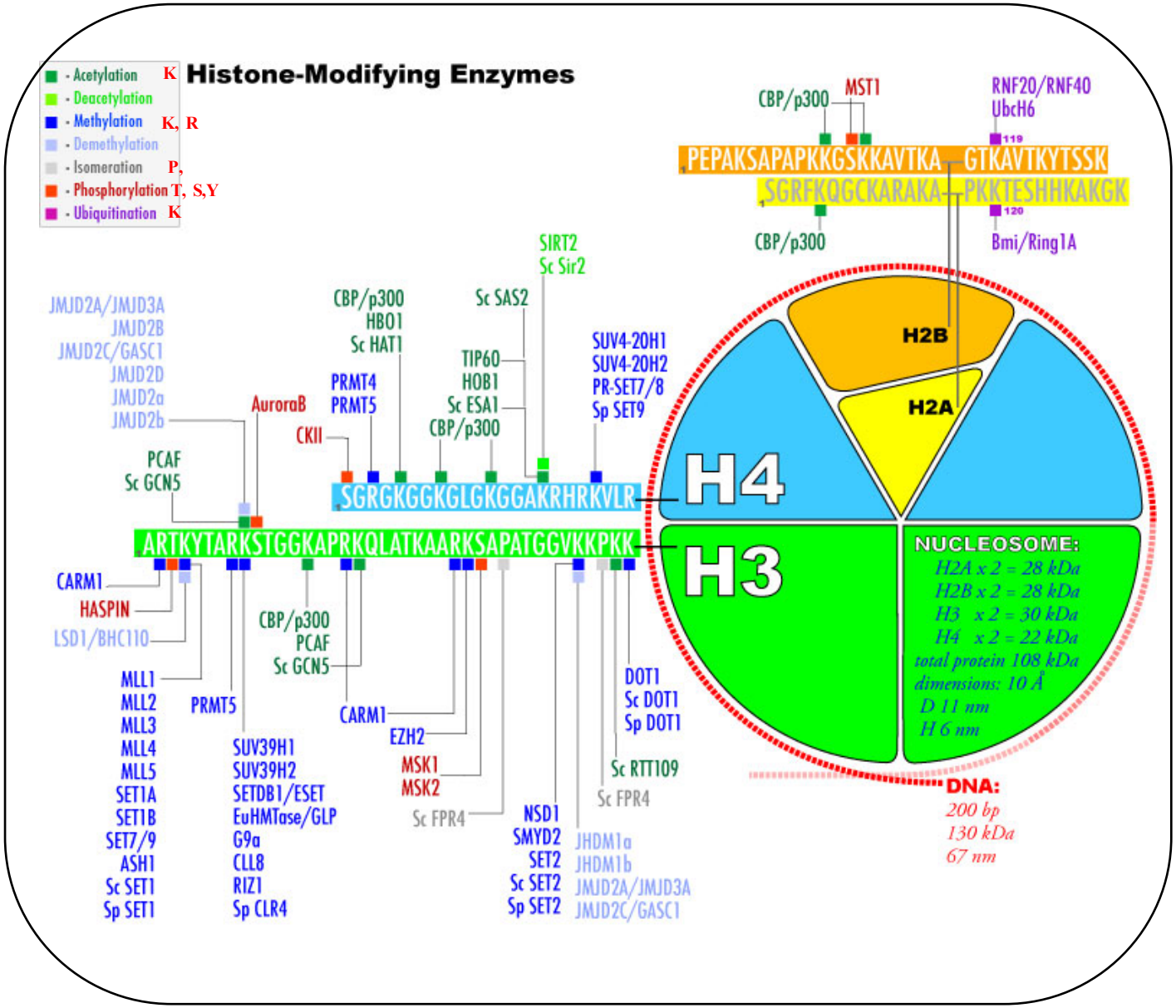
locally restricted

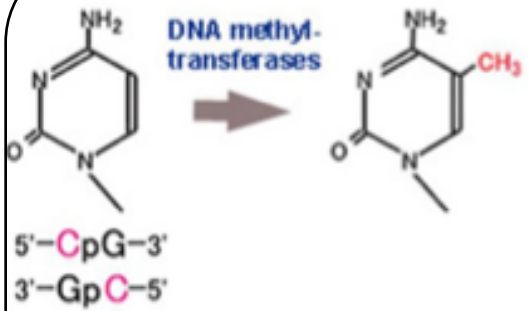


HAT: histone-acetyltransferase

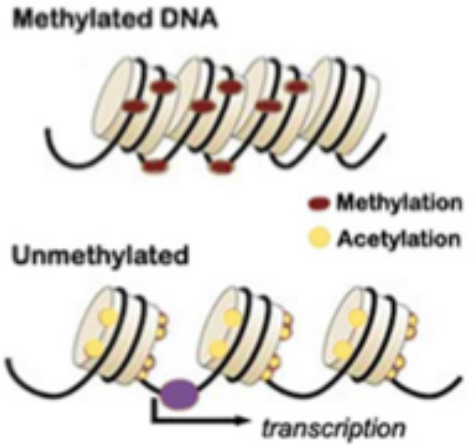
HDAC: histone-deacetylase

DNA METHYLATION AND HISTONE MODIFICATIONS



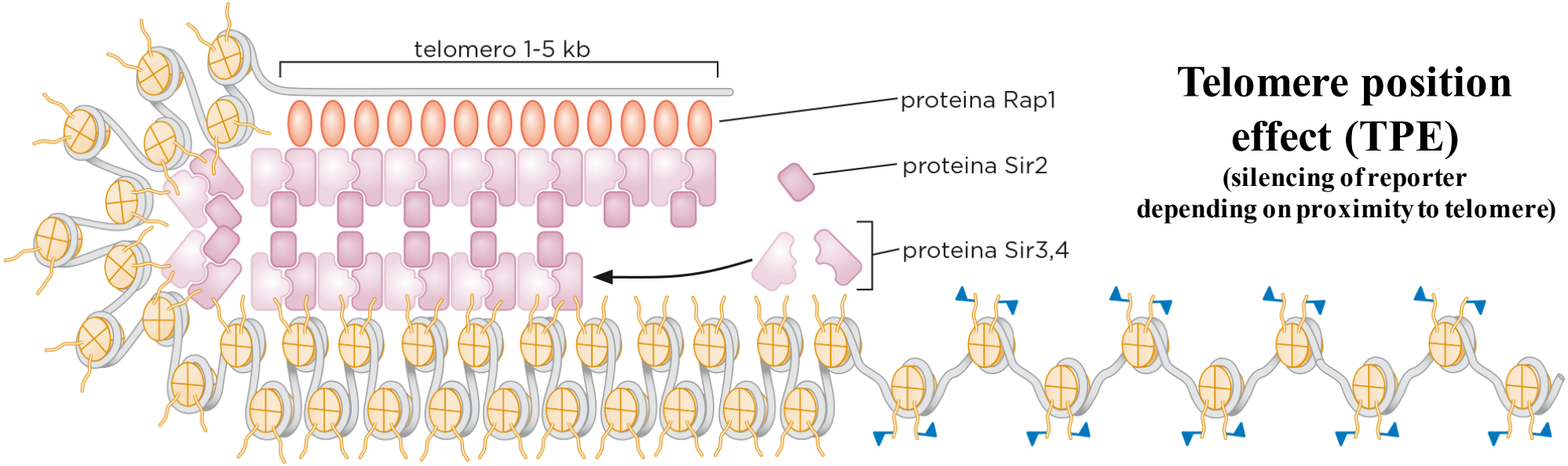


DNA methyltransfererases DNMT1, DNMT3a, DNMT3b act only on CpG di-nuceotides



ROLE MODELS OF LONG-RANGE HETEROCHROMATIN
-- Telomeric heterochromatin in S. cerevisiae --

Observation: Reporter gene inserted into central position in chromsome: EXPRESSED
→ Reporter gene inserted in proximity to chromsome ends: SILENT
→ → make mutatant S. cerevisiae that release reporter from silencing
→ → → identify genes that are mutated = HETEROCHROMATIN PROTEINS
= SILENT INFORMATION REGUALTORS (SIR)



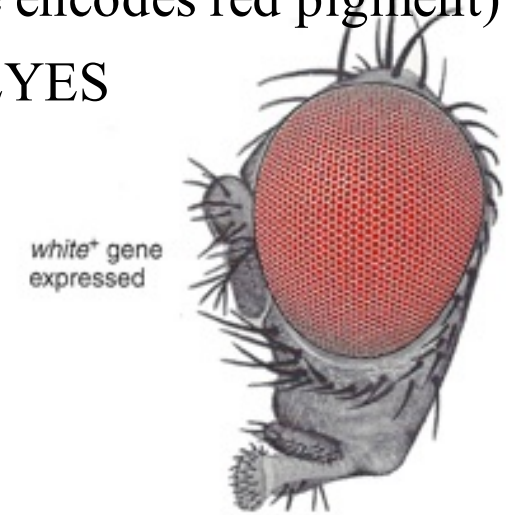
Telomere position effect (TPE)
(silencing of reporter depending on proximity to telomere)

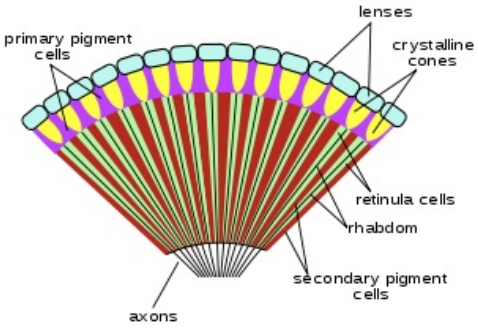
1. Rap1 specifically binds to telomeric repeat sequences ($G_{(2-3)}(TG)_{(1-6)}T$ consensus)
2. Rap1 recruits the SIR complex (SIR2,3,4) SIR2 is a HDAC → silencing of chromatin by histone deacetylation
3. SIR complex spreads towards the centromere (via cooperative binding) until meets region containing H2A variant
4. Htz1 and methylated H3 tails (active regions)

ROLE MODELS OF LONG-RANGE HETEROCHROMATIN
-- Position effect variegation (PEV) in Drosophila melanogaster--

