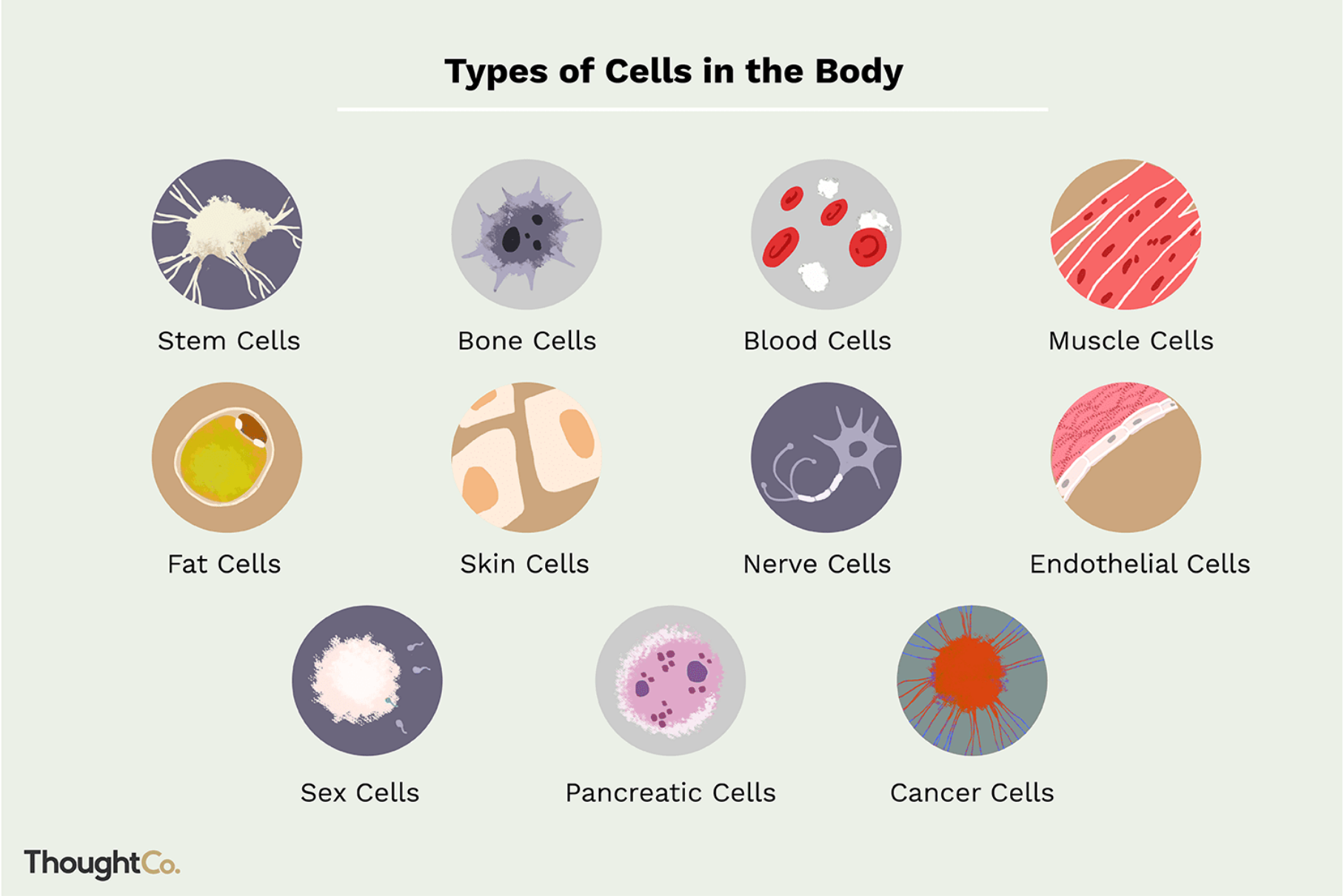
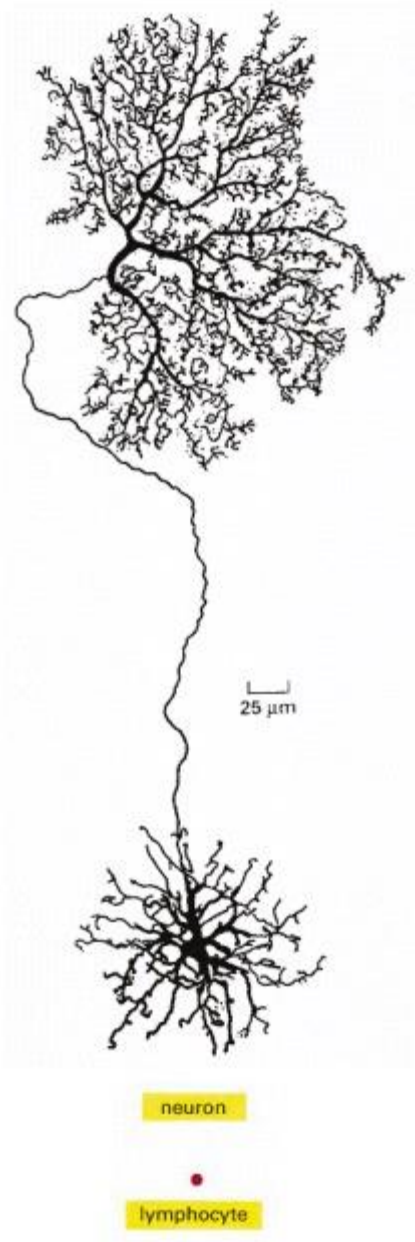
Regolazione dell’espressione genica

Fabrizio Mafessoni – 8-11-2024

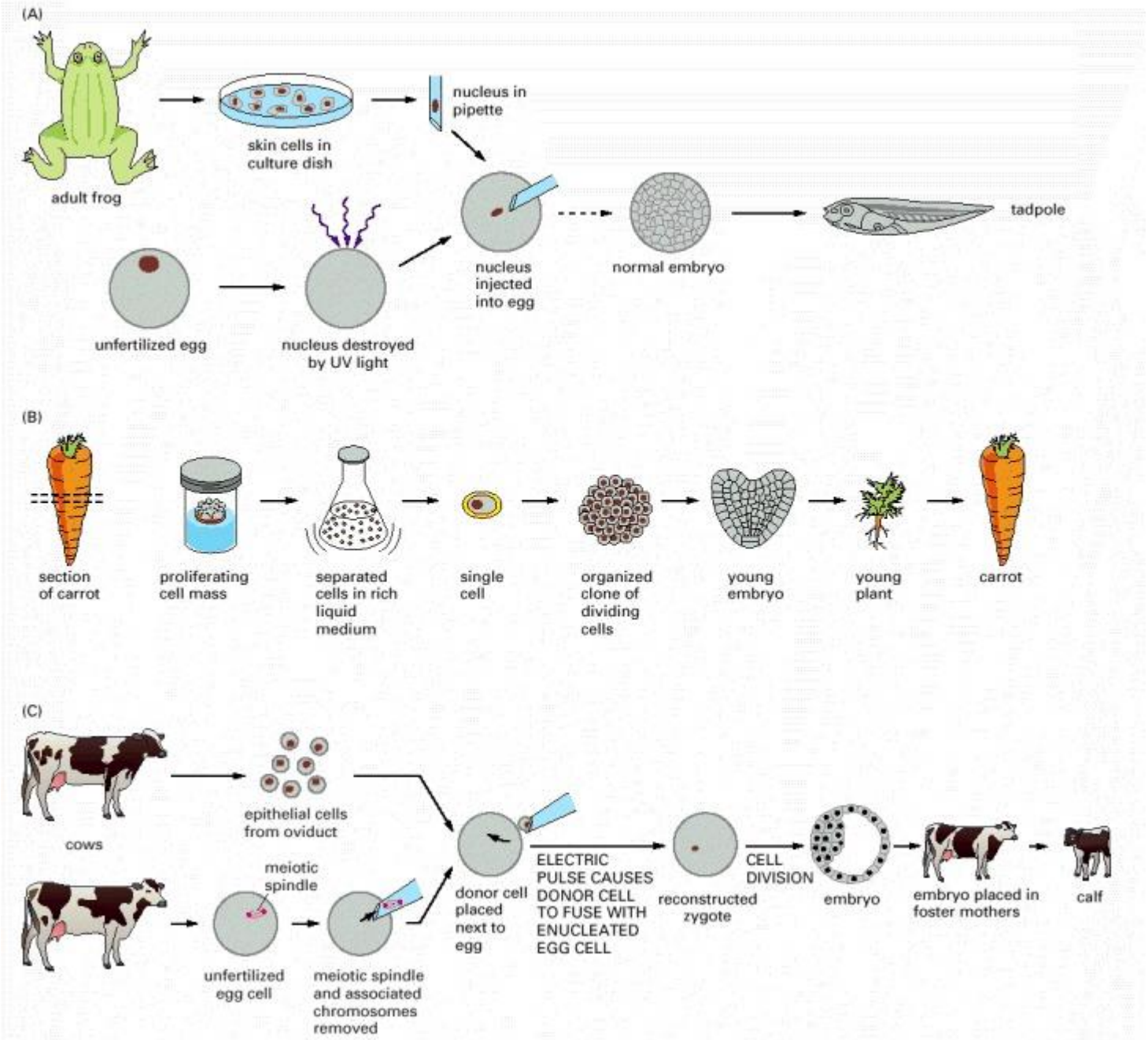
Lo stesso genoma puo’ dare molti tipi cellulari



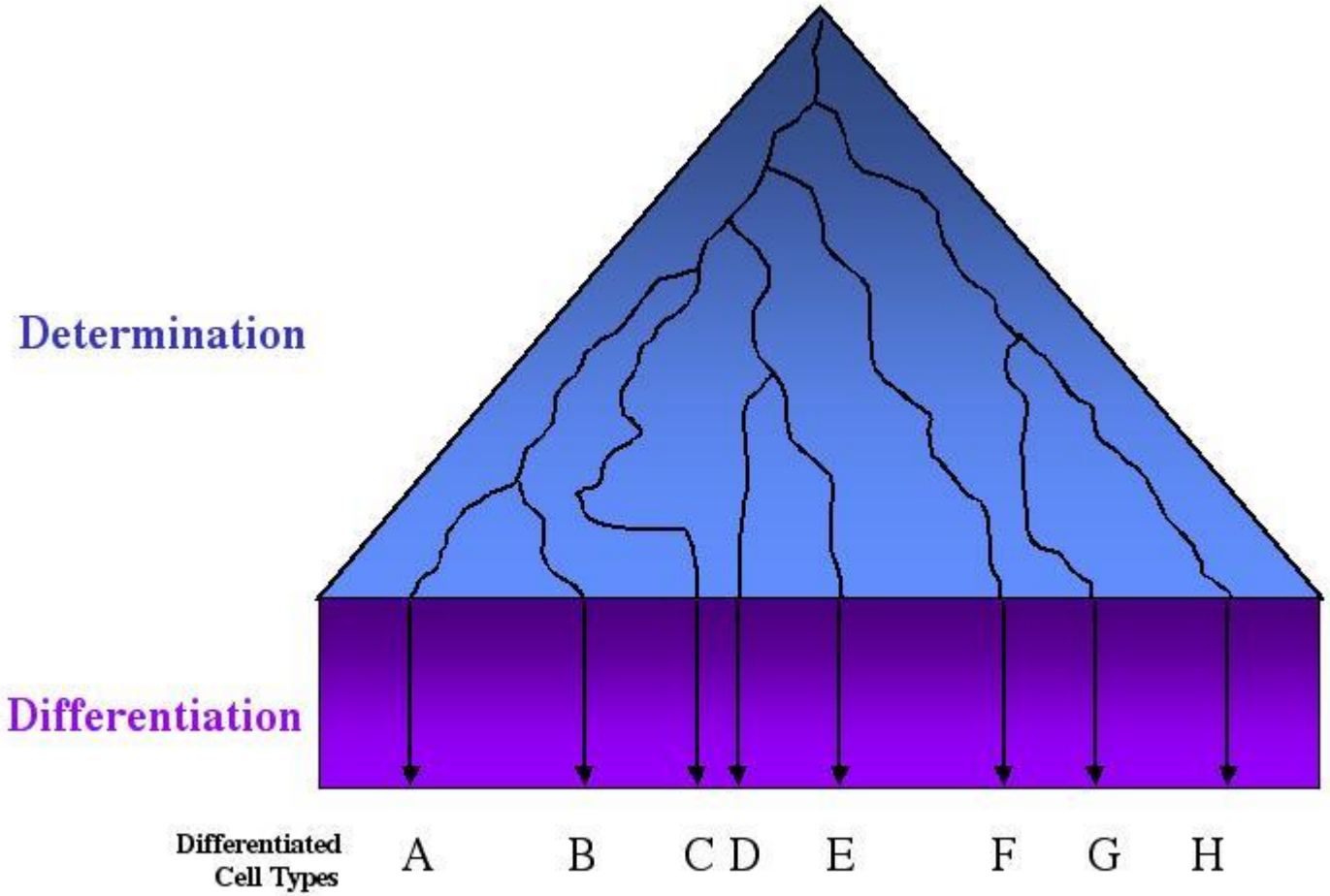
Come avviene?

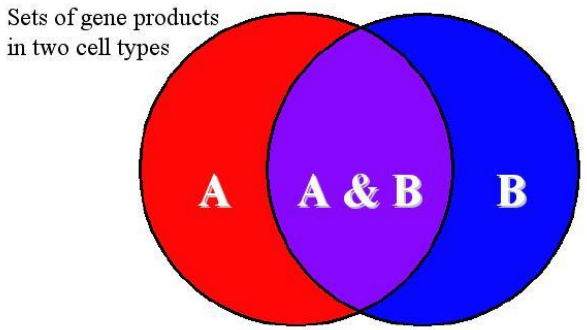


Un nucleo puo' potenzialmente rigenerare un intero organismo



Il processo di differenziazione avviene tramite l'espressione di geni diversi nei vari tipi cellulari

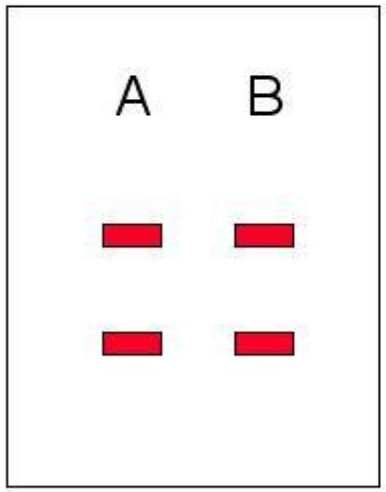




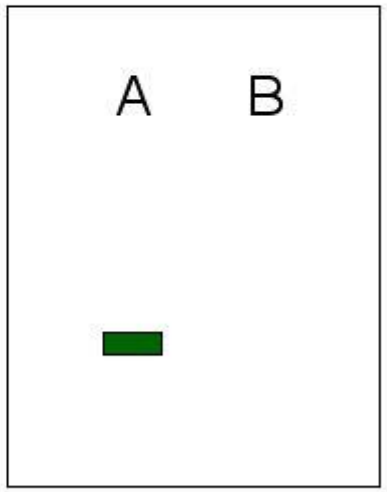
Cell types A & B share a common set of "housekeeping" gene products and a set of unique "luxury" gene products that represent the A or B developmental program

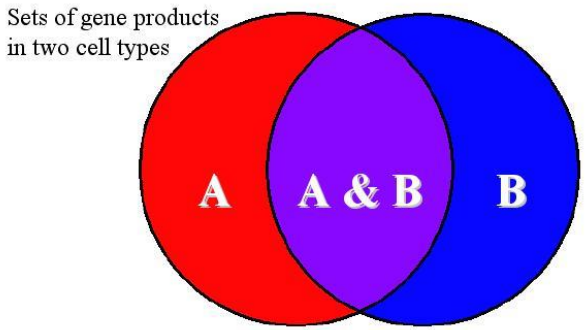
Il processo di differenziazione avviene tramite l'espressione di geni diversi nei vari tipi cellulari

DNA from two tissues (A & B) probed with a particular gene fragment on a Southern blot