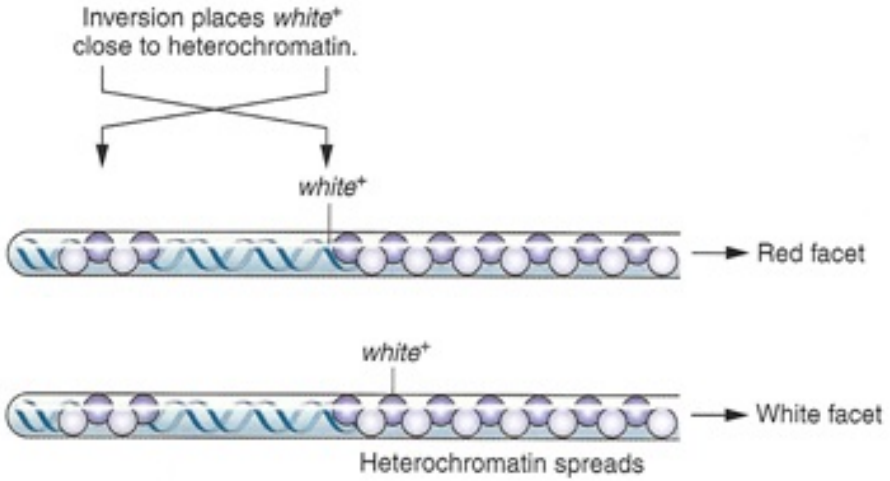
Wild type white gene: RED EYES (white encodes red pigment)

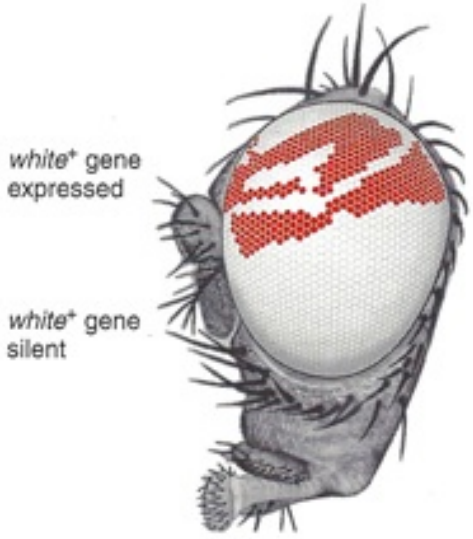
Mutant or silenced white gene: WHITE EYES





-30000 ommatidia



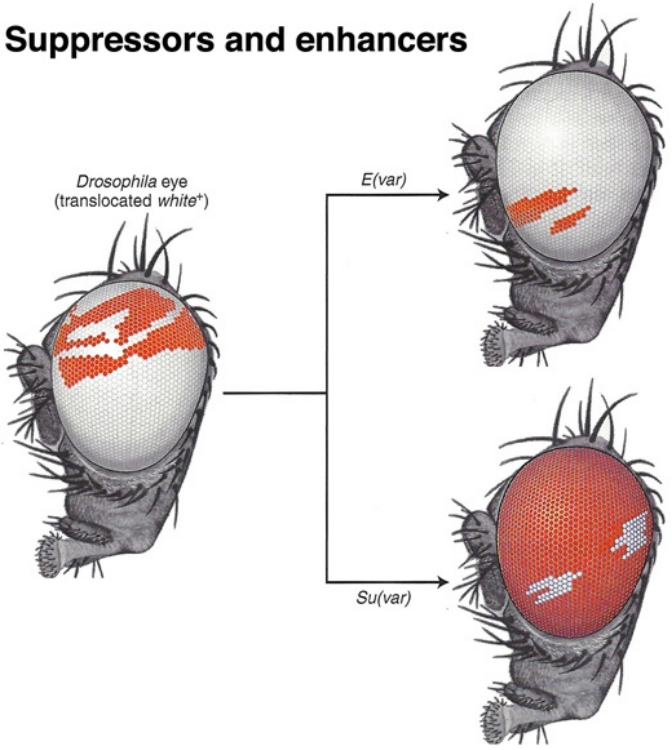


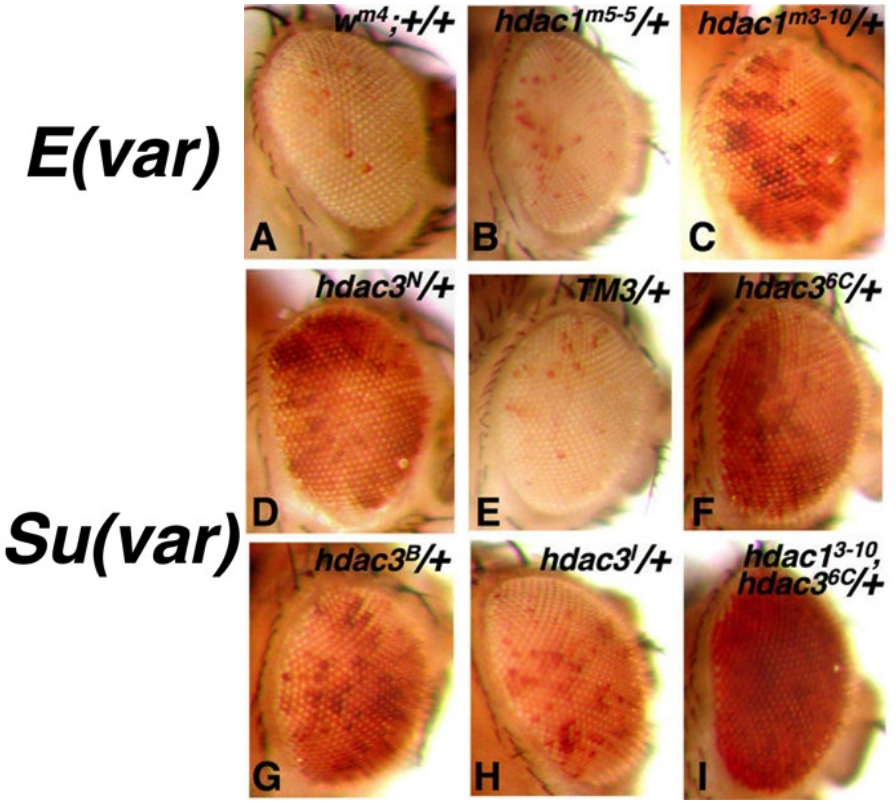
Variegated pigmentation of the eye

→Compact heterochromatin: majority unpigmentated (white gene silenced)

→ less compact HC: more pigmentation (white gene active)

ROLE MODELS OF LONG-RANGE HETEROCHROMATIN
-- Position effect variegation (PEV) in Drosophila melanogaster--





Additive effects of two *Su(var)* mutations

Su(var) = hdac

***hdac* = histone deacetylase**

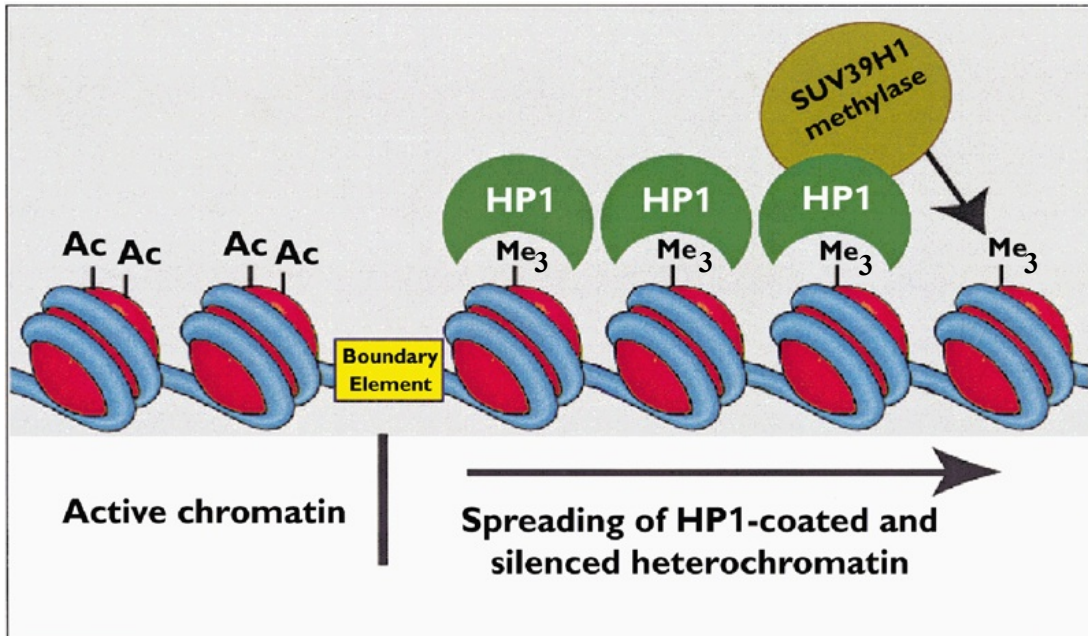
MAJOR HETEROCHROMATINIZING PROTEINS IDENTIFIED

Su(Var)3-9 = H3K9 specific HMTase (in humans SUV39H1 and SUV39H2)
Su(var)205 = HP1 (Heterochromatin protein 1) (in human HP1α,β,γ)

and many others such as HDACs, HATs.

ROLE MODELS OF LONG-RANGE HETEROCHROMATIN
-- Position effect variegation (PEV) in Drosophila melanogaster--

SUV39H1 and HP1 form heterochromatin at centromeric and telomeric Heterochromatin in flies and vertebrates and SAHFs (note: but also regulate the expression of individual genes)



Suv39h1/2 tri-methylate Lysine 9 on histone H3

↓

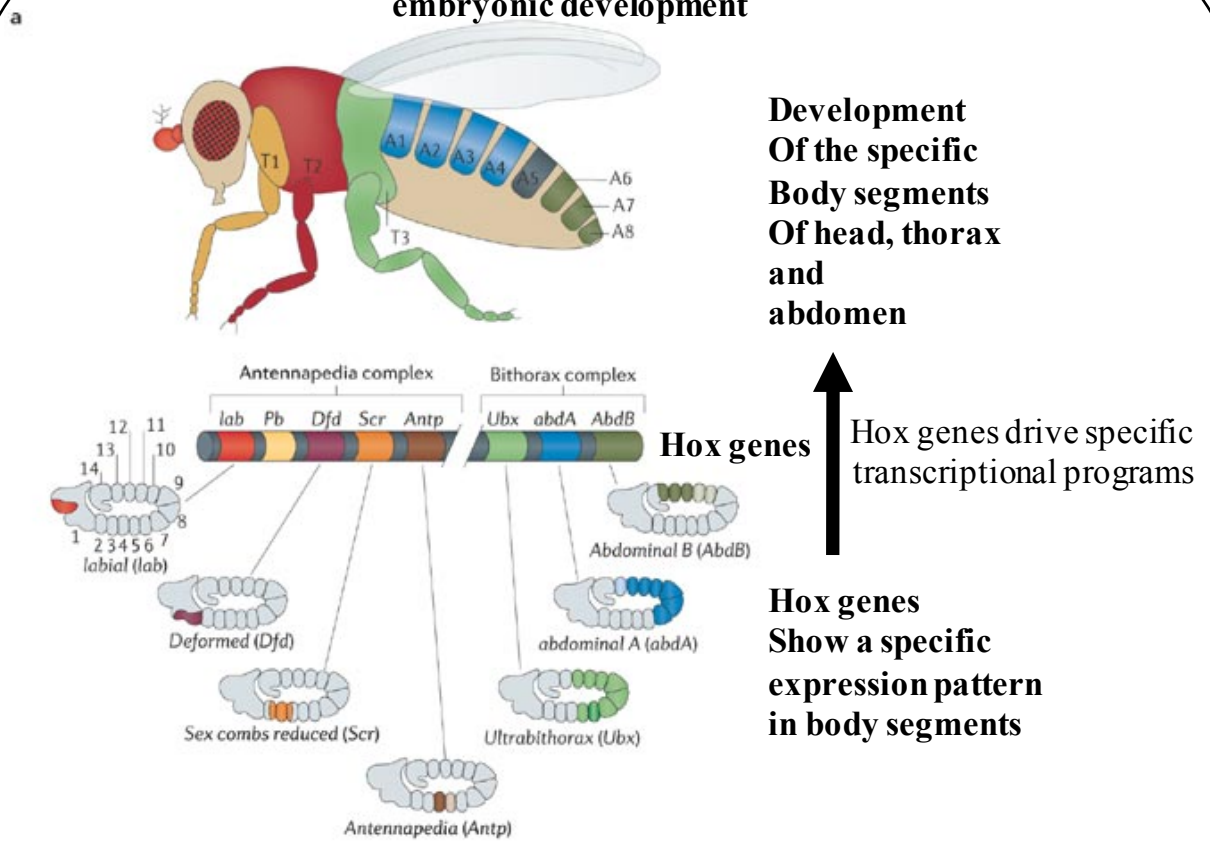
tri-methylated Lysine 9 on histone H3 recruits HP1

↓

heterochromatin

POLYCOMB GROUP GENE- DEPENDENT SILENCING

Polycomb group genes control fly development by repressing Hox (homeotic) genes in different segments during embryonic development

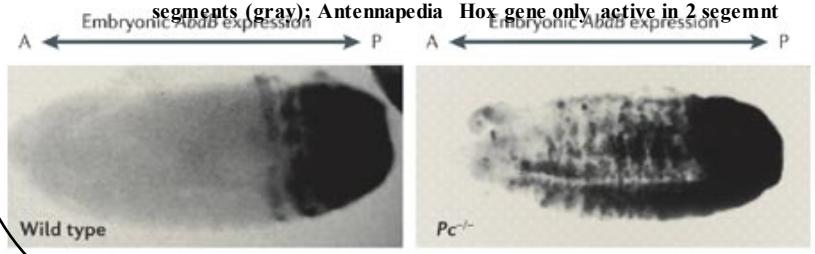


Development Of the specific Body segments Of head, thorax and abdomen

Hox genes drive specific transcriptional programs

Hox genes Show a specific expression pattern in body segments

b **Example: Polycomb represses Hox gene in other segments (gray); Antennapedia Hox gene only active in 2 segemnt**



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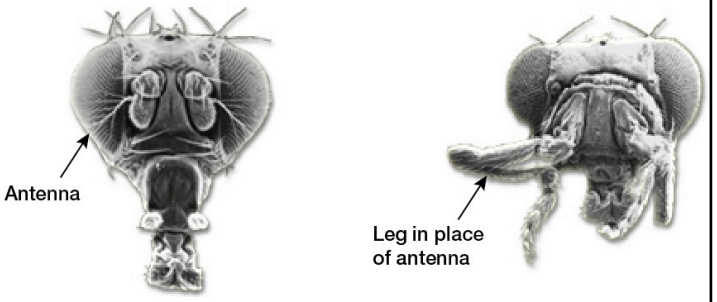
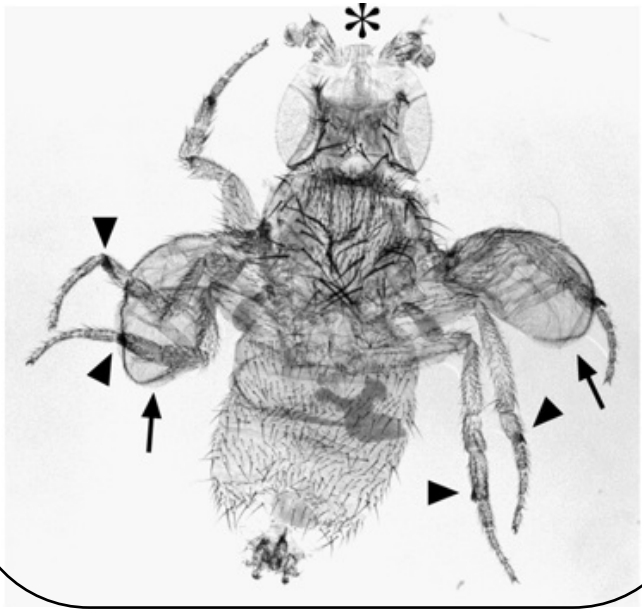
Polycomb mutants recapitulate Hox gene mutations

Wild type

Mutation in Antennapedia gene



Su(z)12 mutant (polycomb group gene) with antennapedia phenotype



POLYCOMB GROUP GENE- DEPENDENT SILENCING

Polycomb represive complex 1 (PRC1)

CBX, PHC, BMI1, **RING1A,B**

Ring1a/b ubiquitin-ligase

Ubiqitylates H2A119

↓

chromatin repression

Polycomb represive complex 2 (PRC2)

EED, PCL, SUZ12, **EZH2**

EZH2 is a Lysine-histone methyltransferase

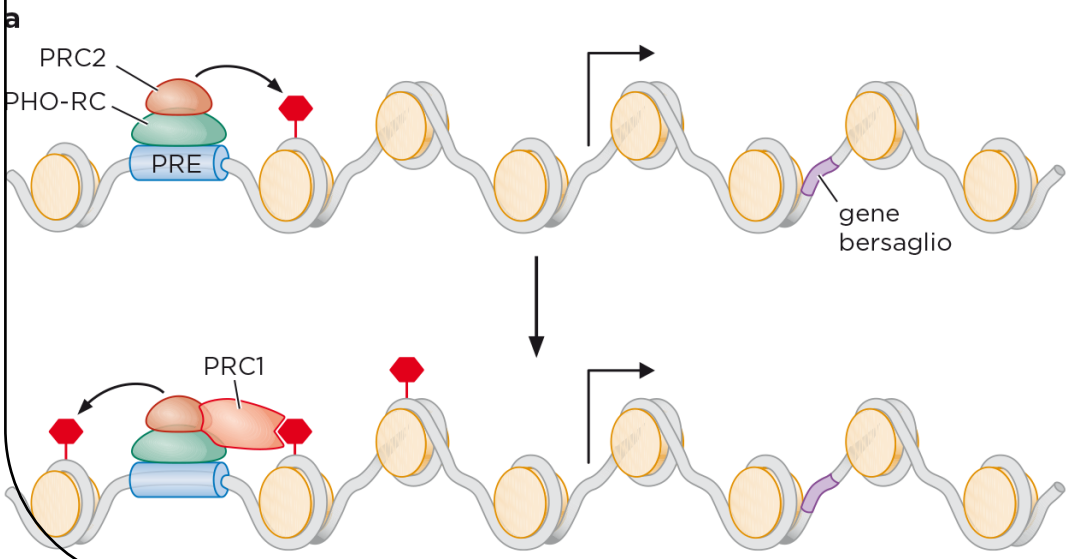
Acts on H2K27 → H2K27me3

↓

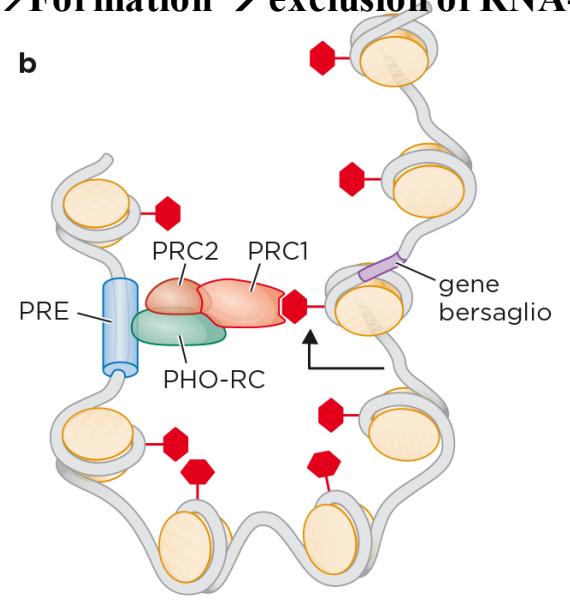
chromatin repression

PRC2 is recruited by DNA elements (polycomb responsive elements) that are localized in vicinity of promoters