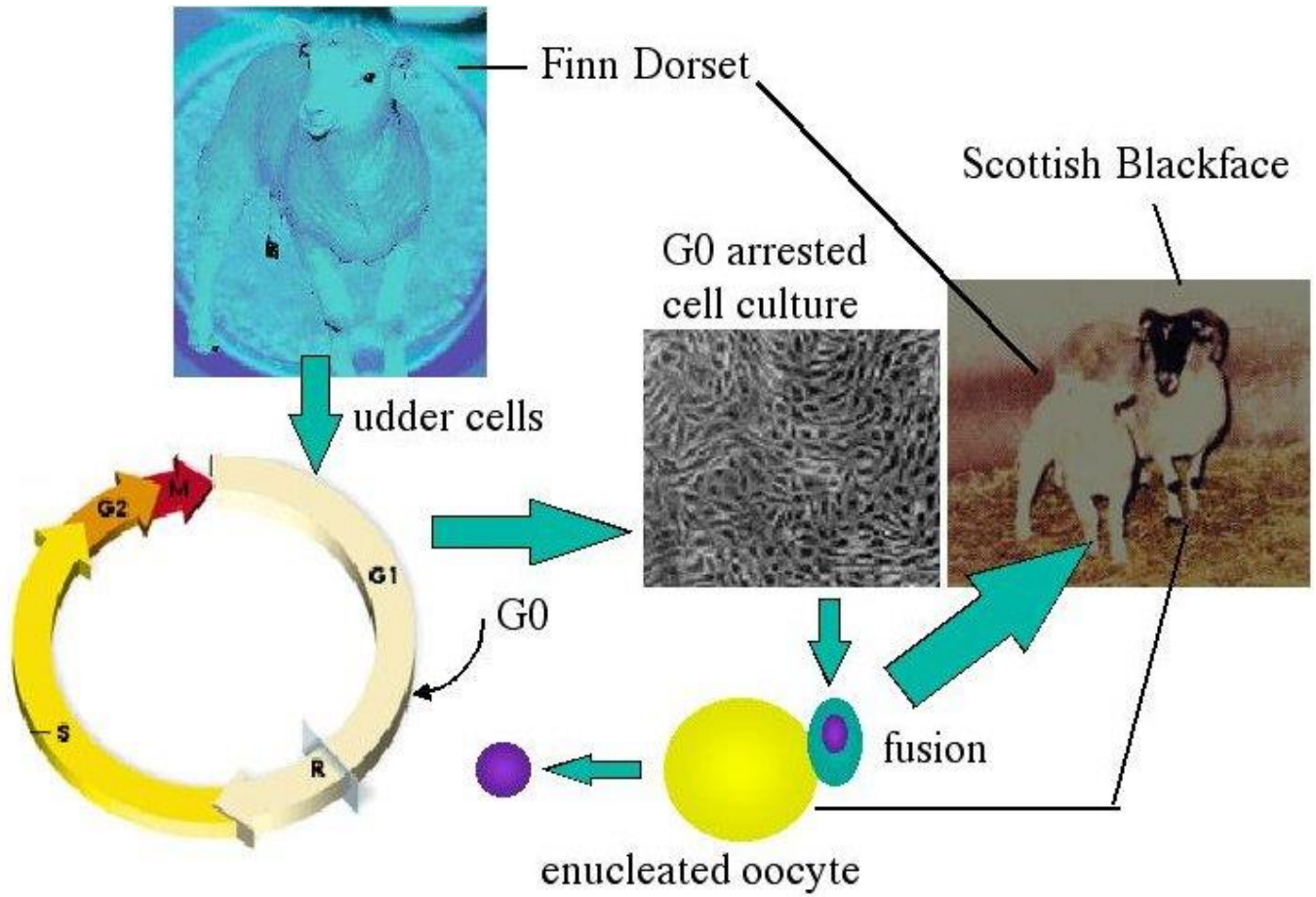
RNA from two tissues (A & B) probed with a particular gene fragment on a Northern blot

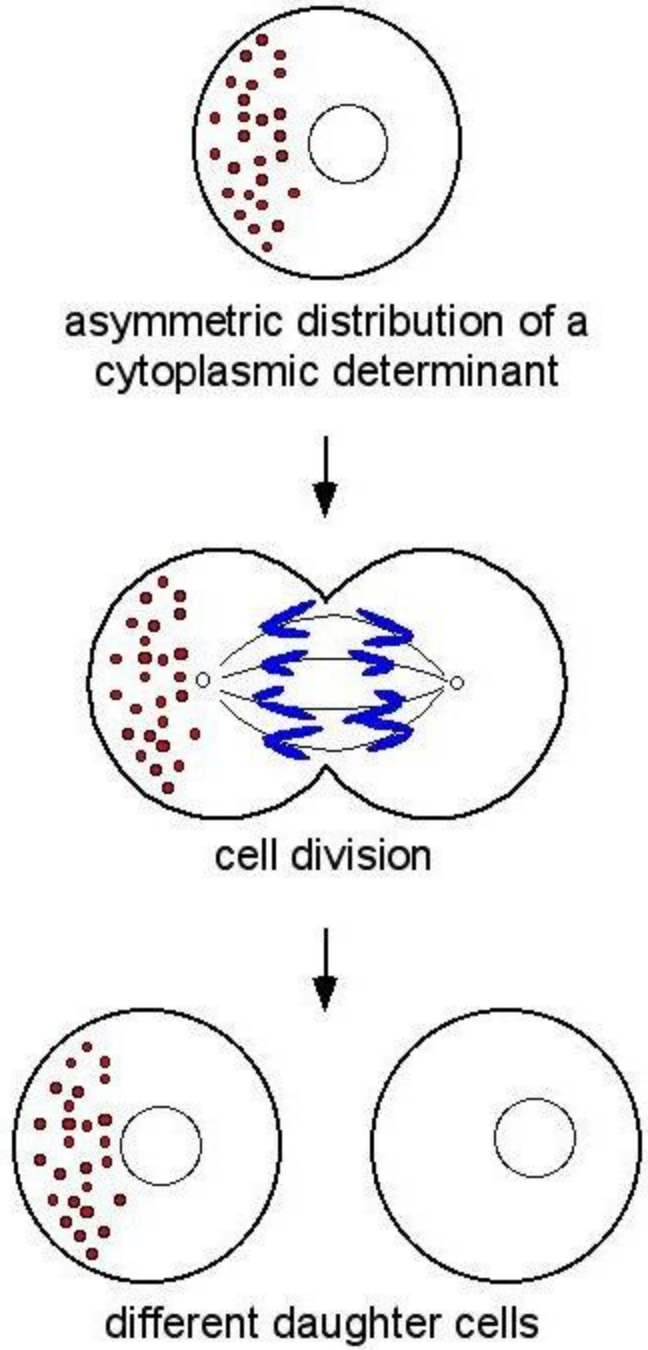




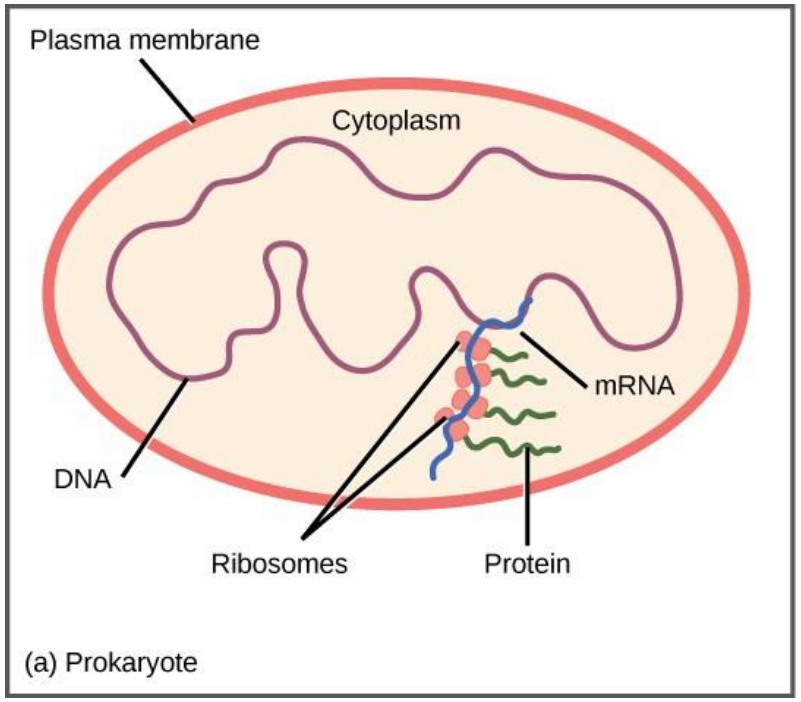
Cell types A & B share a common set of "housekeeping" gene products and a set of unique "luxury" gene products that represent the A or B developmental program

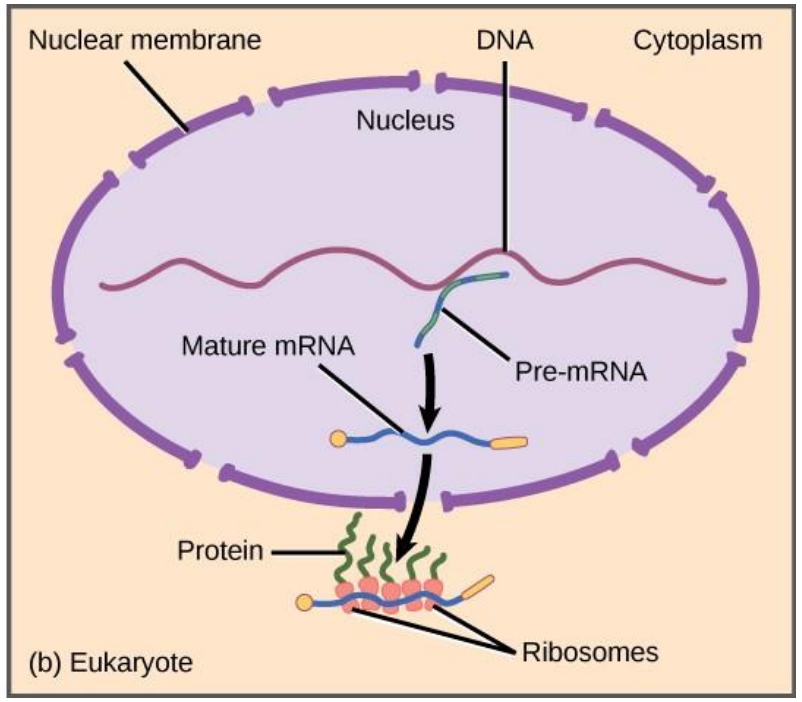
Stesso genoma, fenotipi diversi




asymmetric distribution of a
cytoplasmic determinant
cell division
different daughter cells

Differenze tra regolazione eucariotica e procariotica





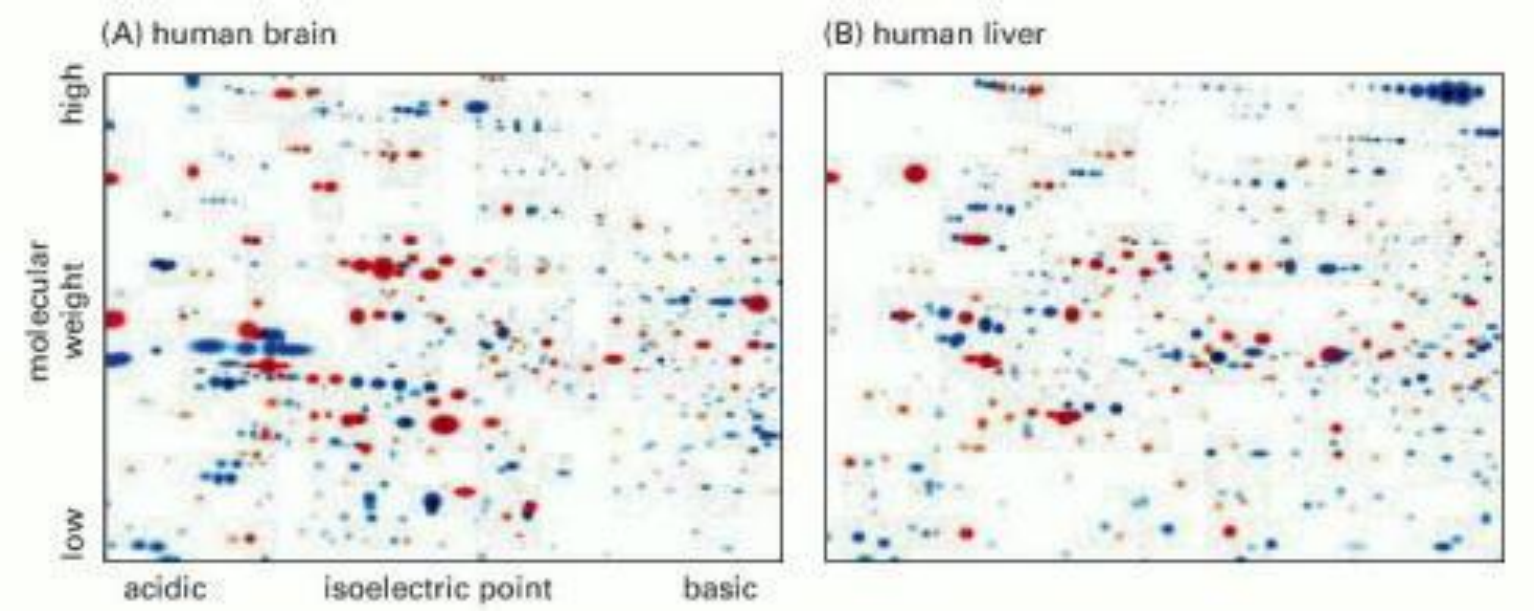
Procarioti:

- In mancanza di nucleo, trascrizione traduzione avvengono quasi simultaneamente
- L'espressione genica e' regolata primariamente trascrizionalmente

Eucarioti:

- La trascrizione avviene nel nucleo precedentemente alla traduzione, che avviene nel citoplasma.
- L'espressione genica e' regolata a molti livelli: epigenetico, trascrizionale, post-trascrizionale, traduzionale, post-traduzionale, e a livello di trasporto nucleare.

Differenze tra regolazione eucariotica e procariotica



Procarioti:

- In mancanza di nucleo, trascrizione traduzione avvengono quasi simultaneamente
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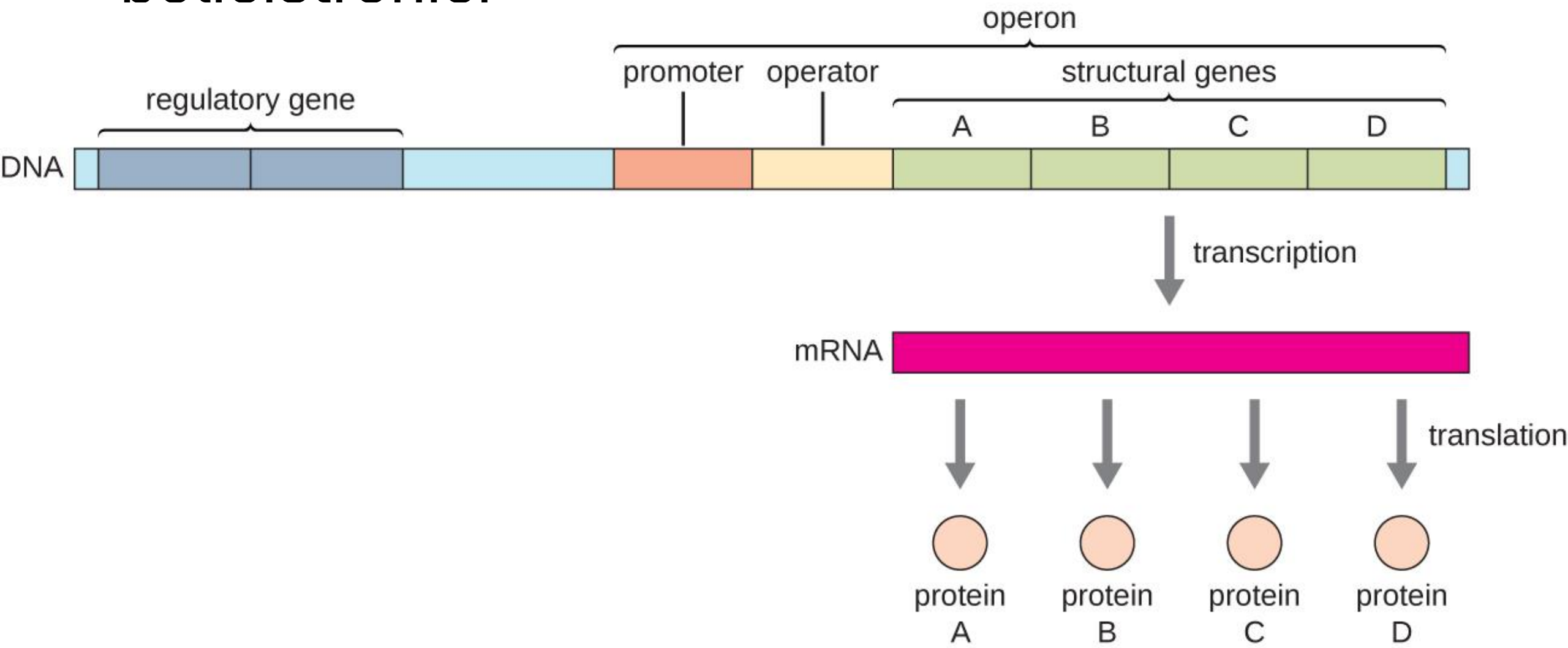
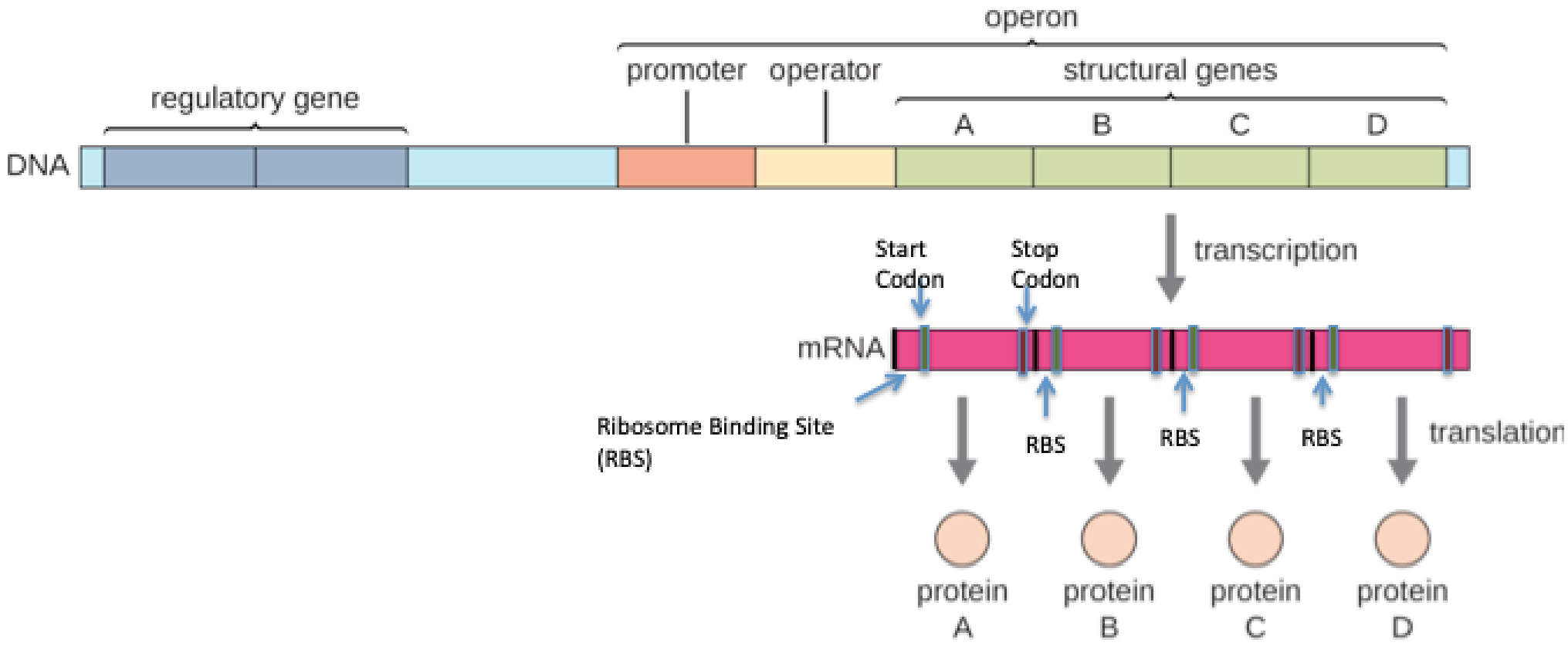
Gli operoni batterici sono spesso policistronici