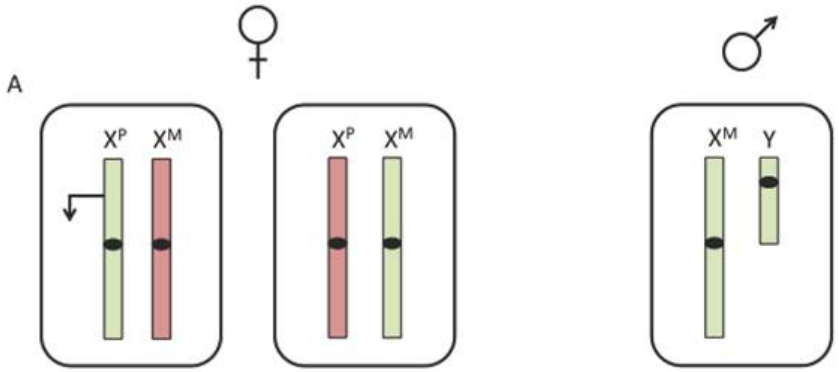
PRC2 recruited by PRE (polycomb responsive elements) →mediates H3K27me3→ PRC1 recruitment → silencing of promoter



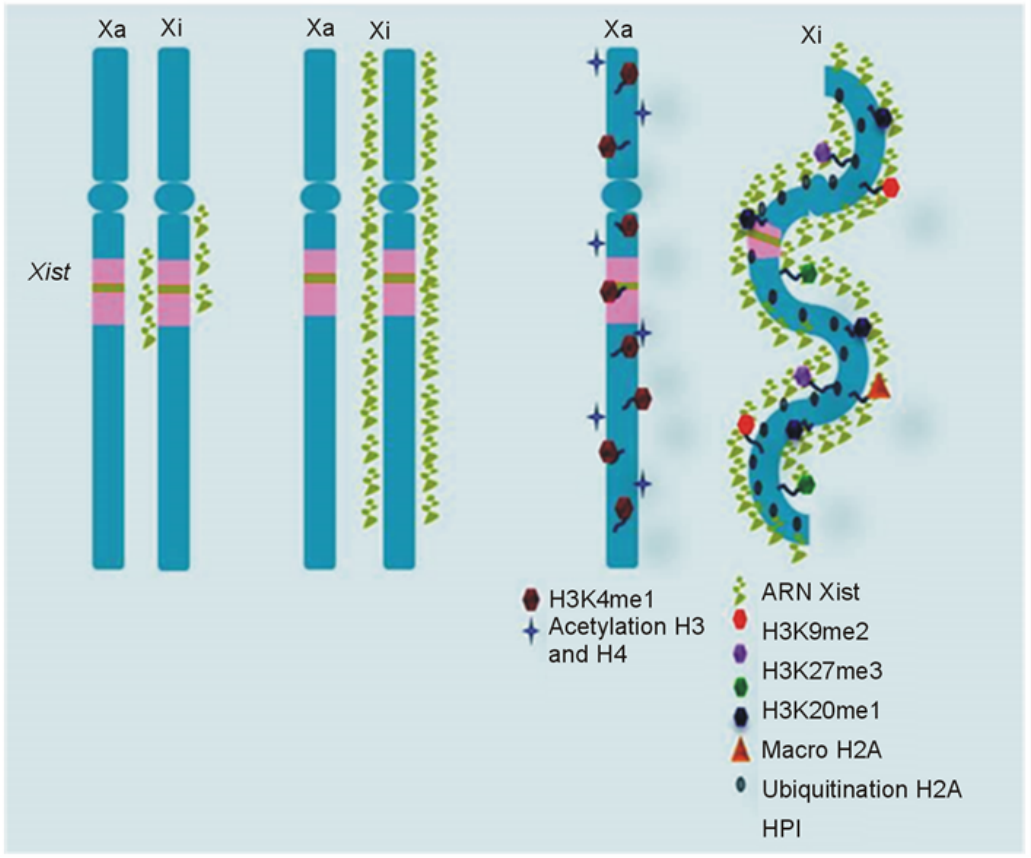
PRC2 recruited by PRE (polycomb responsive elements) →mediates H3K27me3→ PRC1 recruitment → loop →Formation → exclusion of RNA-Pol (silencing)



POLYCOMB GROUP GENE- DEPENDENT SILENCING

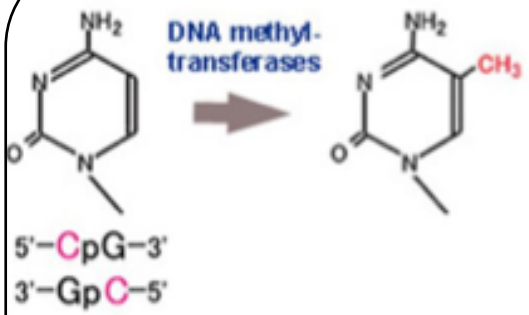


X-inactivation = dosage compensation
of X-linked gene expression
Male: XY; Female XX
To compensate X linked gene expression
Between males and females, female cells
Inactivate one X by heterochromatinzation
(random process)

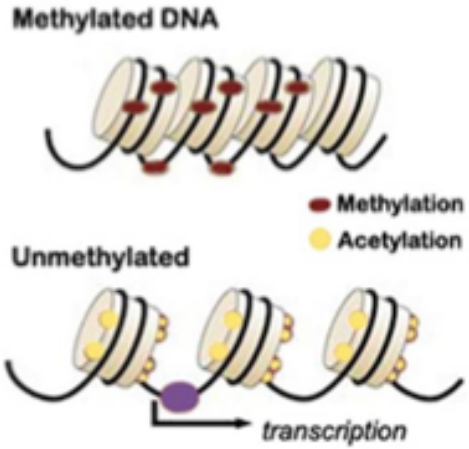


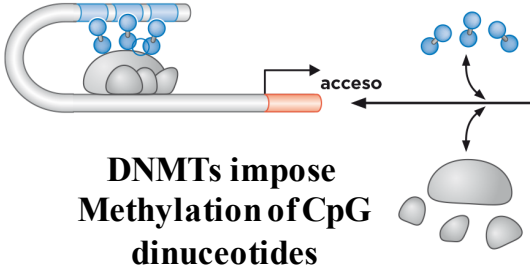
Polycomb induces H2K27me3 along entire X chromosome

DNA METHYLATION



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

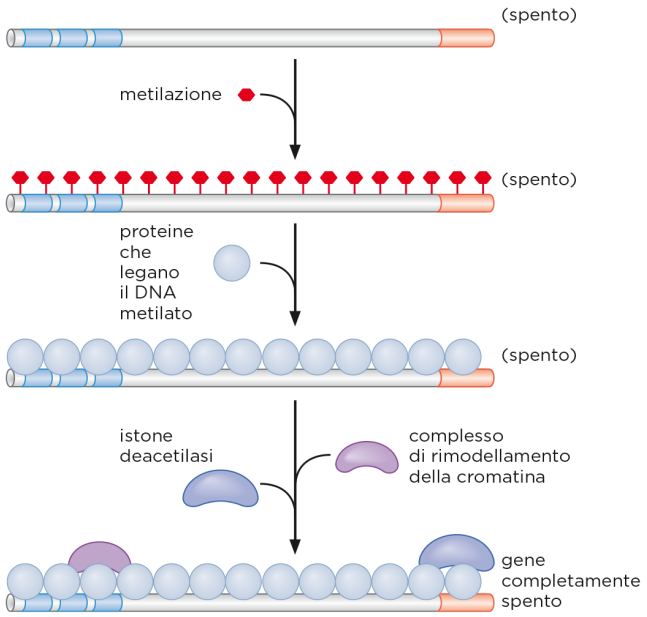




DNMTs impose Methylation of CpG dinuceotides

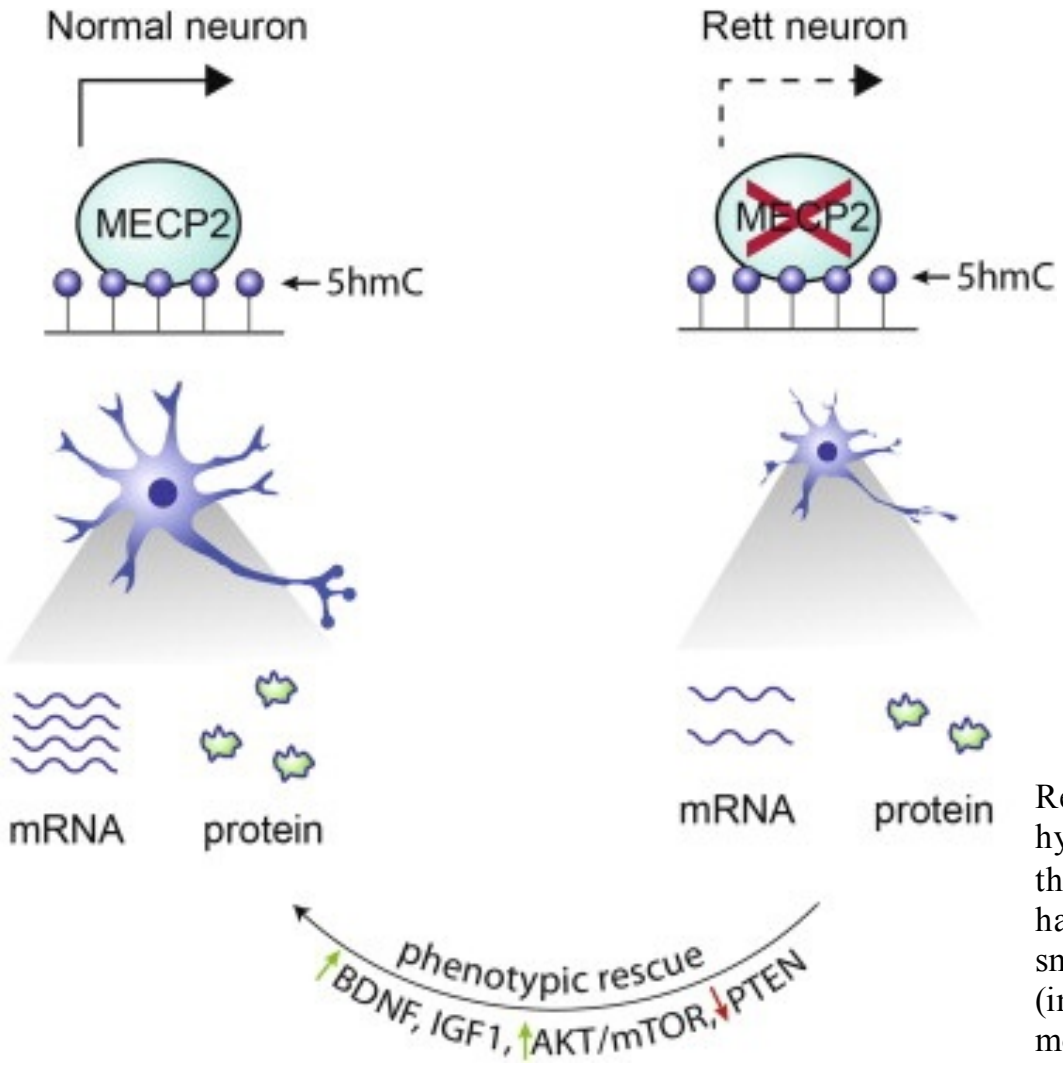
CH_3-CpG bound by specialized proteins (MBP, MeCP2)

Efficeint silencing

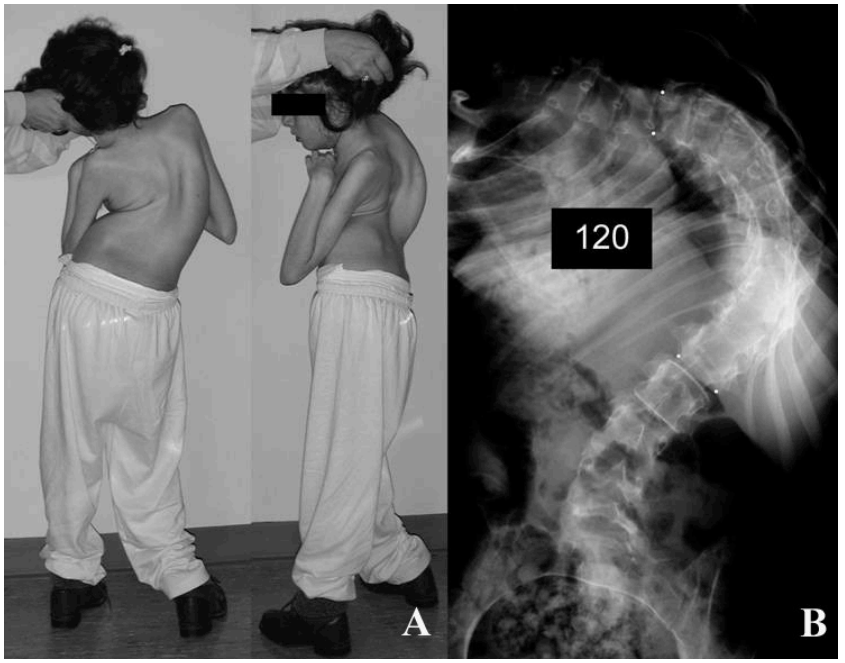


CpG repeats are often localized close to promoters = CpG islands: methylation grade decides on expression levels

RETT SYNDROME A DISEASE RELATED TO EPIGENTIC GENE REGUALTION

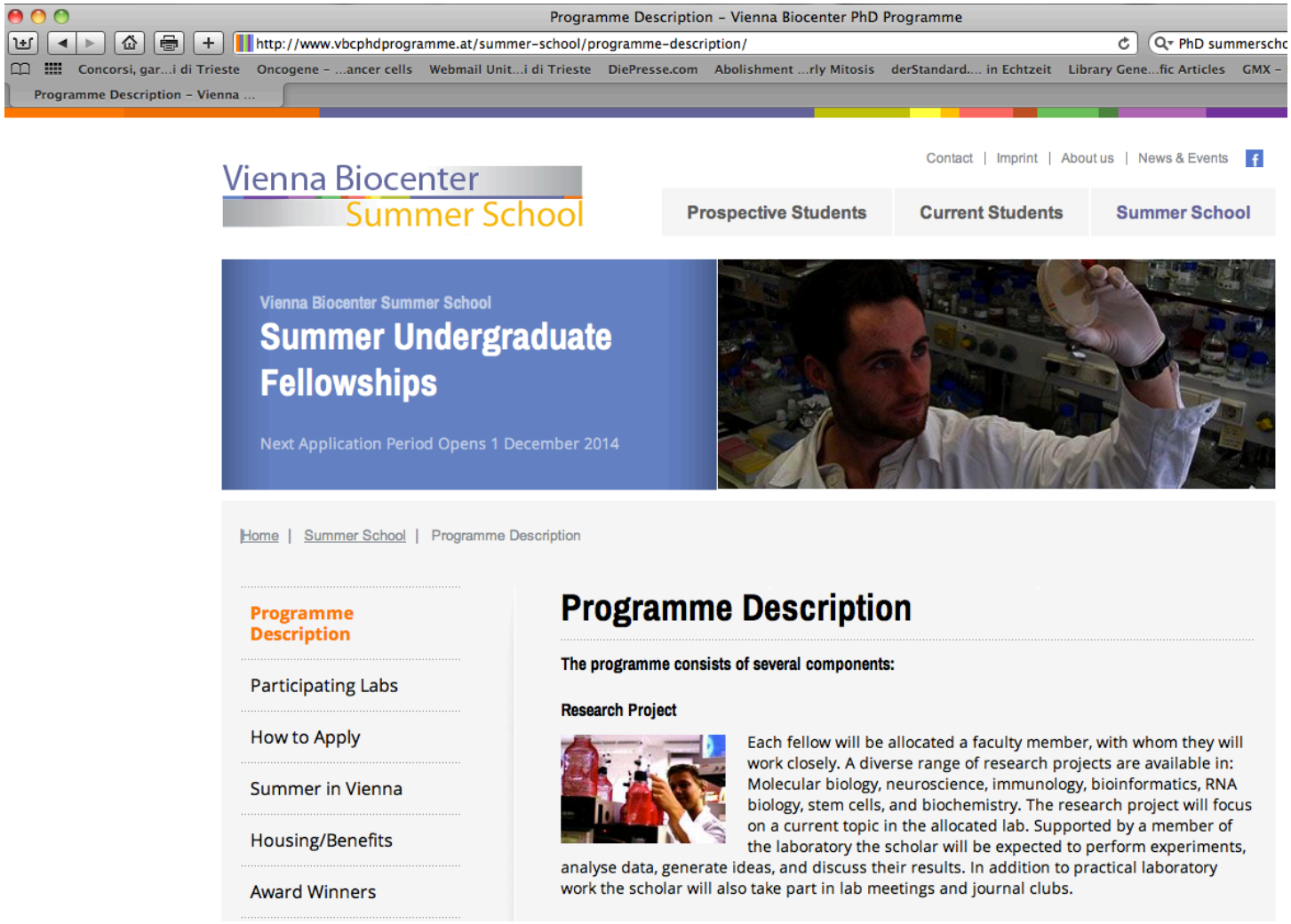


Rett syndrome is caused by mutations in MeCP2



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

START THINKING TO APPLY FOR A SUMMER UNDERGRADUATE SCHOOL IN 2018!!!



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all over europe

-get fellowship
-study and work
in a top environment
- in english language

Controllo dell'espressione genica negli eucarioti

1. **Regulation of transcription**
- Introduction
- Transcription factors – Activators of transcription
- Basic mechanisms of transcriptional activation
- Integration of signals
- Signal transduction

2. **Post-transcriptional gene regulation**
- Chromatin regulation
- **ncRNA - miRNAs**

Small ncRNA and gene/chromatin regulation

micro-RNAs = miRNAs

short interfering RNAs = siRNAs

miRNAs and siRNAs are generated by the same machinery

1. Long precursor RNA

1. Processing into small RNAs by Dicer
siRNAs and miRNAs (21-23 nt) still double-stranded

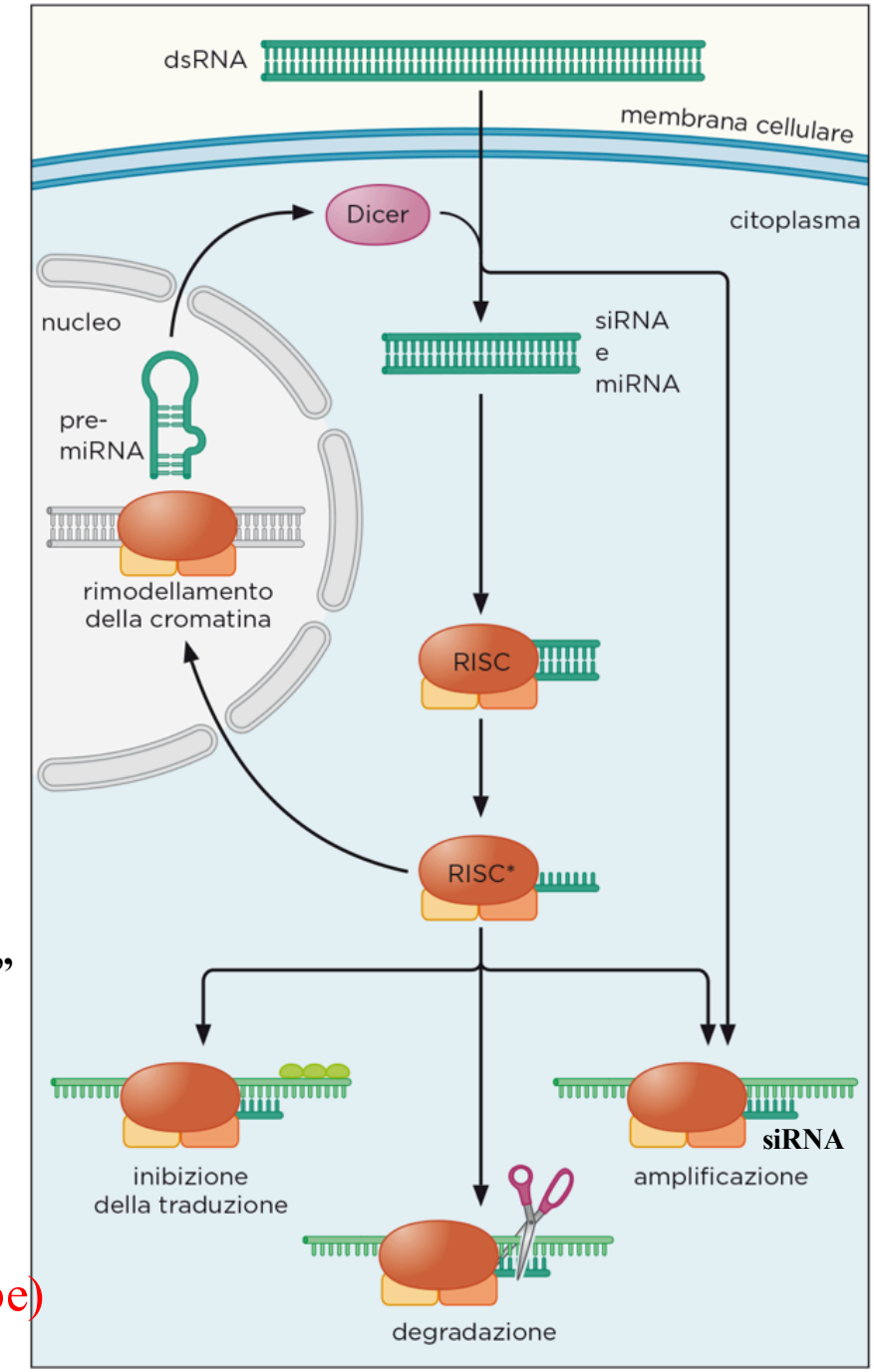
3. Processing by RISC complex
(RNA induced silencing complex)