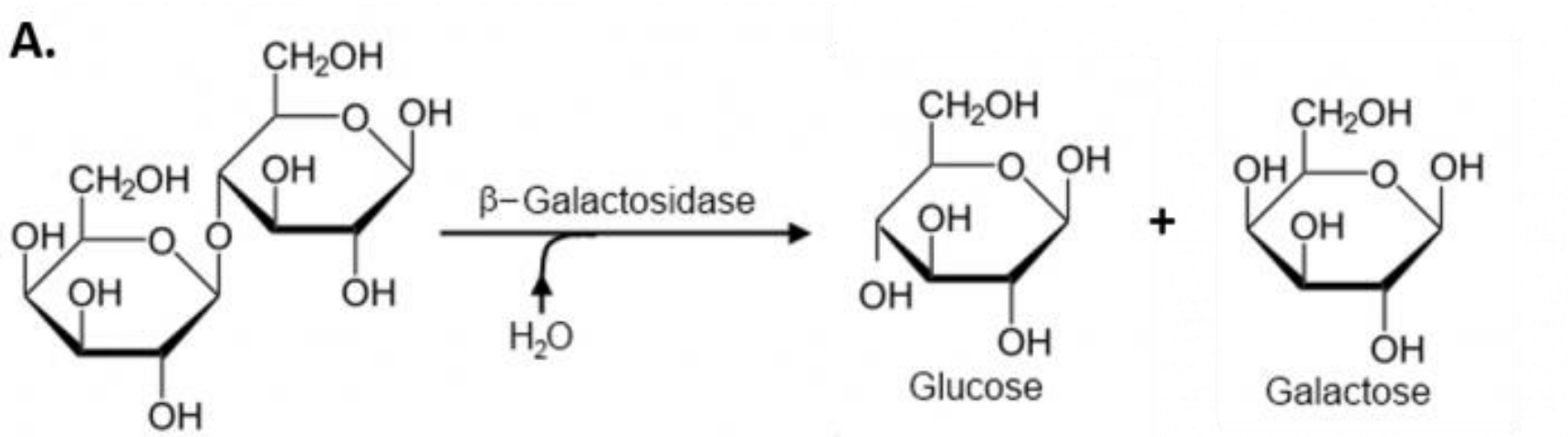


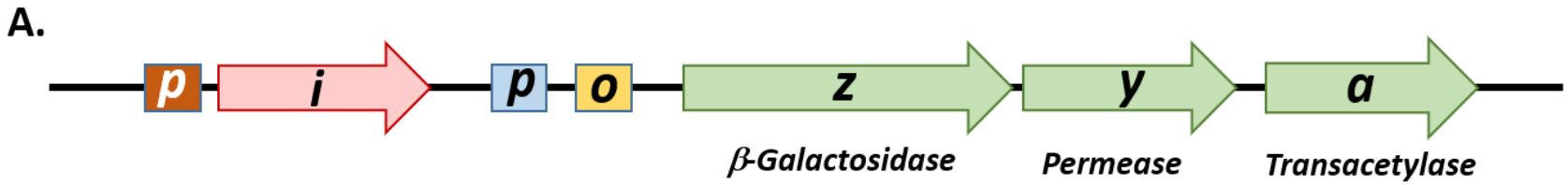
Gli operoni policistronici vengono tradotti da piu ribosomi



L’operone *lac* di *E.coli*

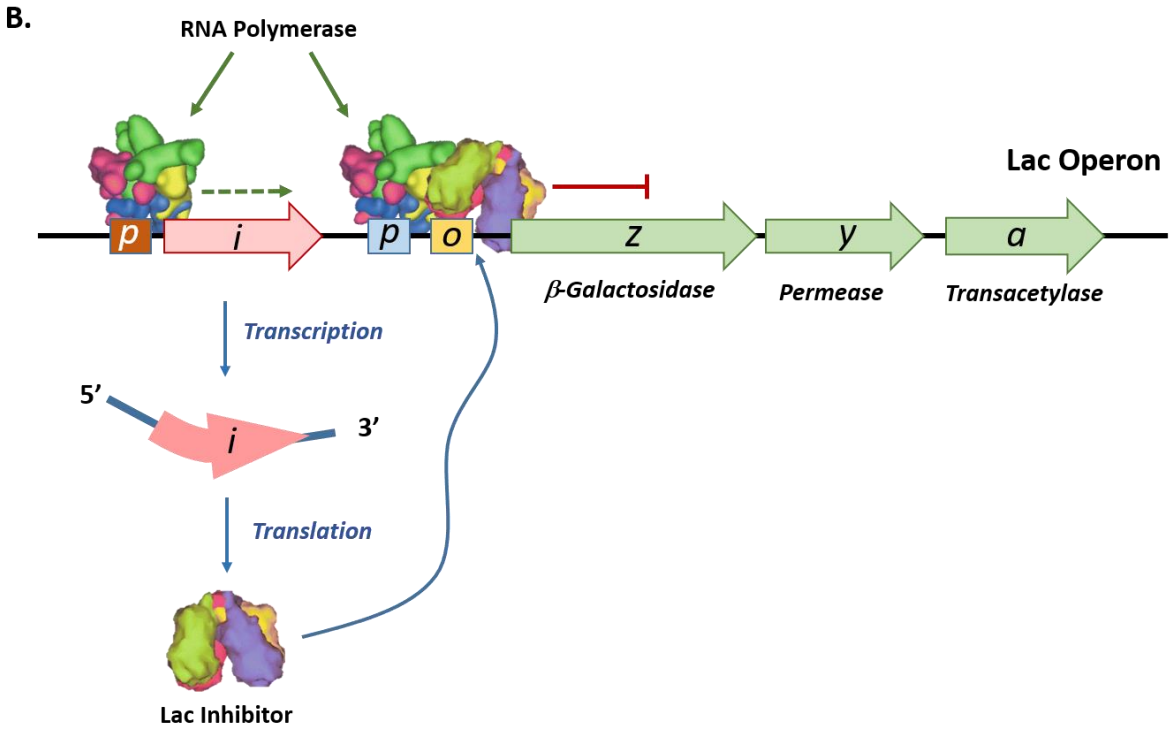
L’operone *lac* di *E.coli*



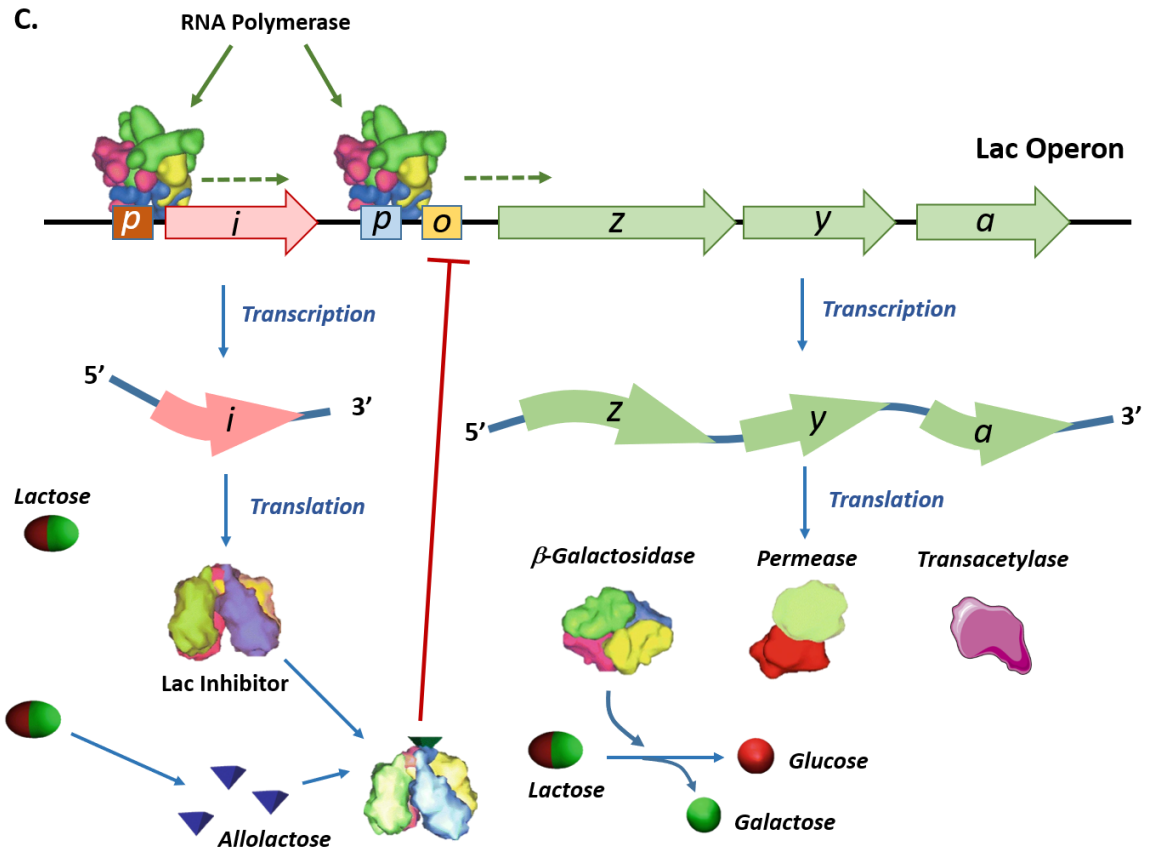


Funzionamento dell'operone *lac*

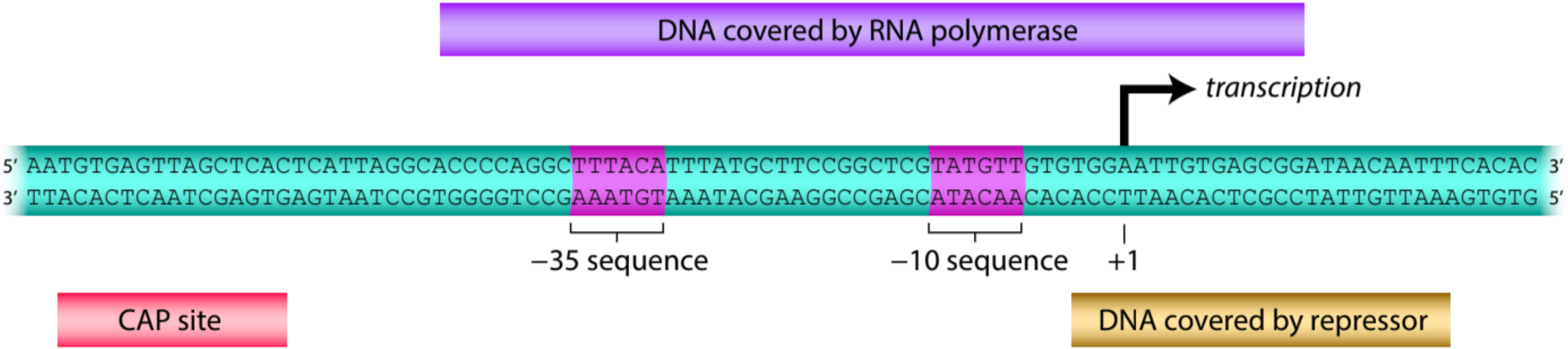
Inibizione dell'operone lac in assenza di lattosio



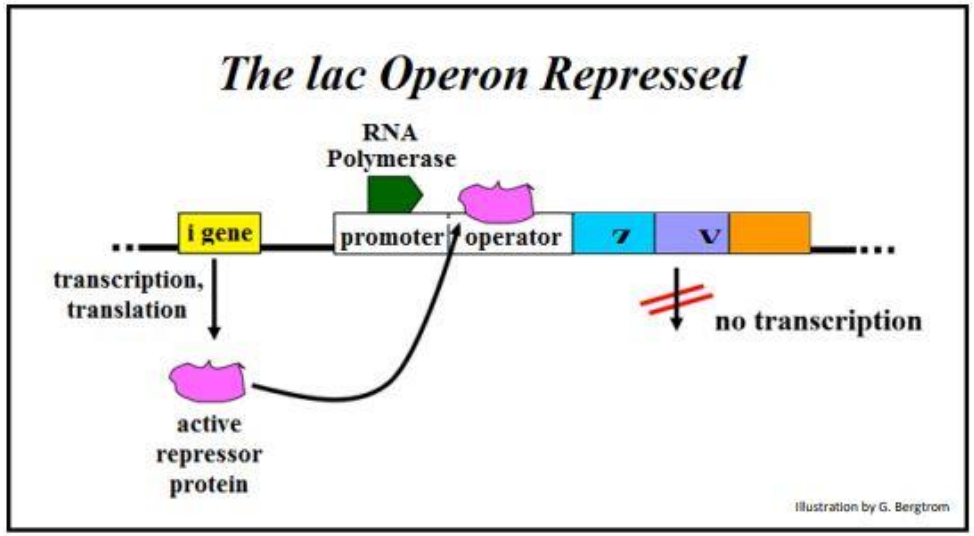
Espressione dell'operone di *lac*

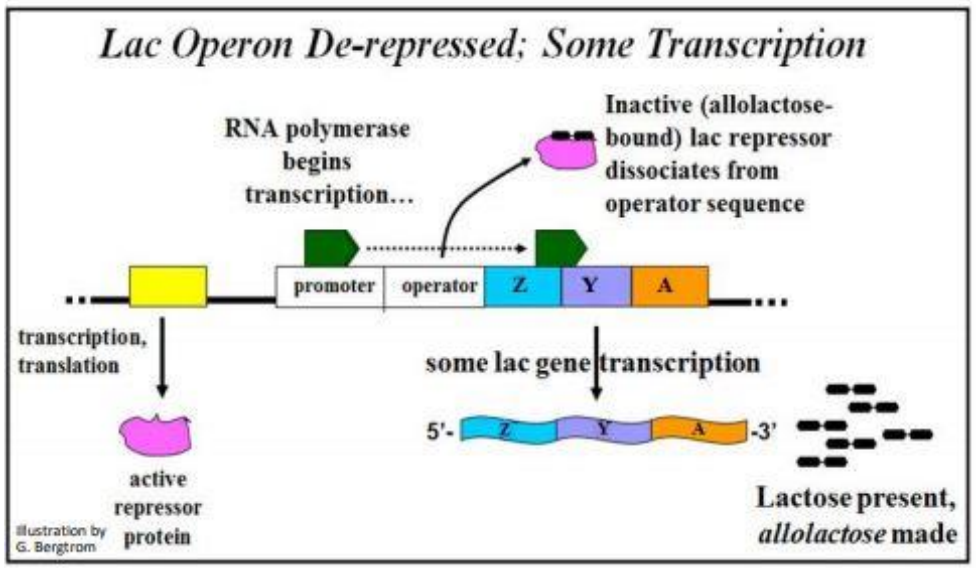


Legame del repressore nell'operone *lac*



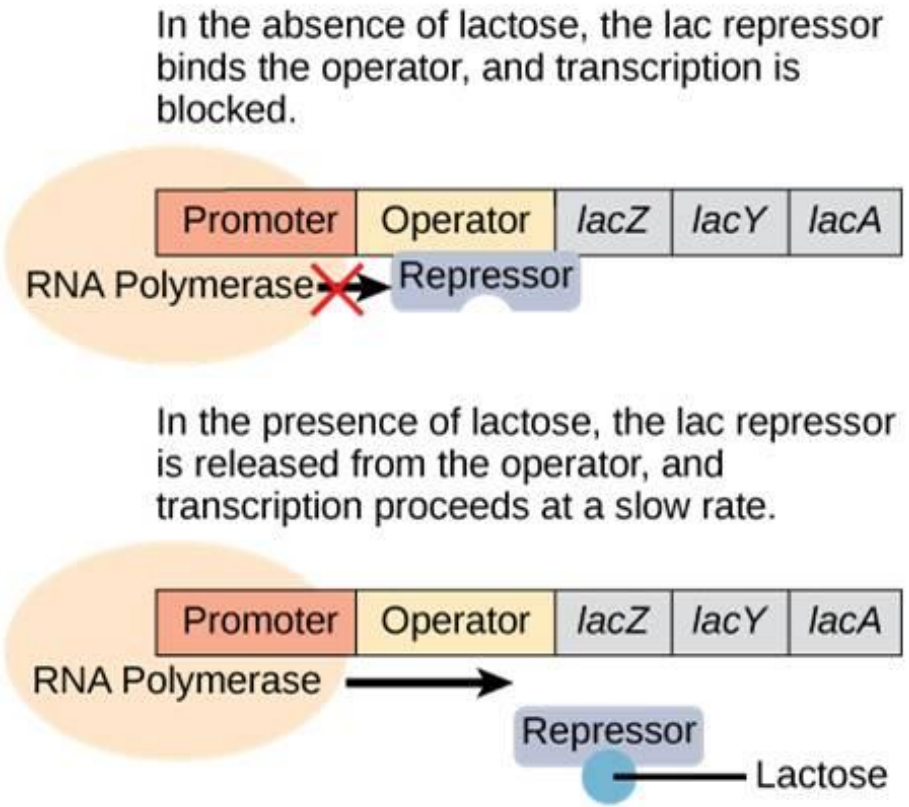
La regolazione positiva dell'operone *lac* permette vari livelli di espressione