4. guide RNA → regualtory RNA
passenger RNA → will be eliminated

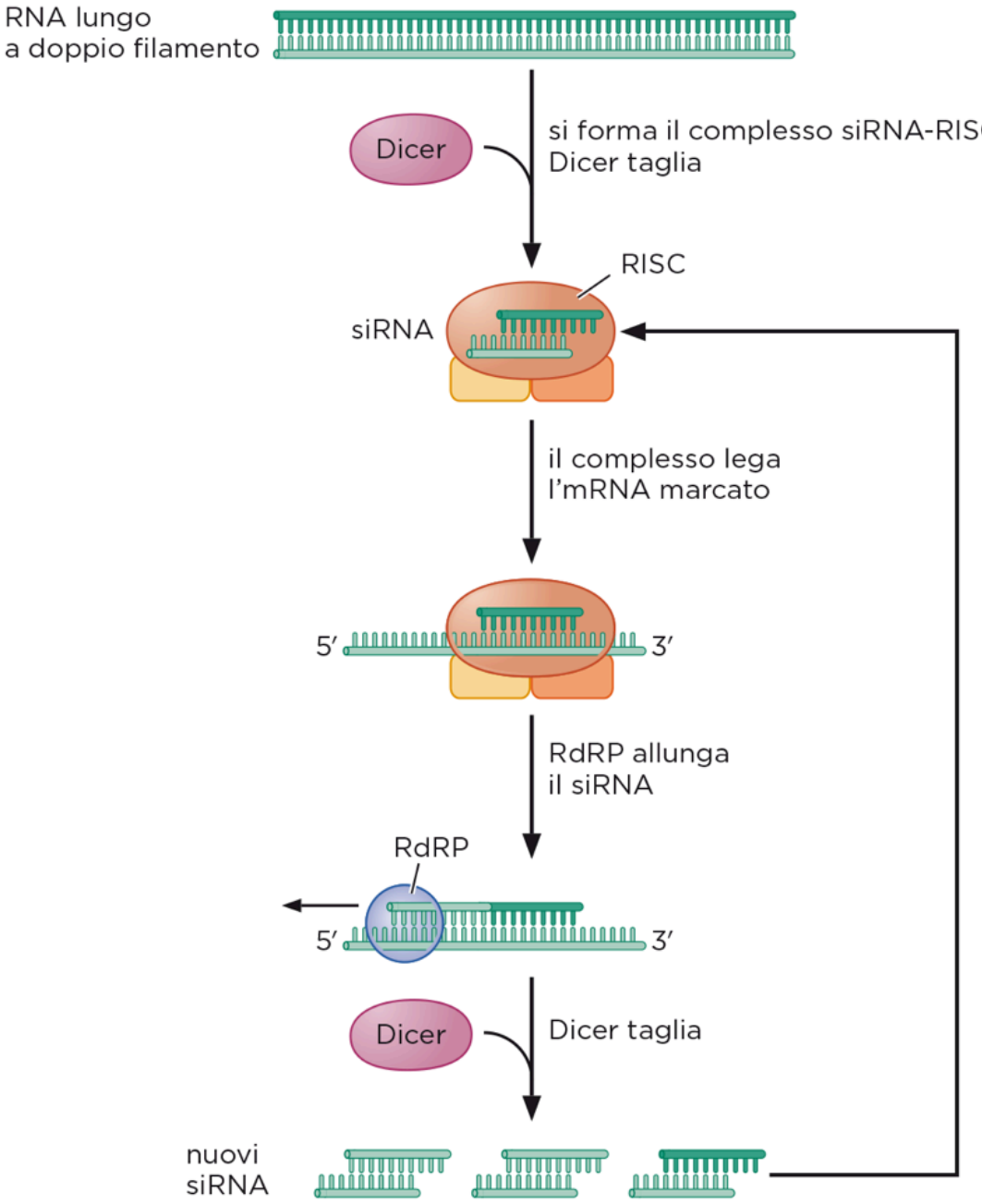
5. RISC complex+guide RNA → regulatory function

A. RNA degradation = siRNA effect (cutting = "slicing"
B. inhibition of mRNA translation =mRNA effect
C. transfer to nucleus and chromatin regulation = siRNA mediated silencing

miRNAs: always "trans"-acting on mRNAs
siRNAs: mostly "cis" acting on chromatin (S-pombe)



RNA dependent RNA polymerase amplifies siRNAs

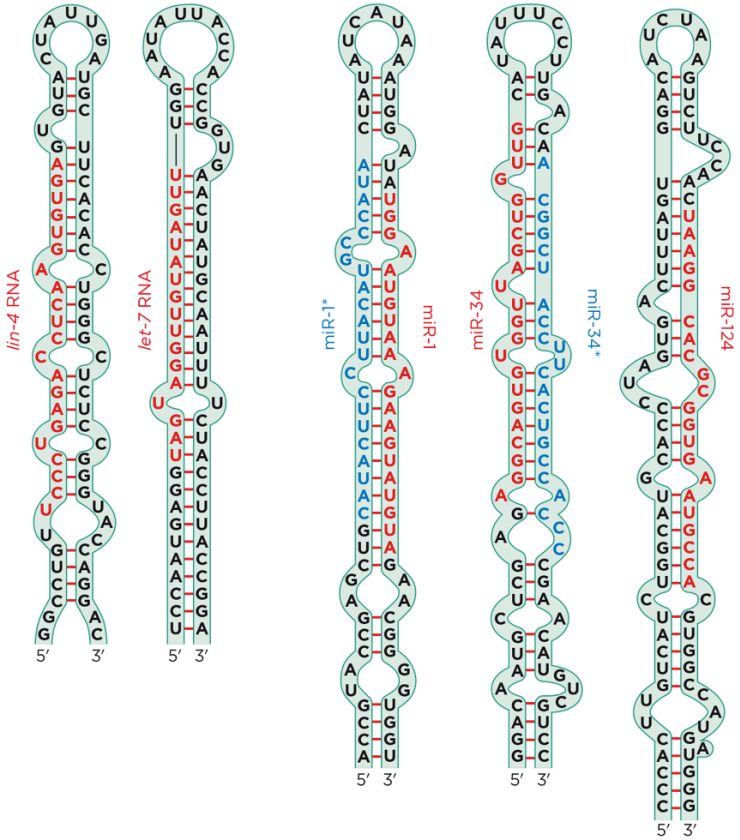


In plants, S. pombe, nematodes, humans???