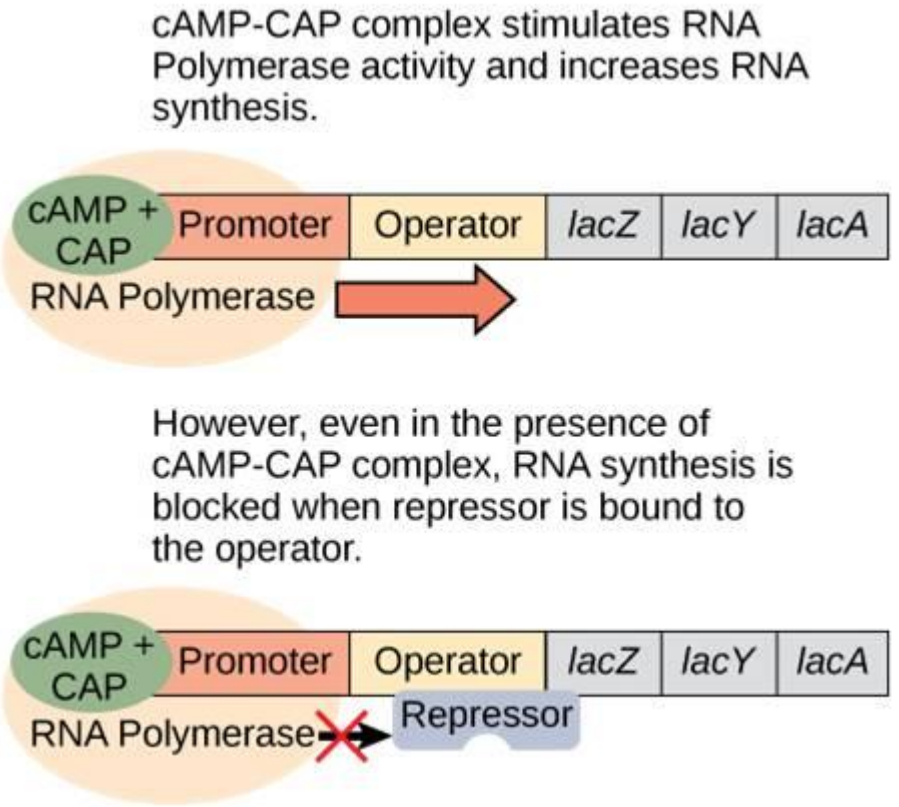


Piu meccanismi regolano l'espressione dell'operone *lac*..

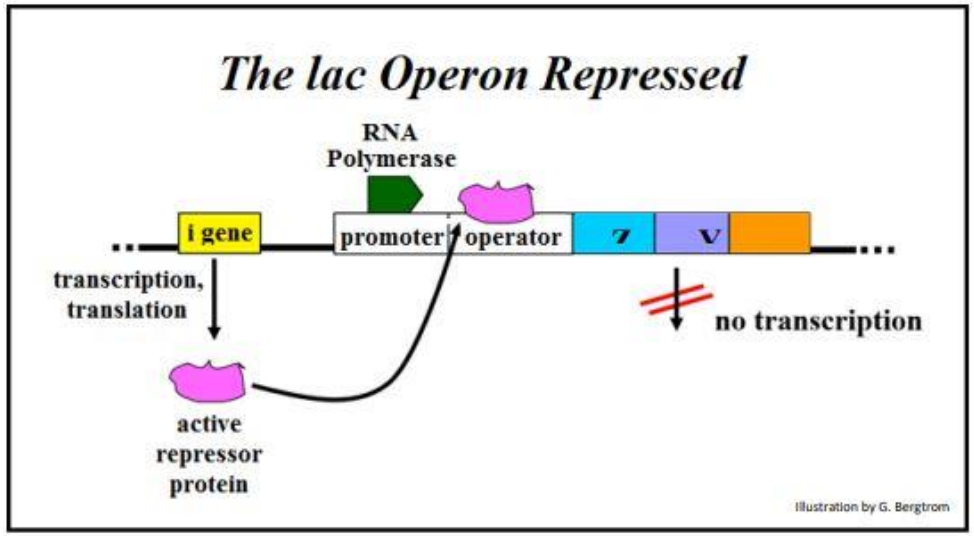
Regolazione negative/inibizione

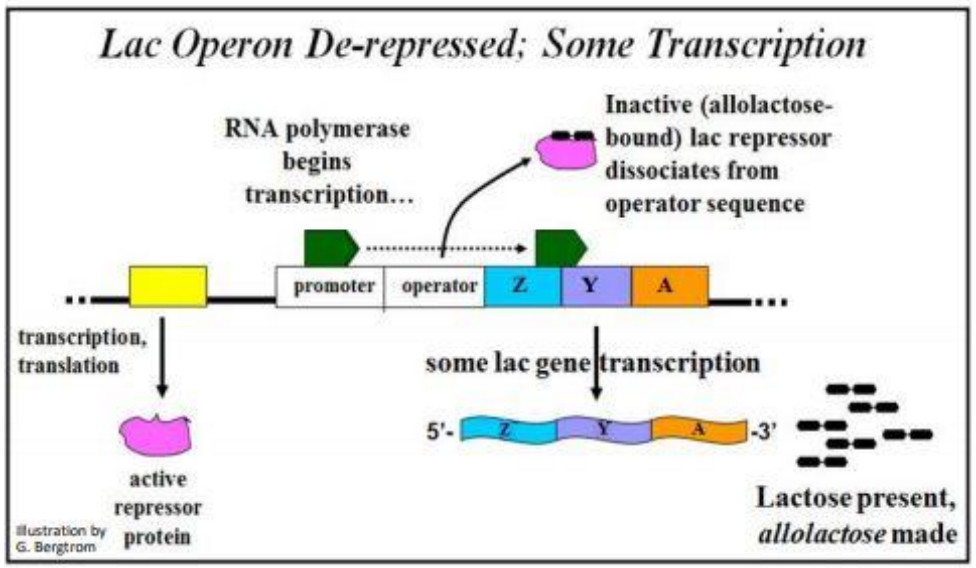


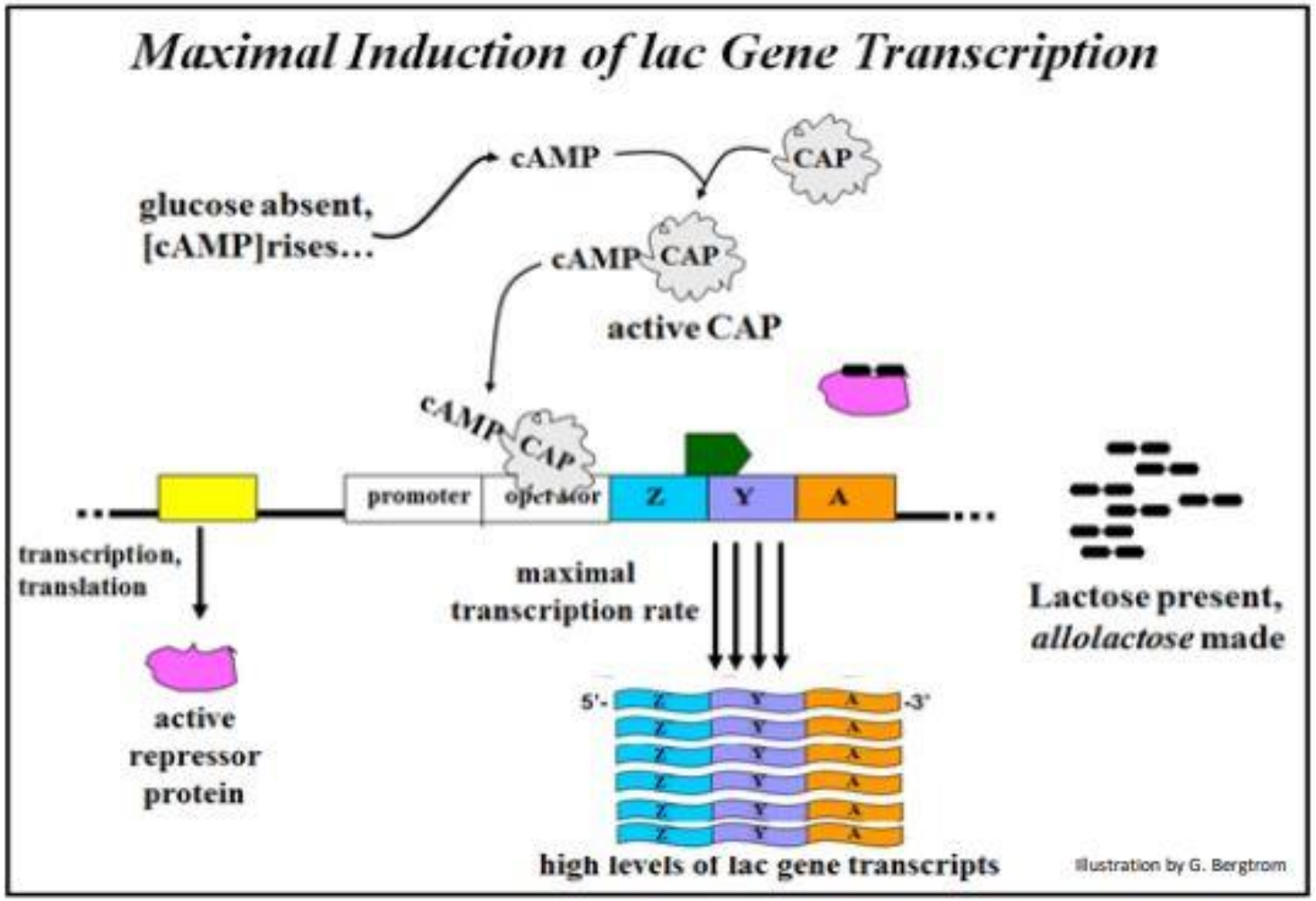
Regolazione positiva



..permettendo vari livelli di espressione dell'operone *lac*, tramite







Qualche domanda

Lactose present?	Repressor bound?	Glucose present?	CAP bound?	Transcription of *lac* operon?
-	Yes	+	No	No
-	Yes	-	Yes	?
+	No	+	No	
+	No	-	Yes	

Qualche domanda

Lactose present?	Repressor bound?	Glucose present?	CAP bound?	Transcription of *lac* operon?
-	Yes	+	No	No
-	Yes	-	Yes	No
+	No	+	No	?
+	No	-	Yes	

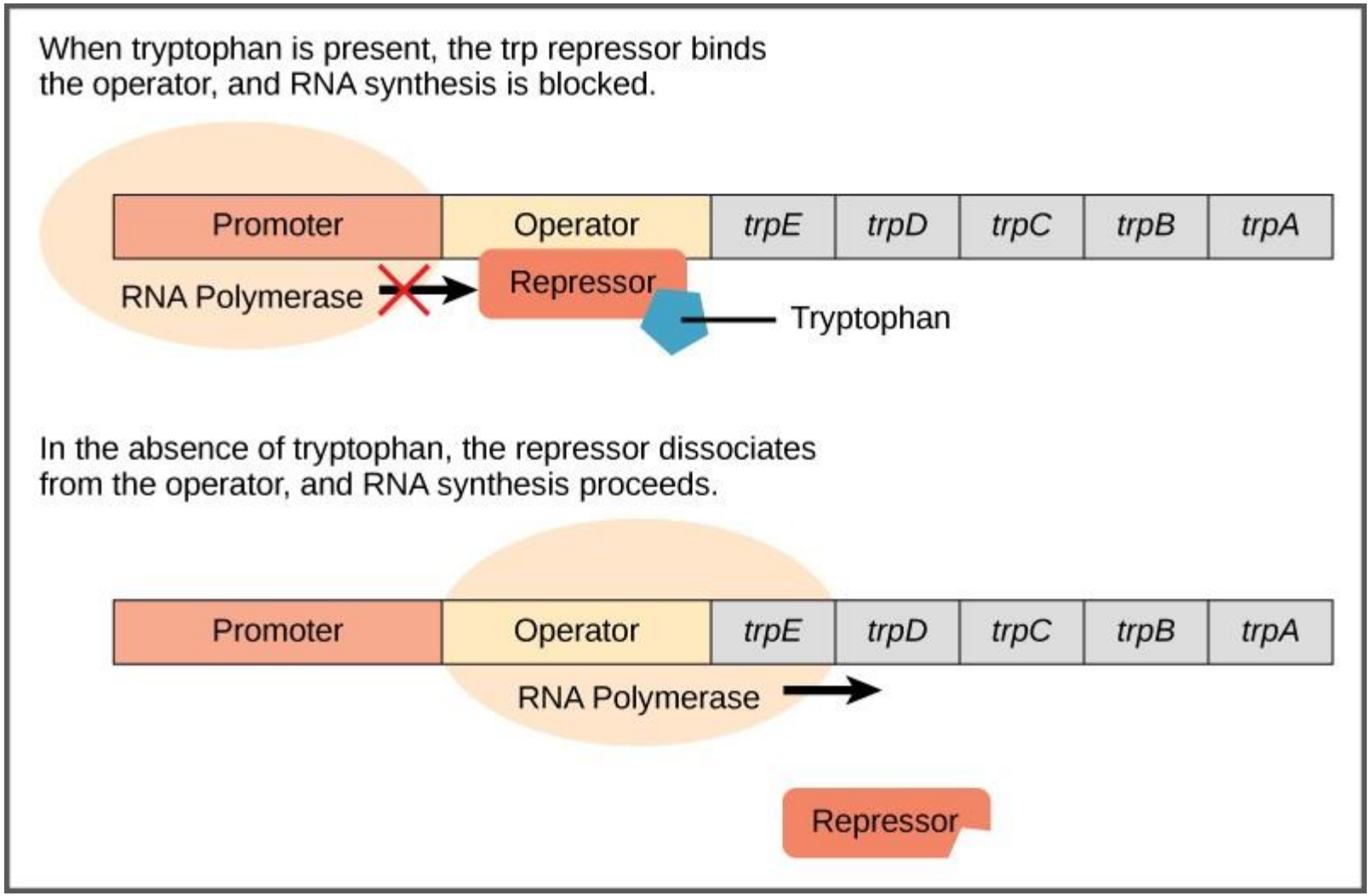
Qualche domanda

Lactose present?	Repressor bound?	Glucose present?	CAP bound?	Transcription of *lac* operon?
-	Yes	+	No	No
-	Yes	-	Yes	No
+	No	+	No	Some
+	No	-	Yes	

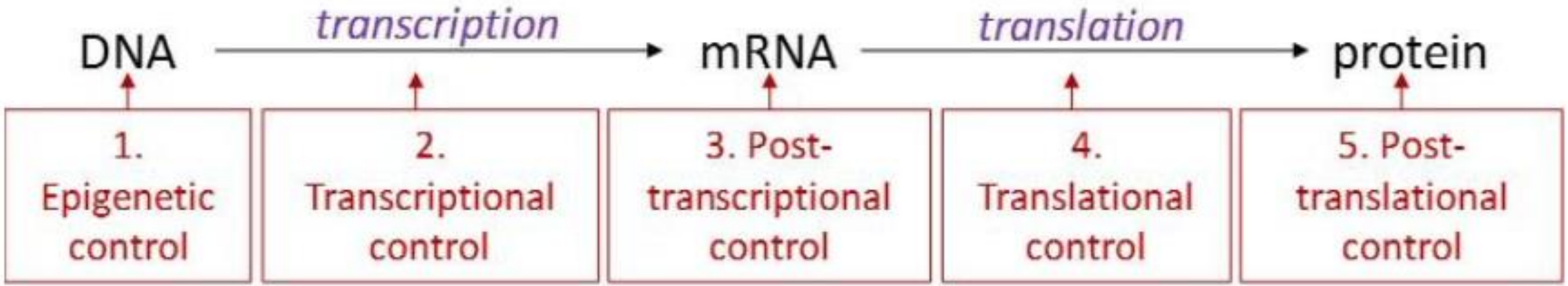
Qualche domanda

Lactose present?	Repressor bound?	Glucose present?	CAP bound?	Transcription of *lac* operon?
-	Yes	+	No	No
-	Yes	-	Yes	No
+	No	+	No	Some
+	No	-	Yes	Lots

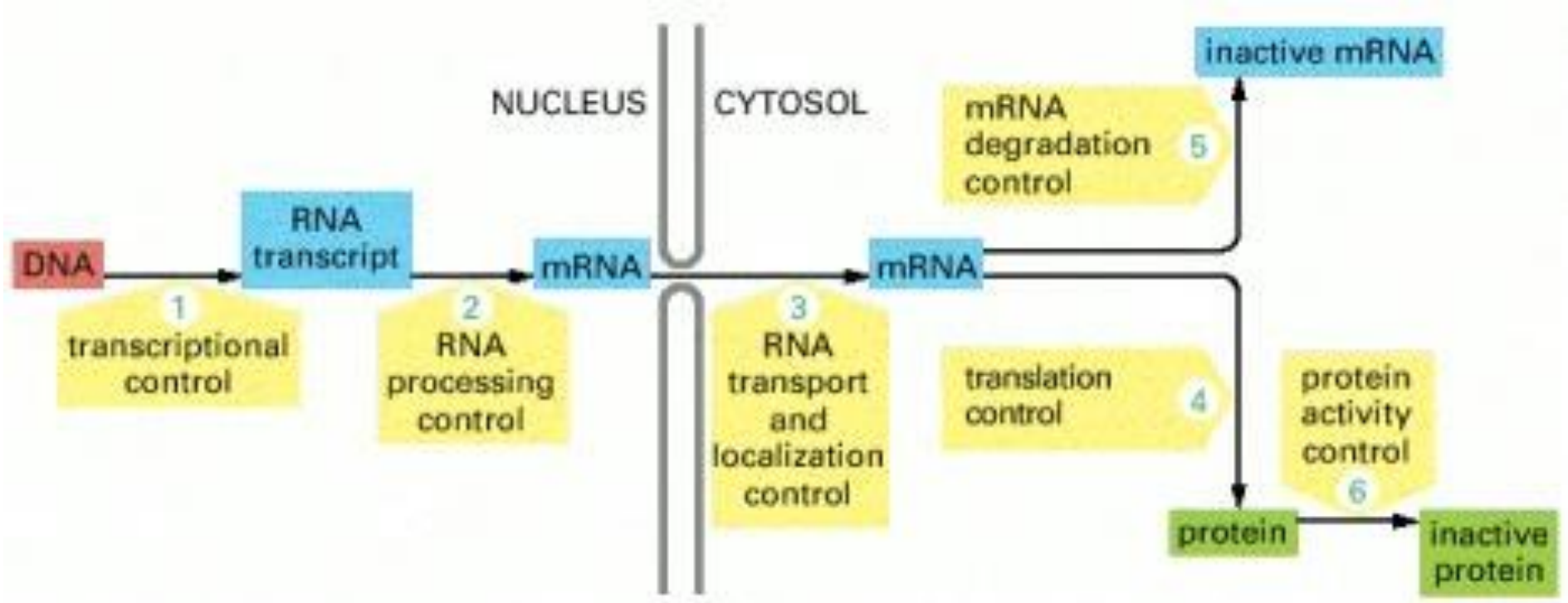
L’operone del triptofano



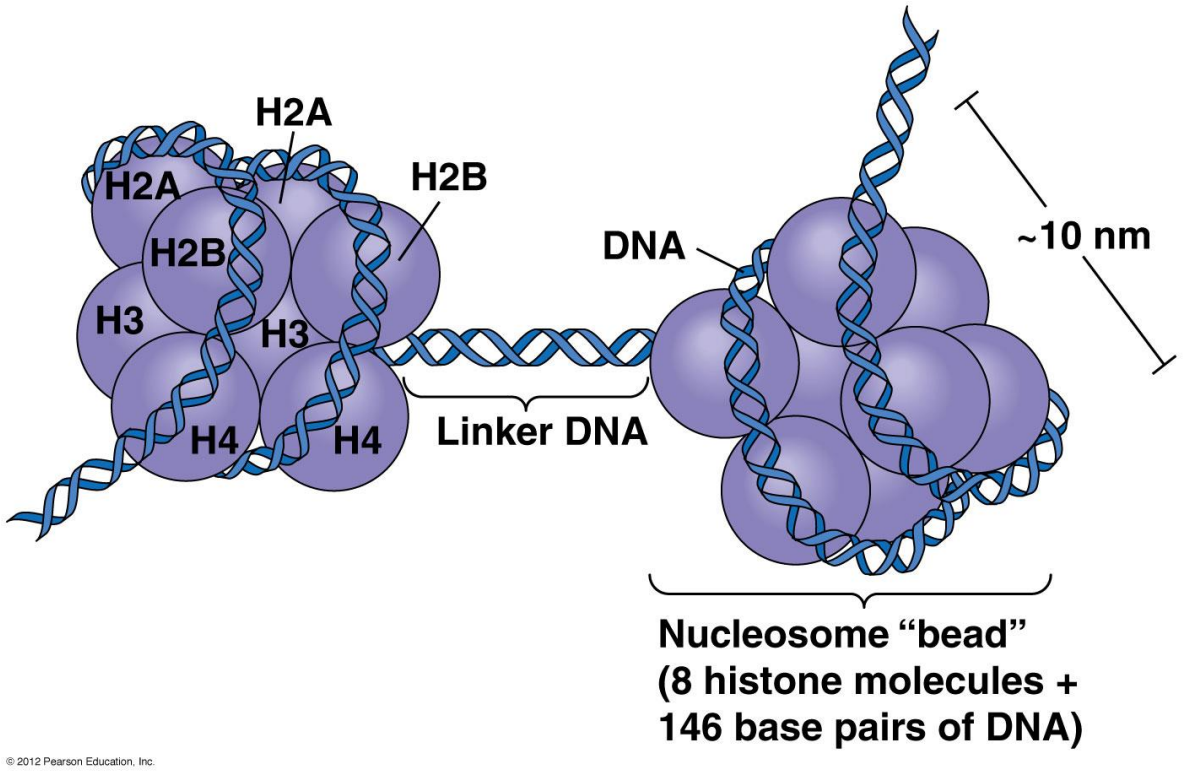
Regolazione dell’espressione genica negli eucarioti

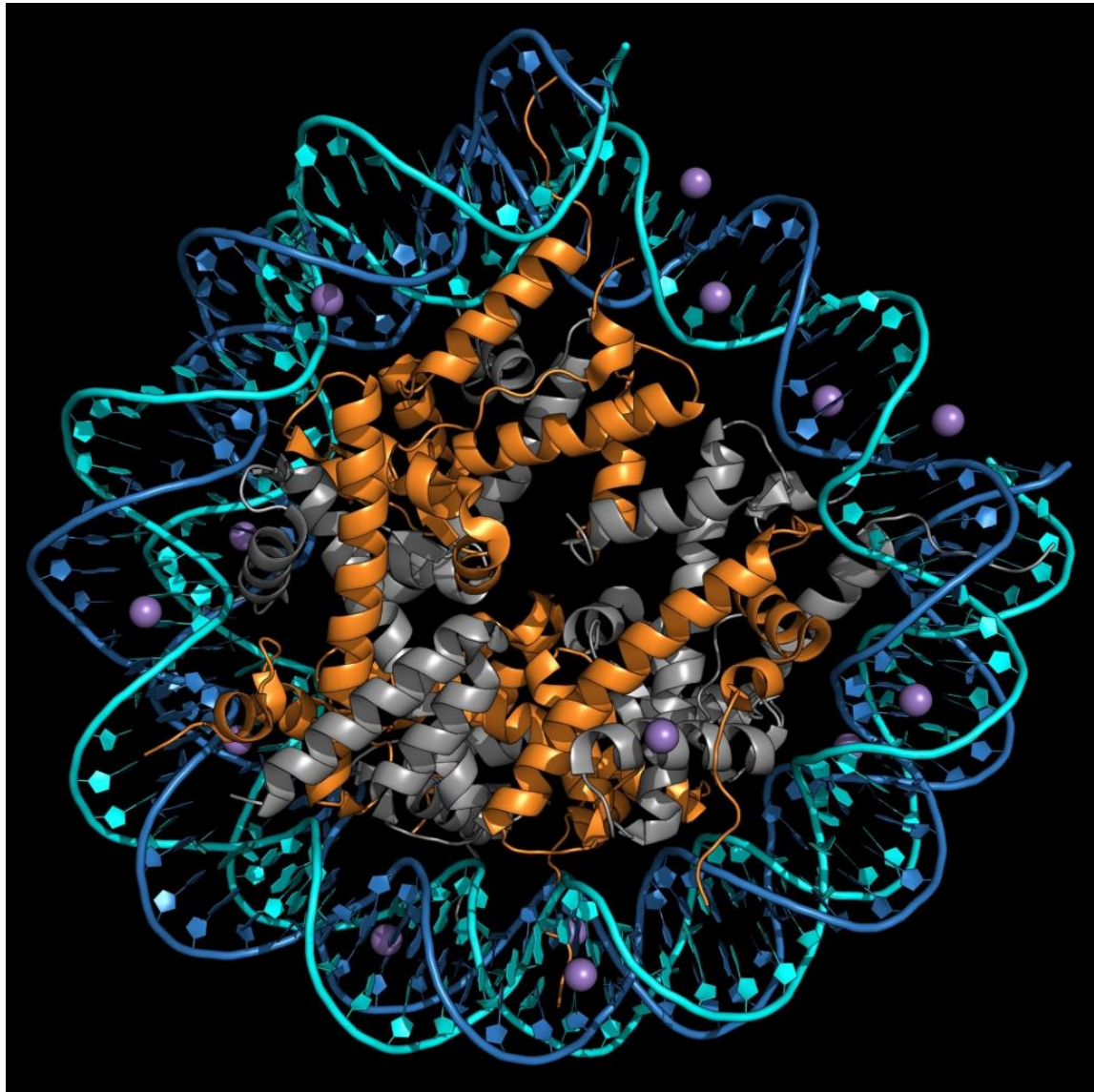


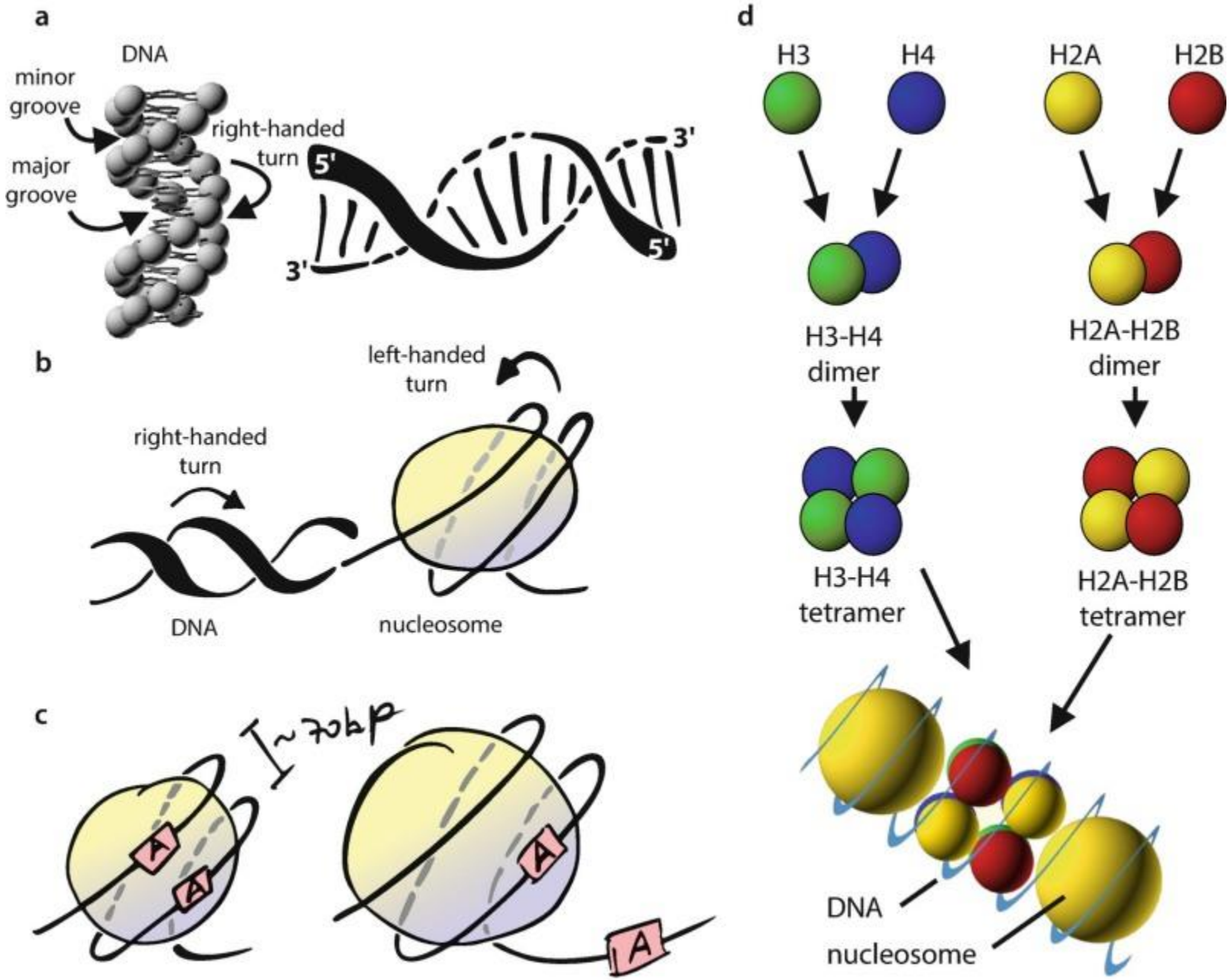
Regolazione dell’espressione genica negli eucarioti