miRNA dependent regulation of gene expression

One strand generates miRNA

both strand Generate miRNA



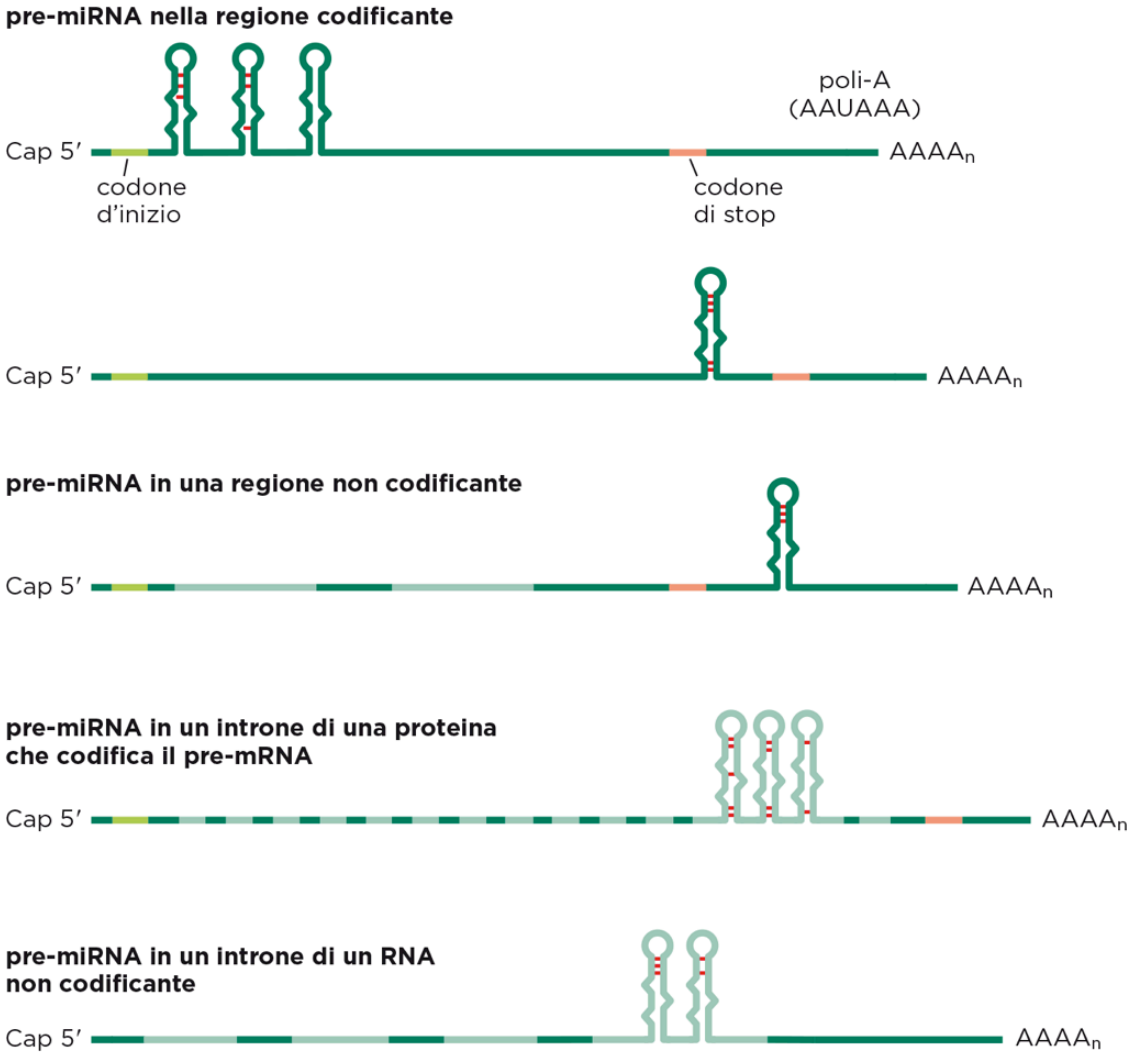
pri-miRNA (primary miRNA

↓ **Drosha**

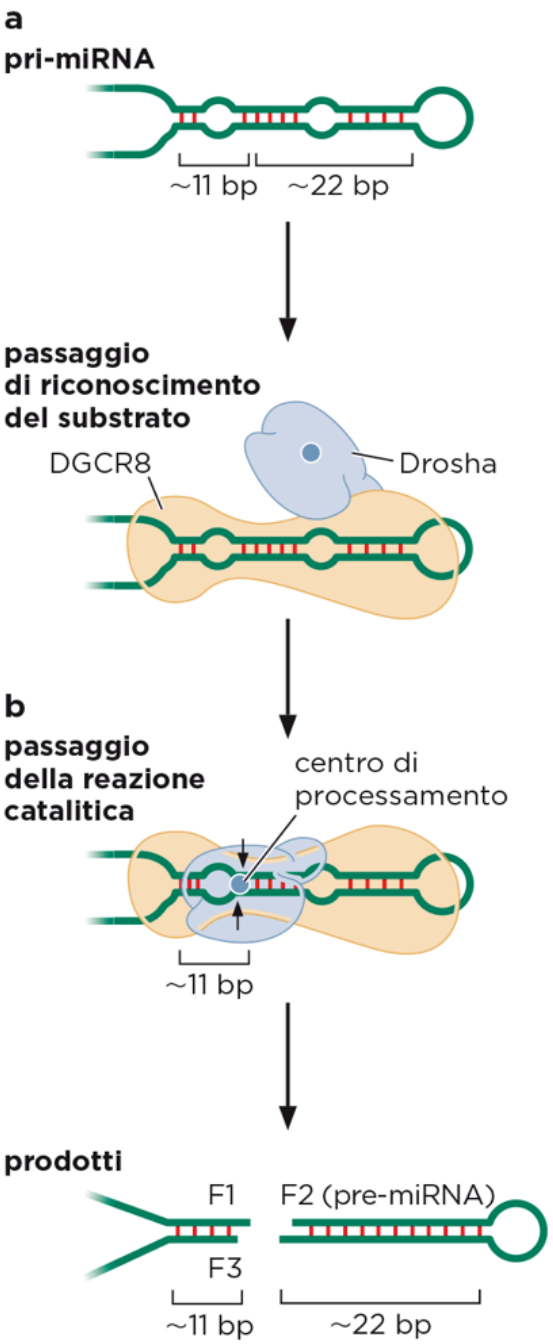
pre-miRNA (precursor miRNA)

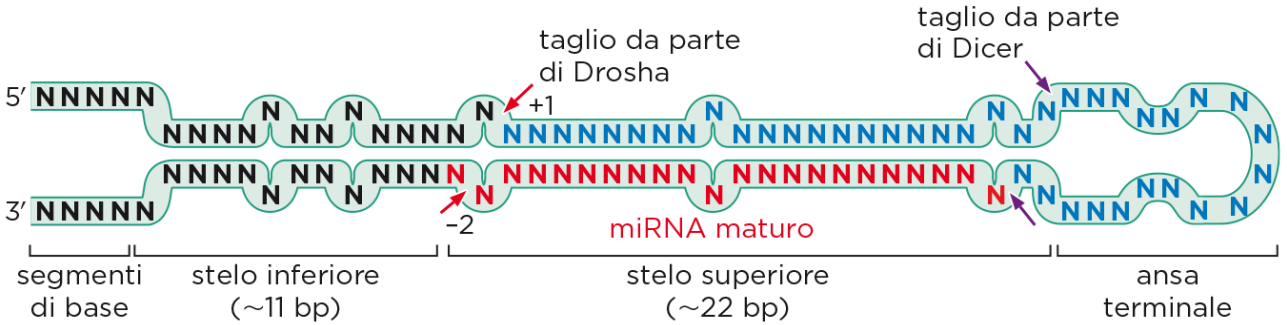
↓ **Dicer**

miRNA (mature miRNA)



miRNA generation - DROSHER





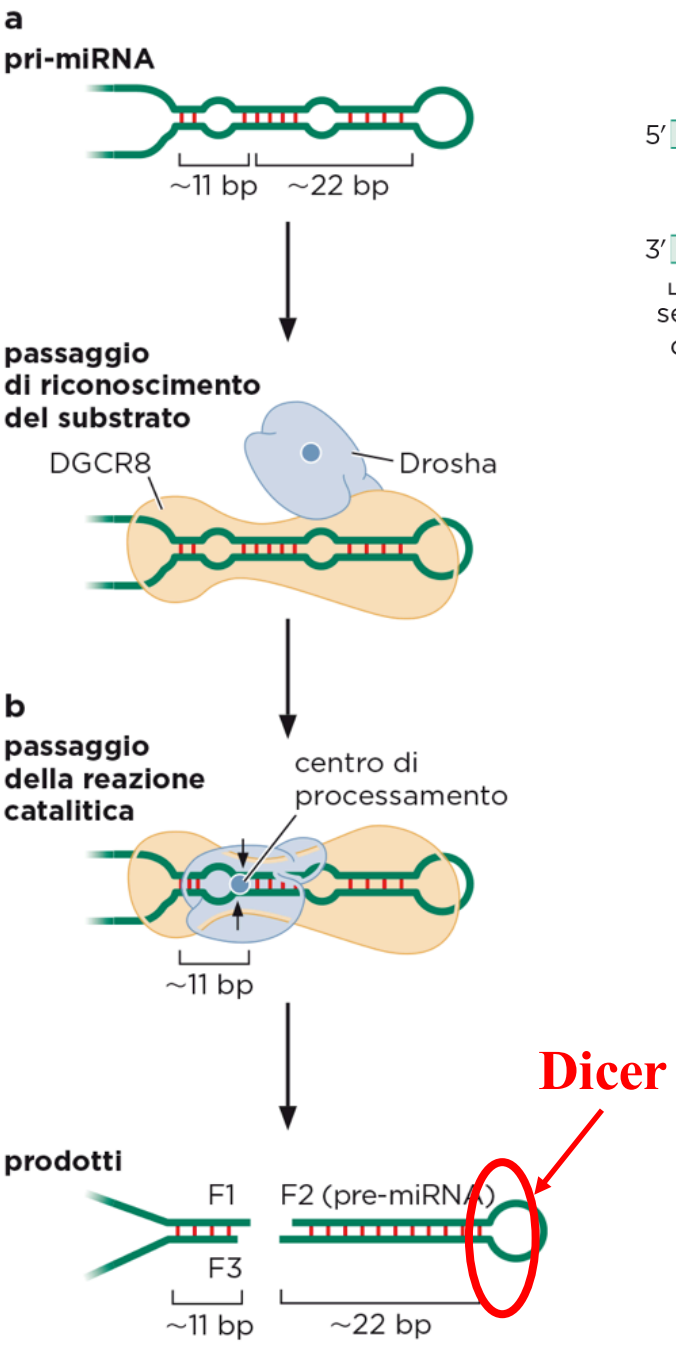
Drosha, Dicer: **Type III RNases**: cut 2 RNA strands in RNA douplex, leave overhang!!

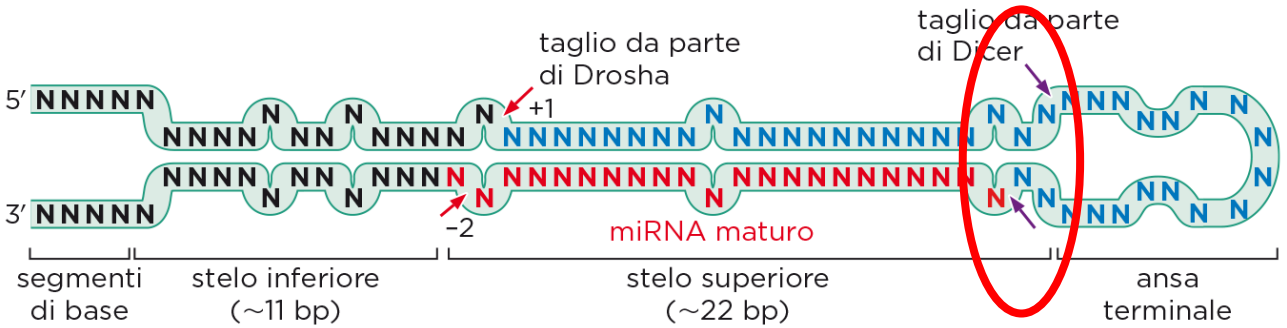
1. Microprocessor (Drosha and DGCR8) generates a 65-70 nt RNA stem loop:

Drosha cuts app. 11 nt after ssRNA-dsRNA contact
5 components: stelo inferiore (11 bp); stelo superiore (22 nt)
ansa terminale; segmenti di base