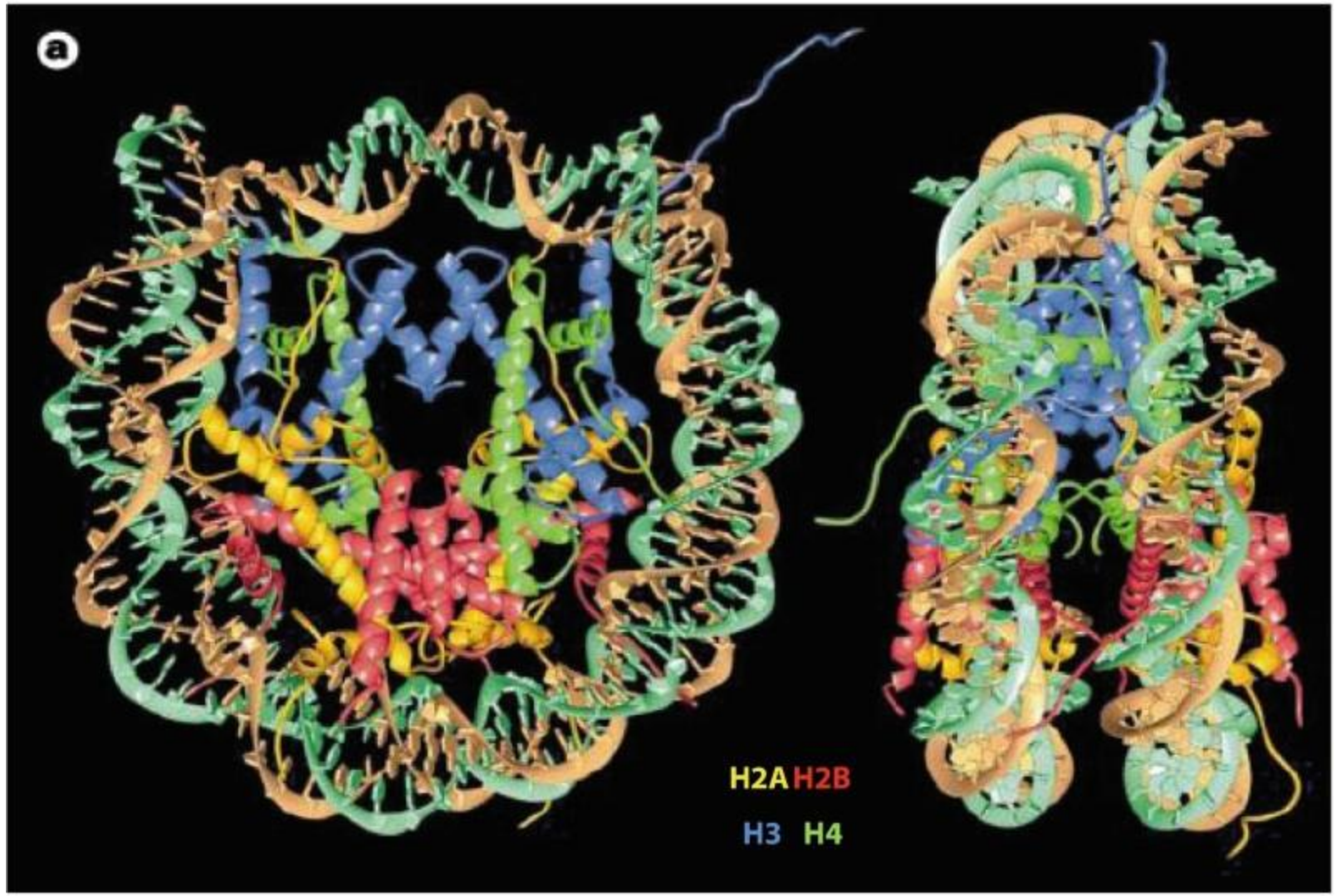


Il DNA eucariotico e' avvolto attorno agli istoni

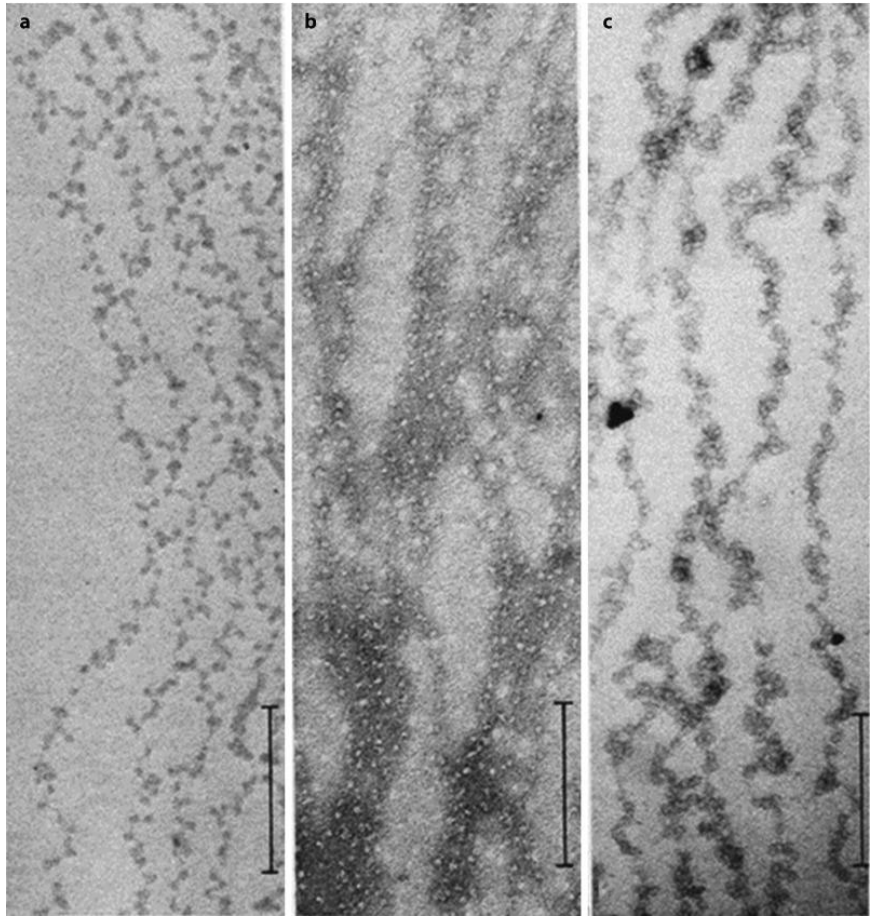




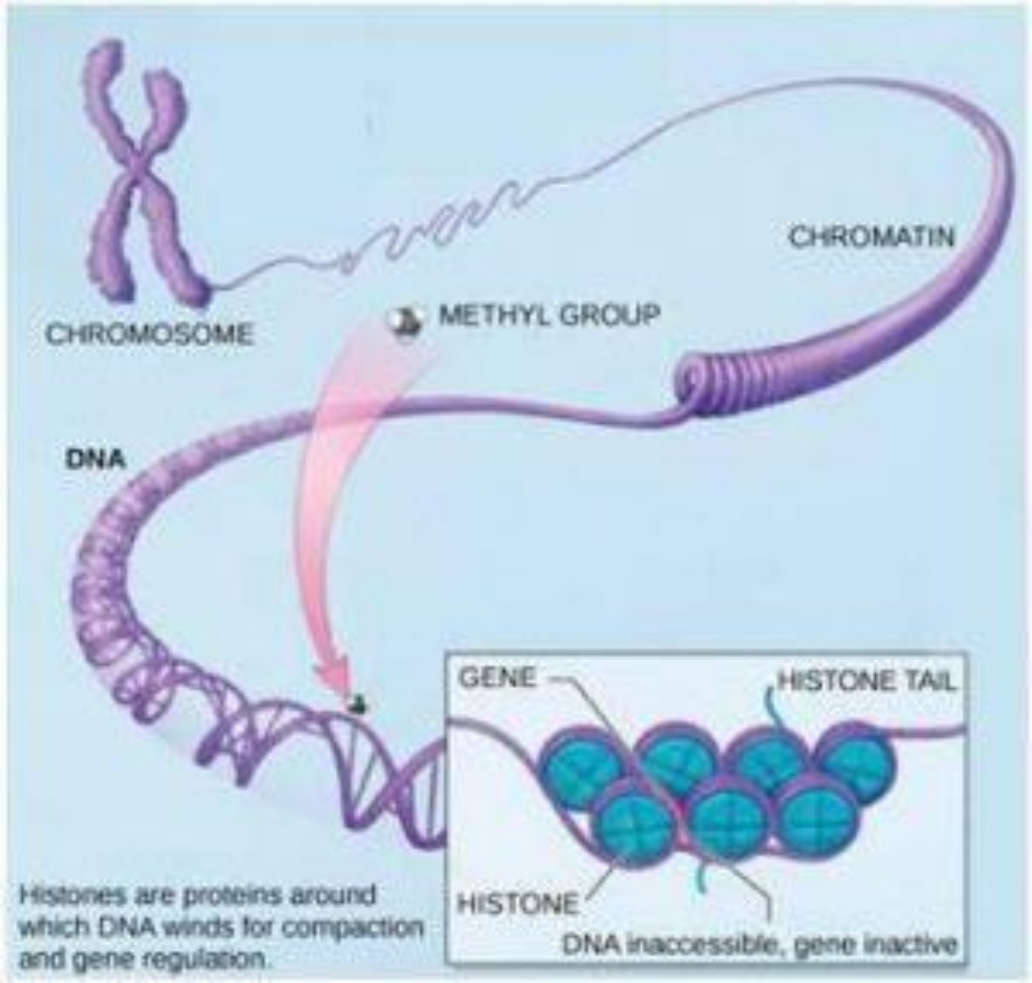

a
DNA
minor groove
major groove
right-handed turn
5'
3'
3'
5'
b
left-handed turn
right-handed turn
DNA
nucleosome
c
~70bp
A
A
A
A
d
H3
H4
H2A
H2B
H3-H4 dimer
H2A-H2B dimer
H3-H4 tetramer
H2A-H2B tetramer
DNA
nucleosome

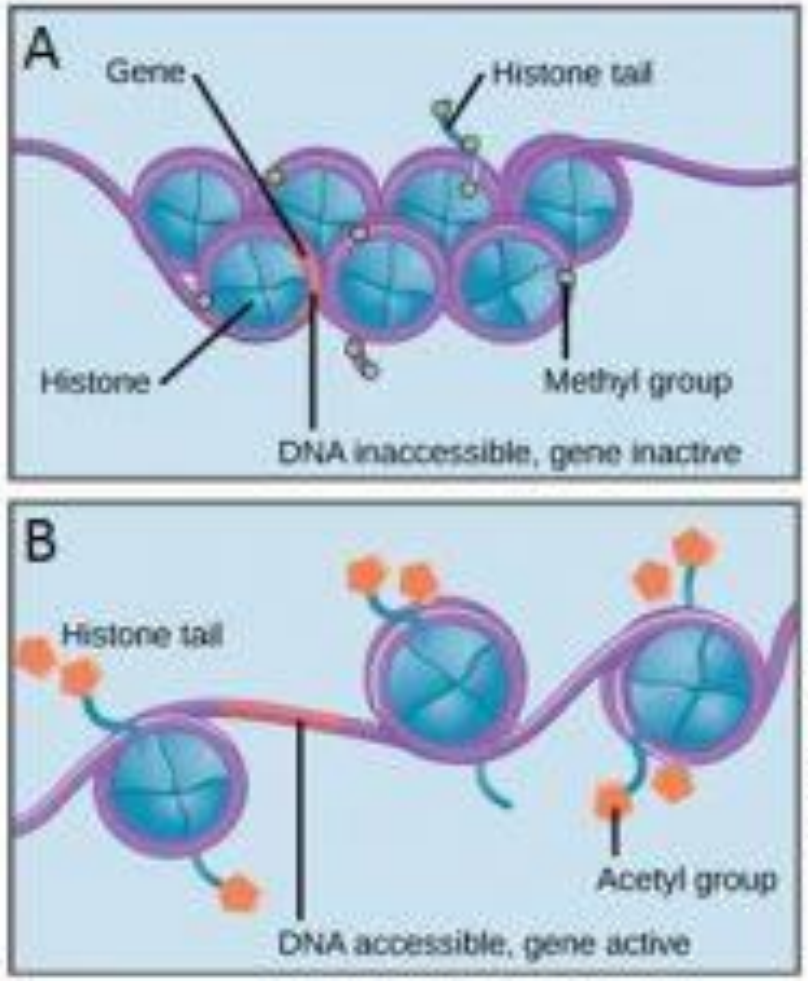

a
H2A H2B
H3 H4
11 nm
5 nm

Eucromatina nel timo di ratto (a,b) e globulo rosso di pollo



Modificazioni degli istoni regolano la compattezza della cromatina

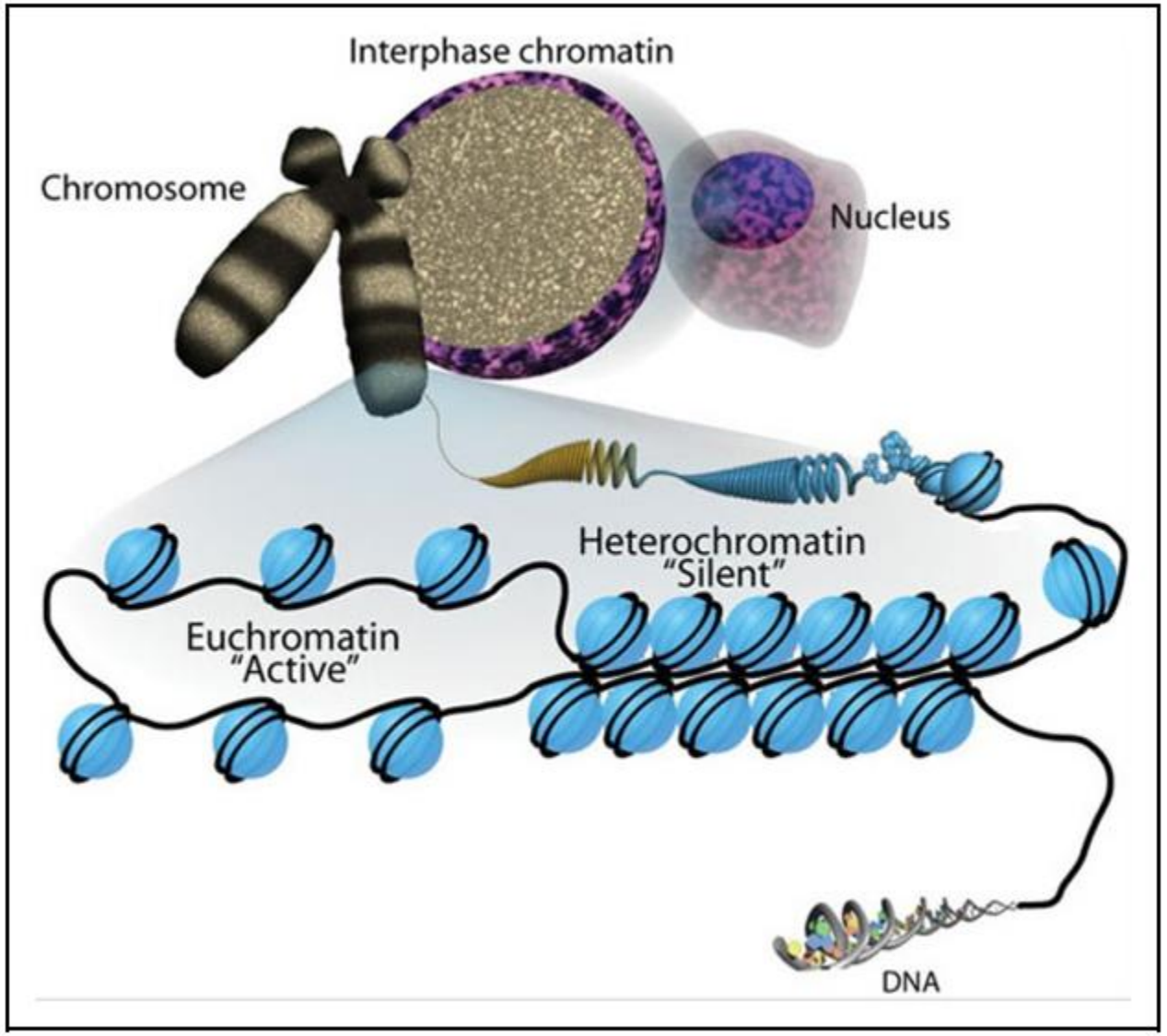




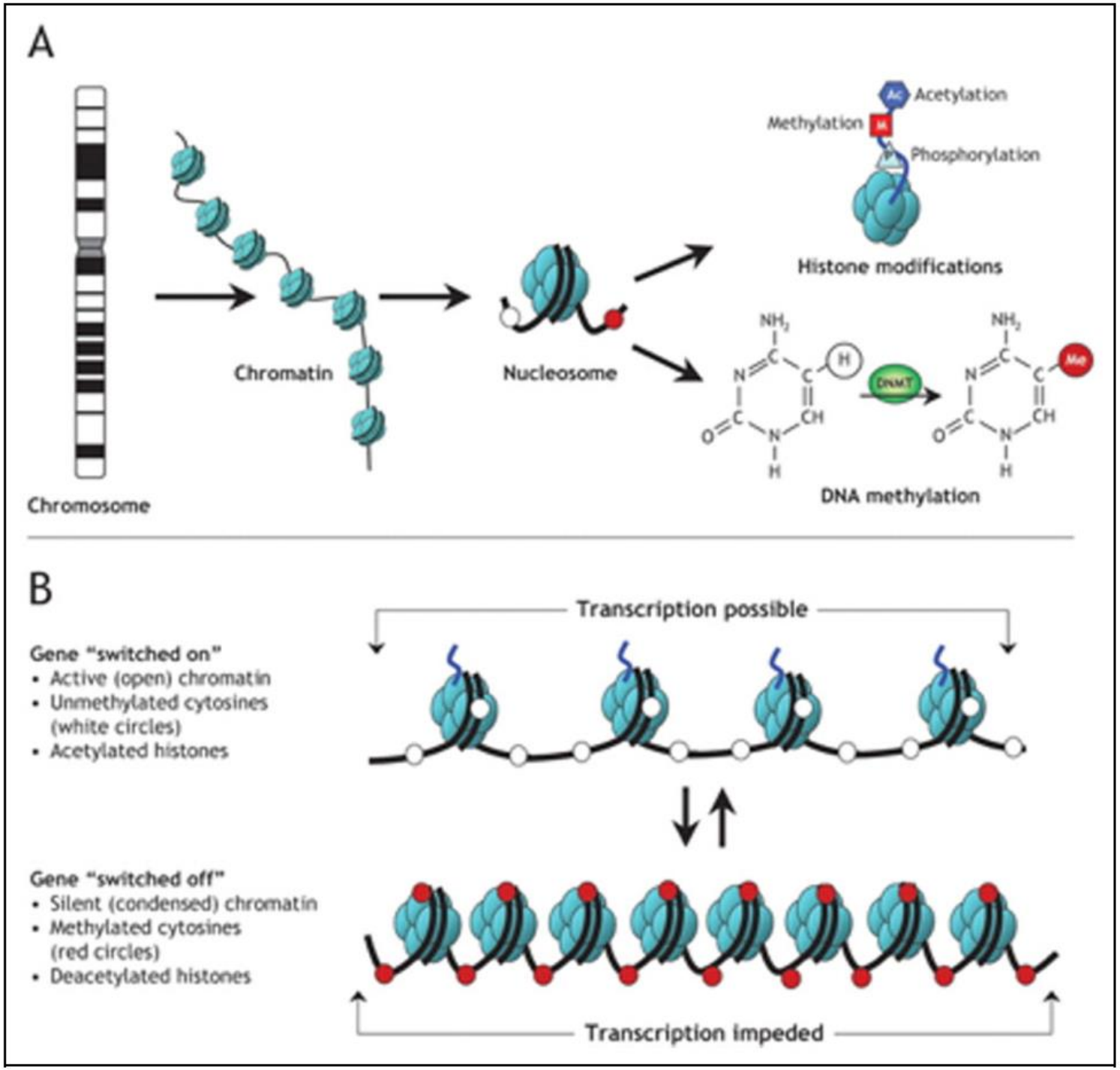
Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

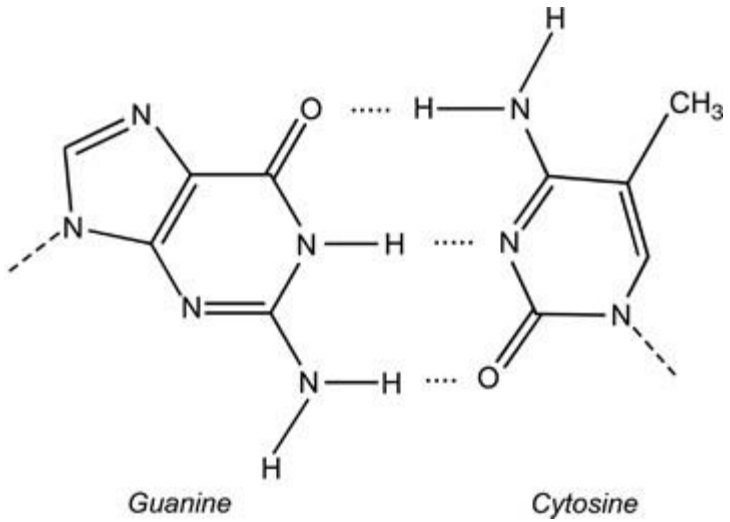
Eterocromatina ed eucromatina

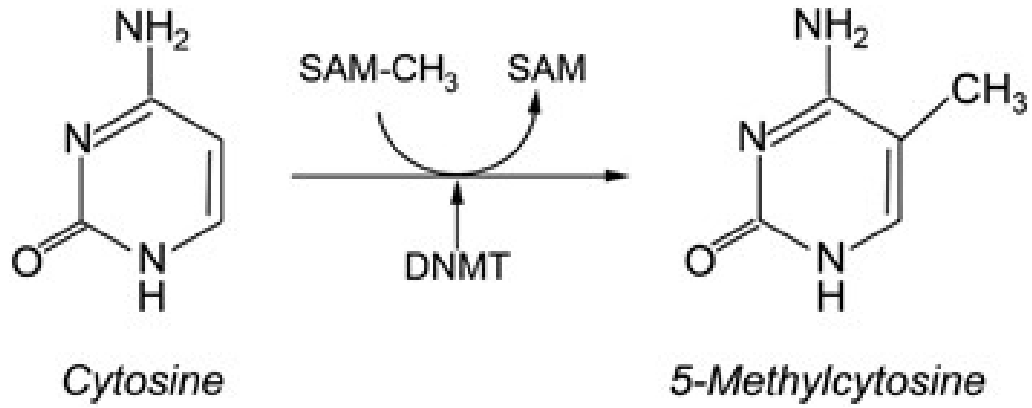


Metilazione del DNA e modificazioni degli istoni sono i due prinipali segnali di regolazione della cromatina



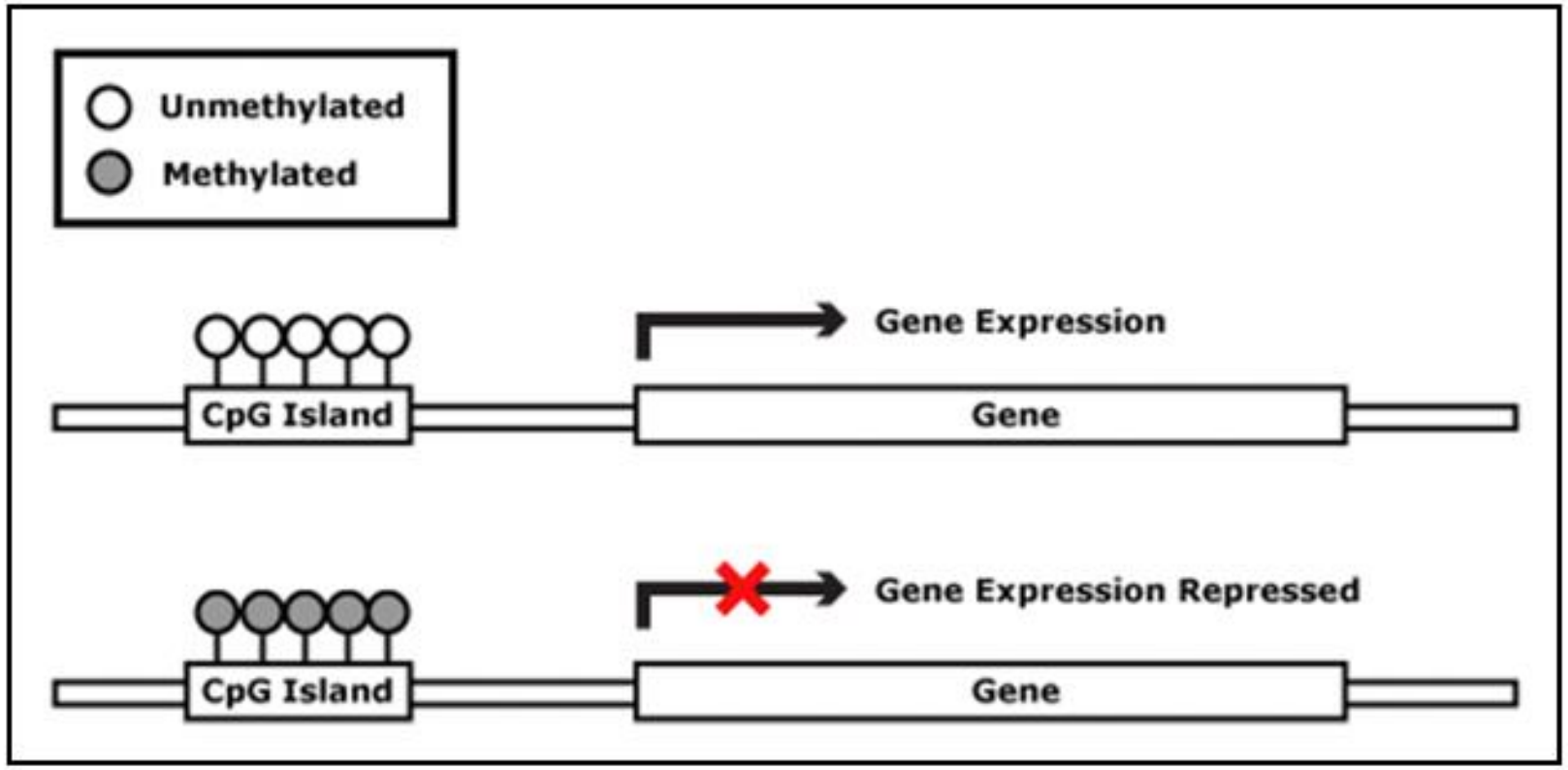
Metilazione del DNA



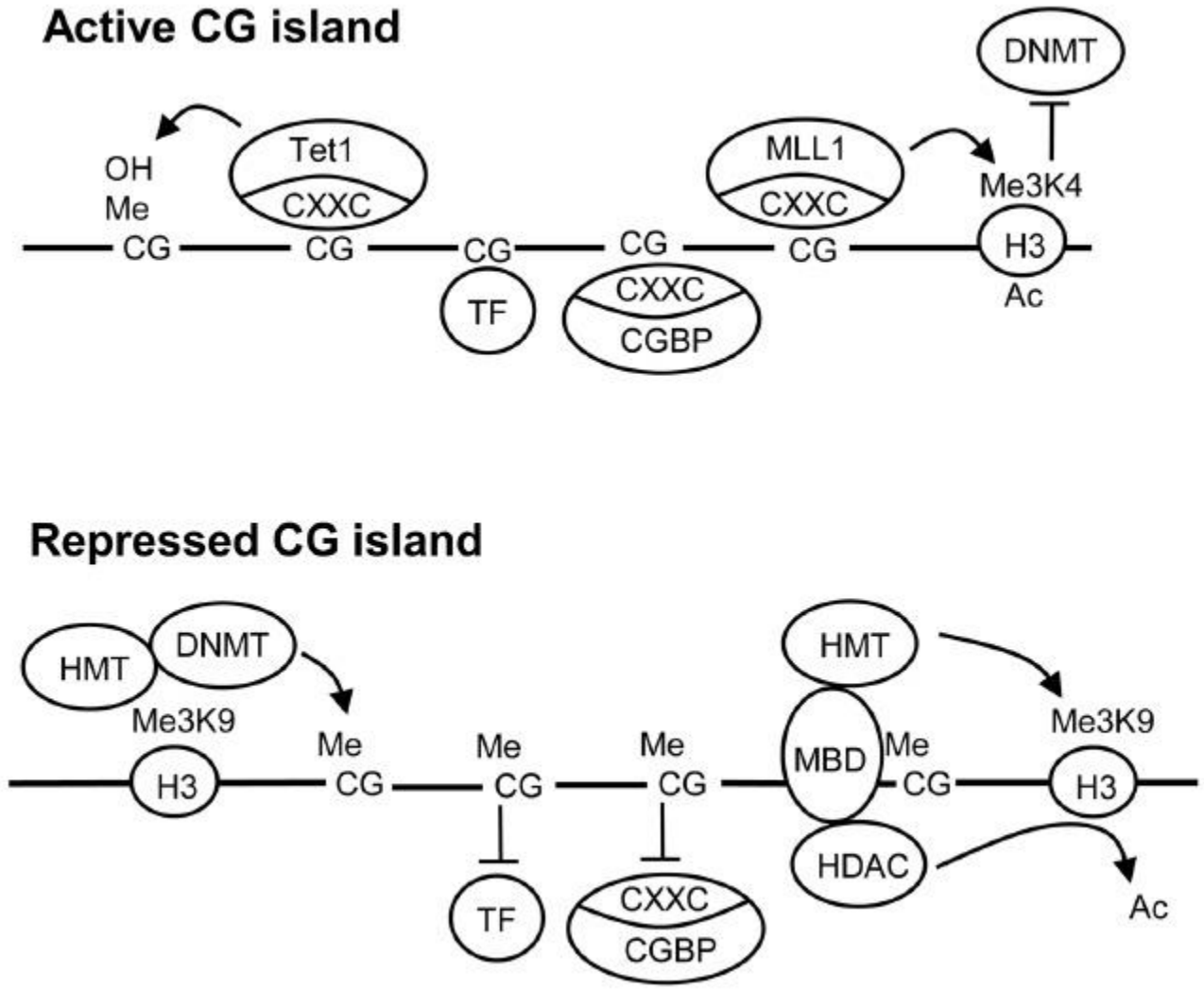


Le citosine sono metilate da enzimi DNA-metiltransferasi (DNMT)