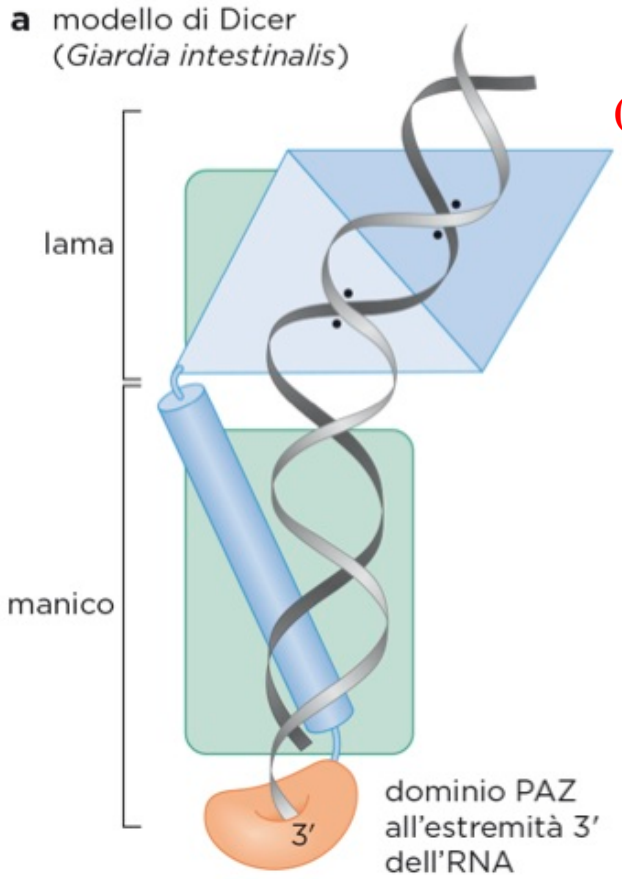
2. Transfer to cytoplasma

miRNA generation - DICER





Dicer



DICER processes all dsRNAs (NOT SEQUENCE SPECIFIC)

2 RNase domains: act on ALL dsRNAs

manico interacts with dsRNA

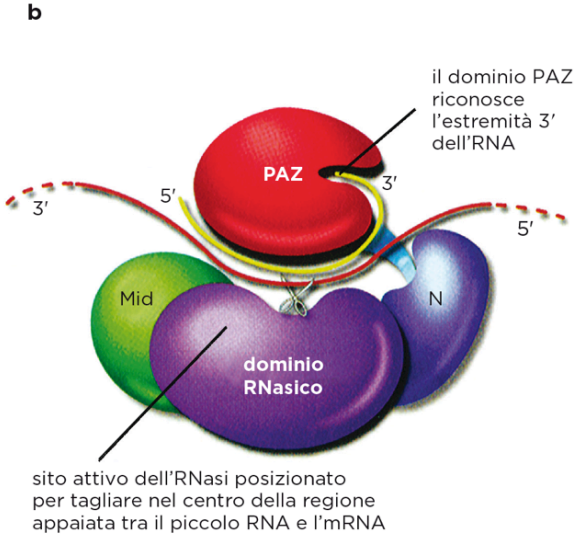
PAZ interacts with dsRNA

Regulation of gene expression by miRNAs

```
hsa-miR155        3'-UGGGGAUAGUG-----CUAAUCGUAAUU-5'
                     |   || |:::          |||||||
Human          5'GUAAUUUAAAACUUUUGU-----UUAAAGCAUUAC--------AGUAU----------3'
Chimpanzee     5'GUAAUUUAAAACUUUUGU-----UUAAAGCAUUAC--------AGUAU----------3'
Cow            5'------CAAAA-UUCUGU-----UUAAAGCAUUAC--------AACAU----------3'
Rabbit         5'--------AAAUUUUGGU-----UUAAAGCAUUAU--------UU-------------3'

Mouse          5'AUGAUAAAGCAUUAUGGUGGUGGUGGGGCAGUGAGGAGG
Rat            5'AAGAUGAAGCAUUAUUGU----GUGUGUGUGUGAG----
```

Seed sequence: pos 2-8 in miRNA (5' → 3')



One strand of pre-miRNA is incorporated into the RISC complex (RNA induced Silencing complex) = guide strand
Passenger strand degraded by RISC complex

Base pairing miRNA/siRNA – target RNA
(seed seqeunce in miRNA is most important for target identification

RNAse domain cleaves target transcript OR translational repression

Regulation of gene expression by miRNAs

Gene regulation

RISC uses the bound guide strand to target complementary 3'-untranslated regions (3'UTR) of mRNA transcripts via Watson-Crick base pairing. RISC can now regulate gene expression of the mRNA transcript in a number of ways.

mRNA degradation

The most understood function of RISC is degrading target mRNA which reduces the levels of transcript available to be translated by ribosomes. There are two main requirements for mRNA degradation to take place:

a near-perfect complementary match between the guide strand and target mRNA sequence, and,
a catalytically active Argonaute protein, called a 'slicer', to cleave the target mRNA.
mRNA degradation is localised in cytoplasmic bodies called P-bodies.

Translational repression

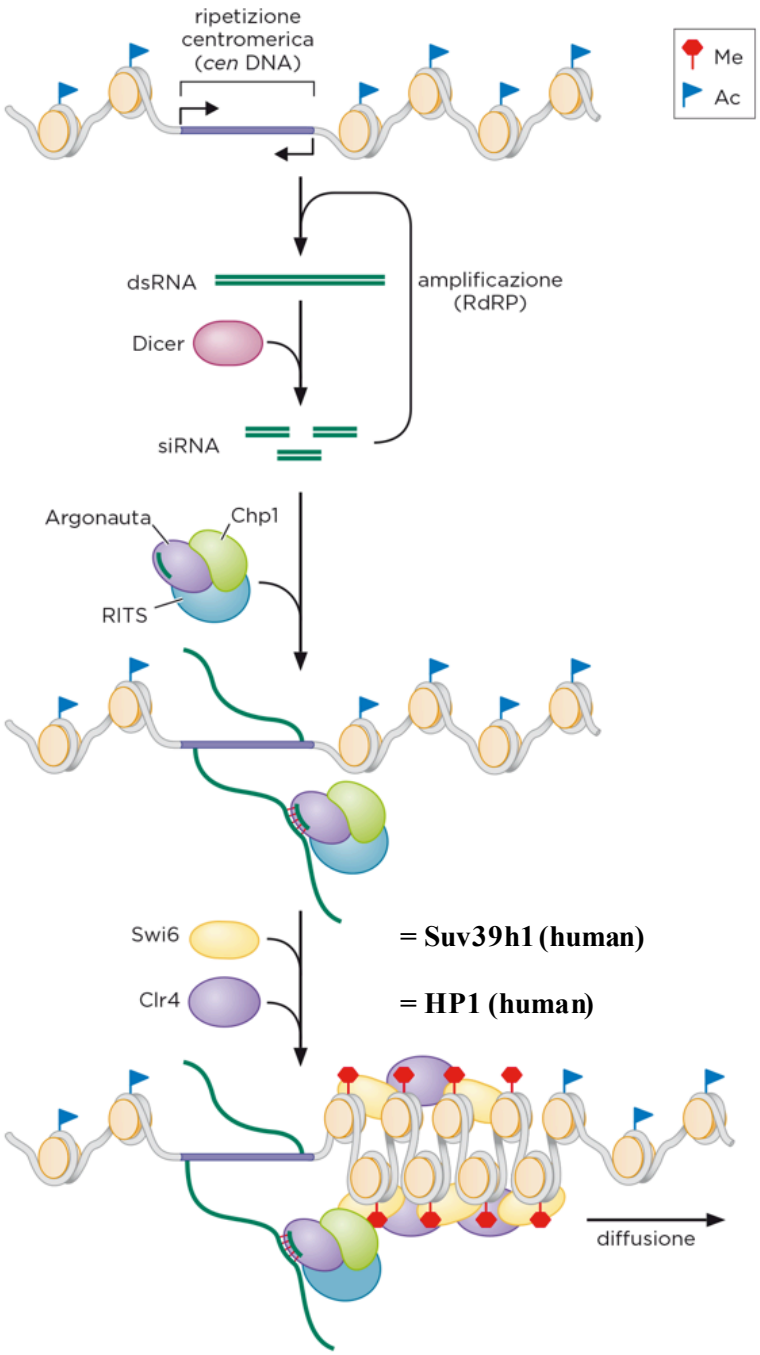
RISC can modulate the loading of ribosome and accessory factors in translation to repress expression of the bound mRNA transcript. Translational repression only requires a partial sequence match between the guide strand and target mRNA.

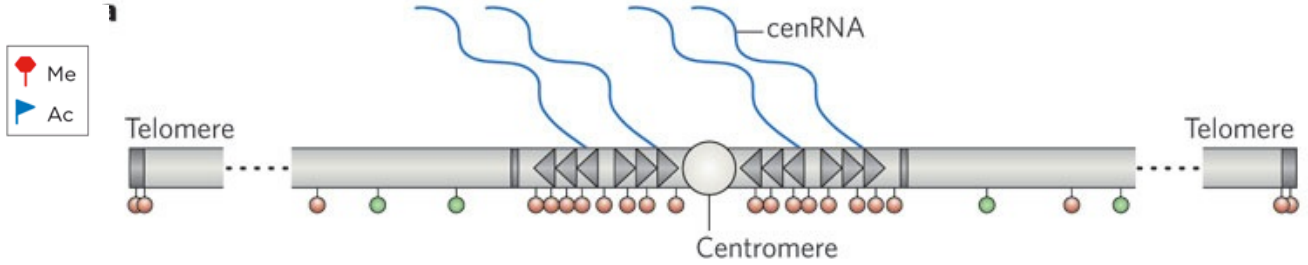
Translation can be regulated at the initiation step by:

preventing the binding of the eukaryotic translation initiation factor (eIF) to the 5' cap. It has been noted RISC can adeadenylate the 3' poly(A) tail which might contribute to repression via the 5' cap.
preventing the binding of the 60S ribosomal subunit binding to the mRNA can repress translation.
Translation can be regulated at post-initiation steps by:

promoting premature termination of translation ribosomes, or,
slowing elongation.
There is still speculation on whether translational repression via initiation and post-initiation is mutually exclusive.

siRNA mediated chromatin regulation (silencing)





Centromeres in S. pombe:

-Heterochromatin (H3K9me3, Clr4 (HP1)

-Reporter genes inserted: repression

-Discovery:

RNAi mutant result in loss of H3K9m3/Clr4 and reactivation of reporter gene that was inserted into centromeric region

=RNAi mediated gene silencing