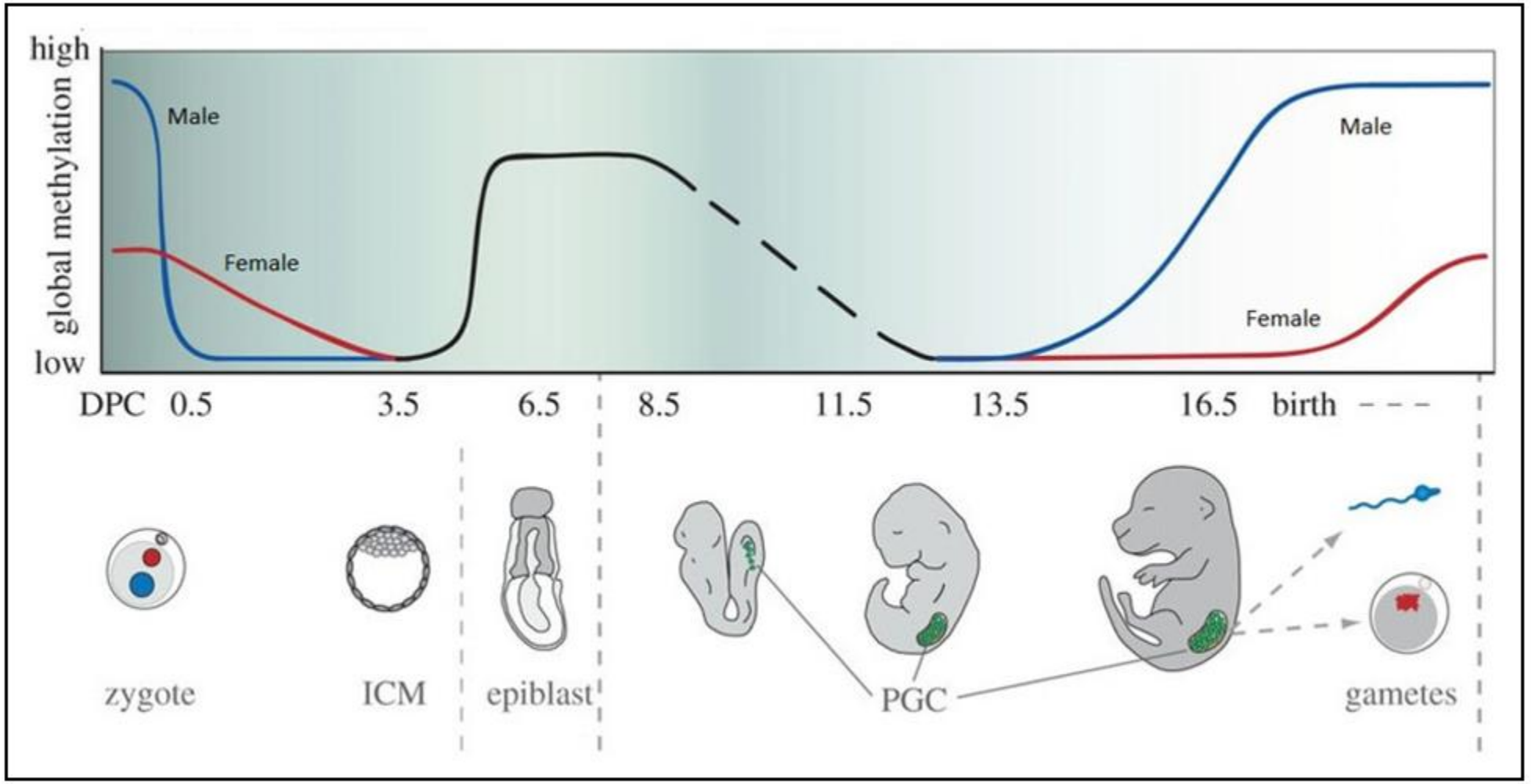
Metilazione del DNA



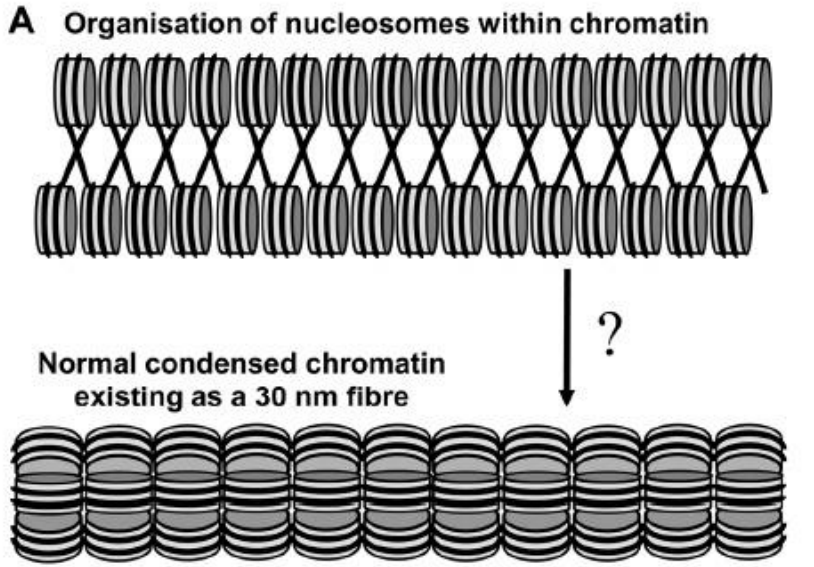
Metilazioni delle isole CpG

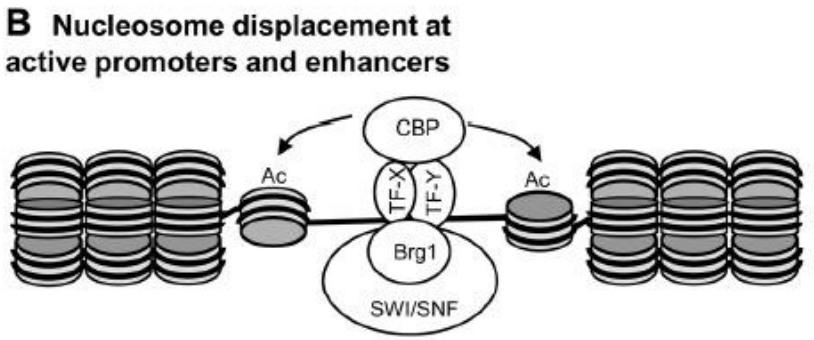


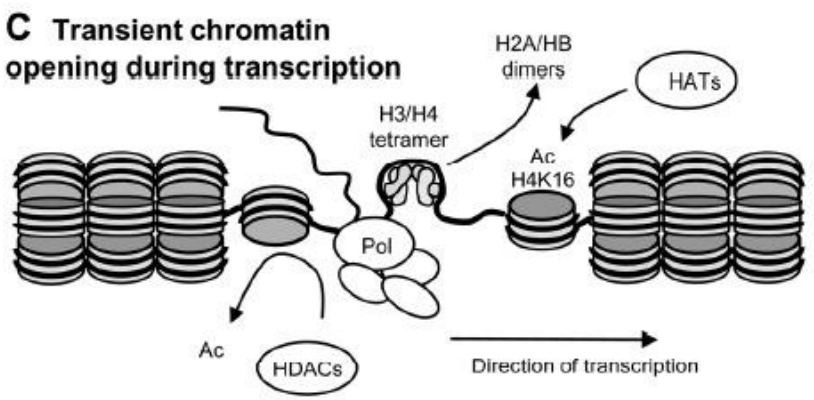
La metilazione del DNA nello sviluppo



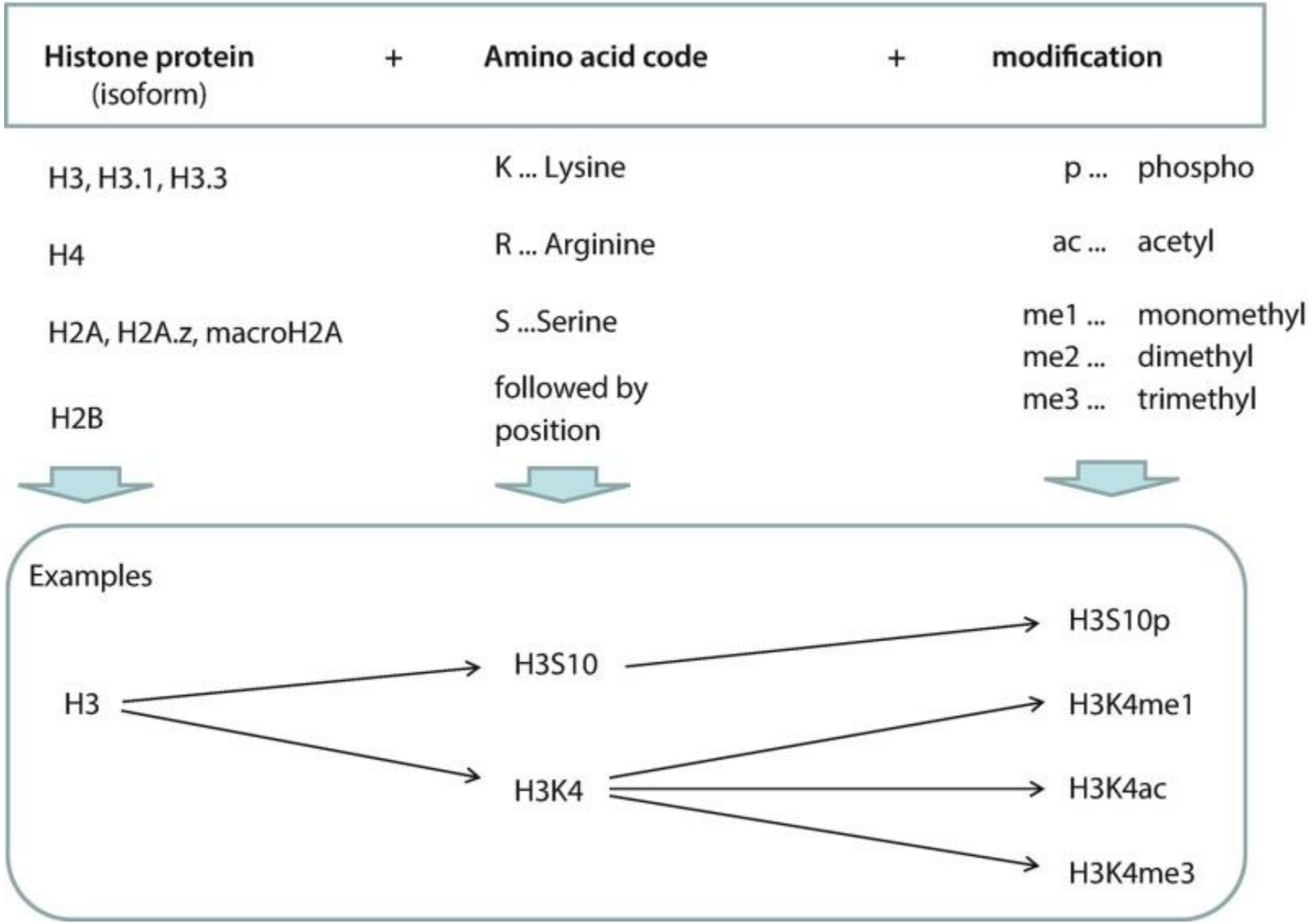
Modificazioni degli istoni



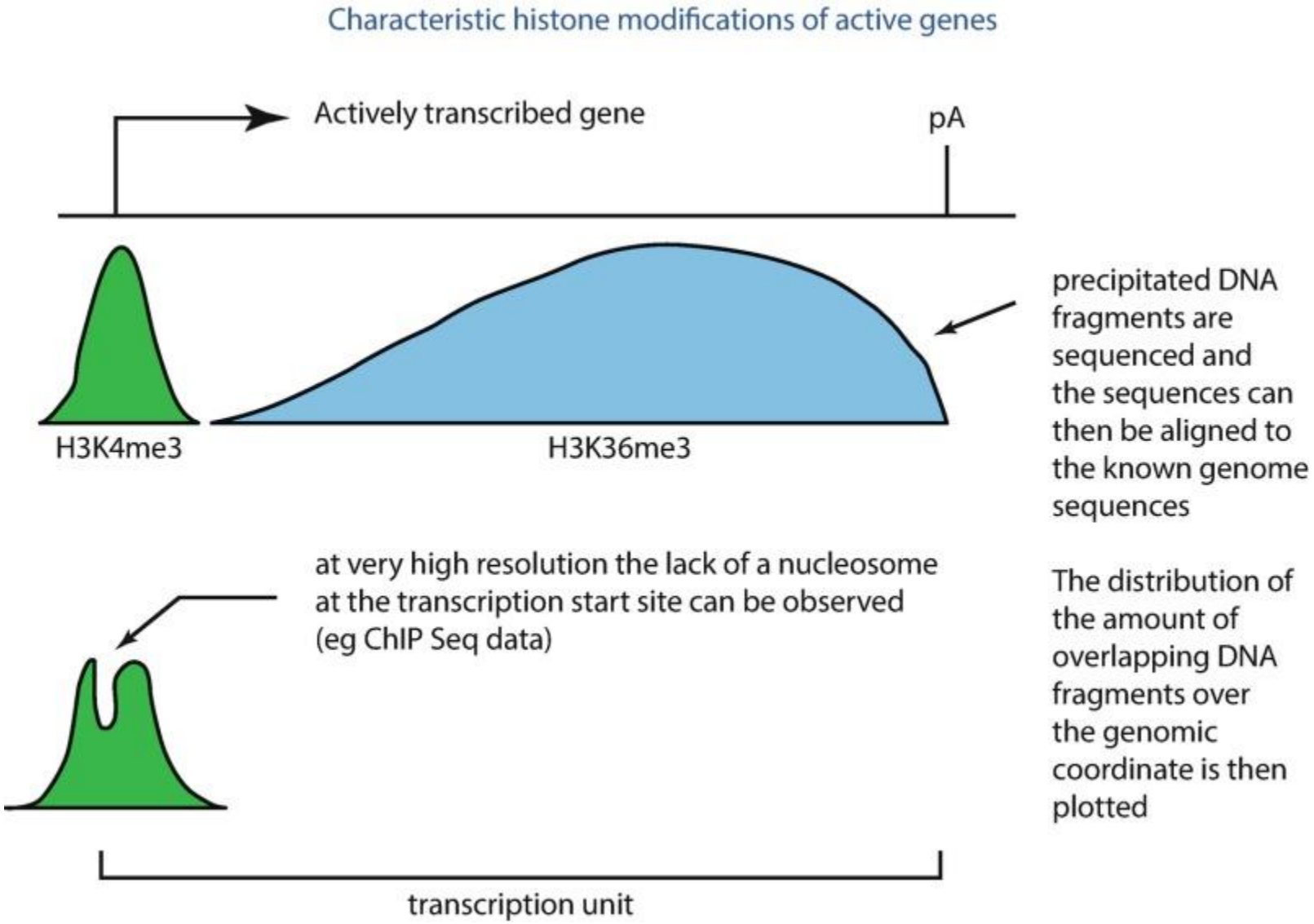




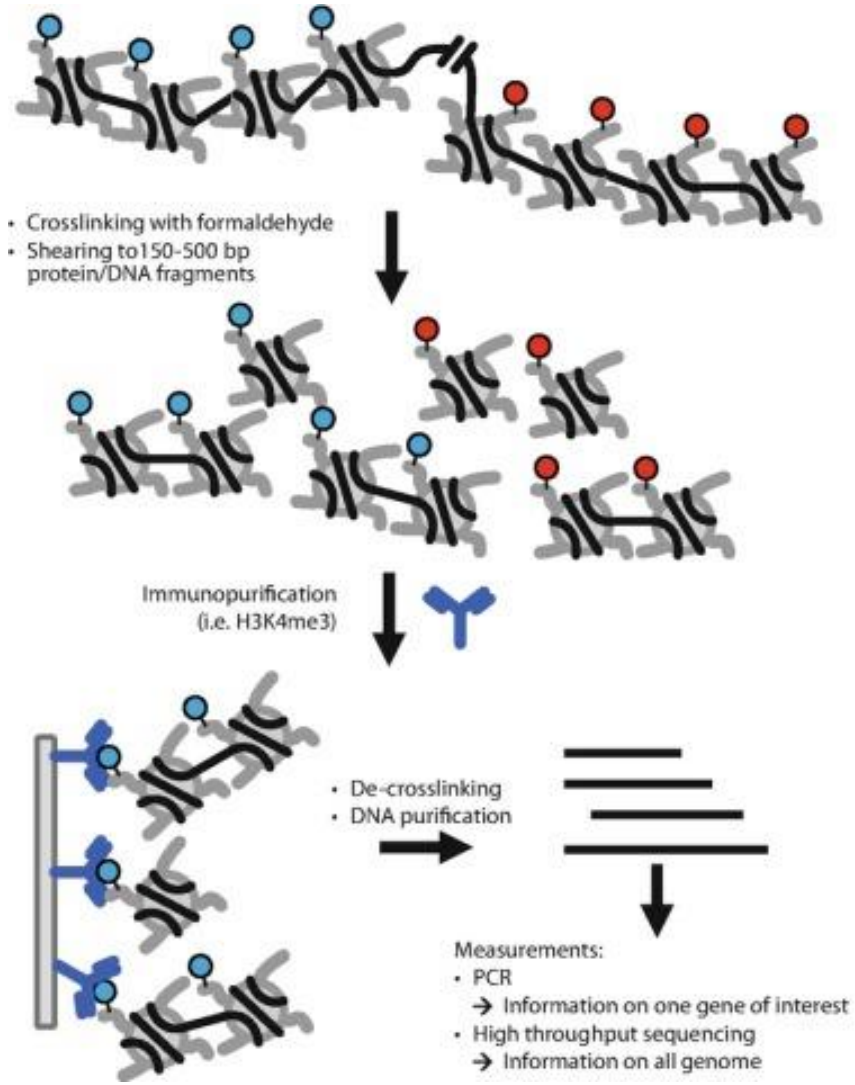
Nomenclatura delle modificazioni degli istoni

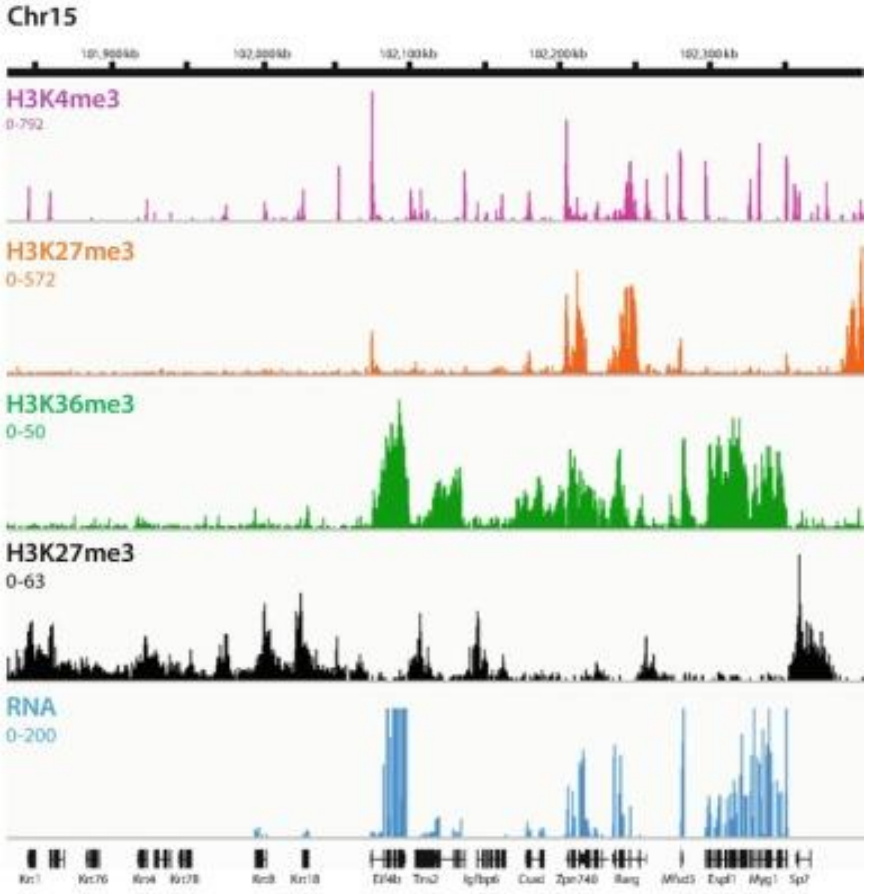


Il sequenziamento del DNA legato agli istoni (con Chipseq) permette la caratterizzazione del ruolo delle modifiche epigenetiche nell'espressione

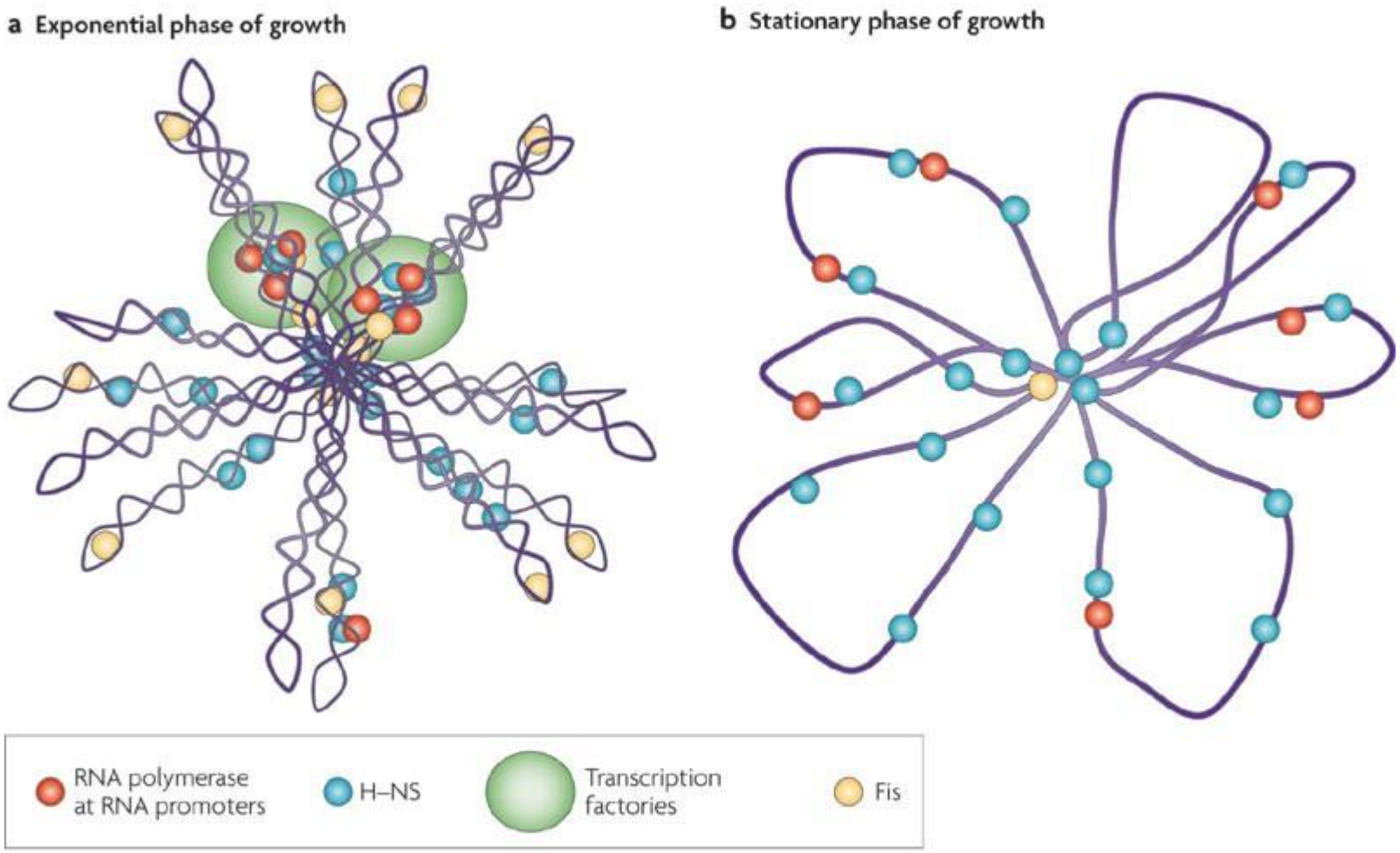


L’immunoprecipitazione della cromatina (ChipSeq)

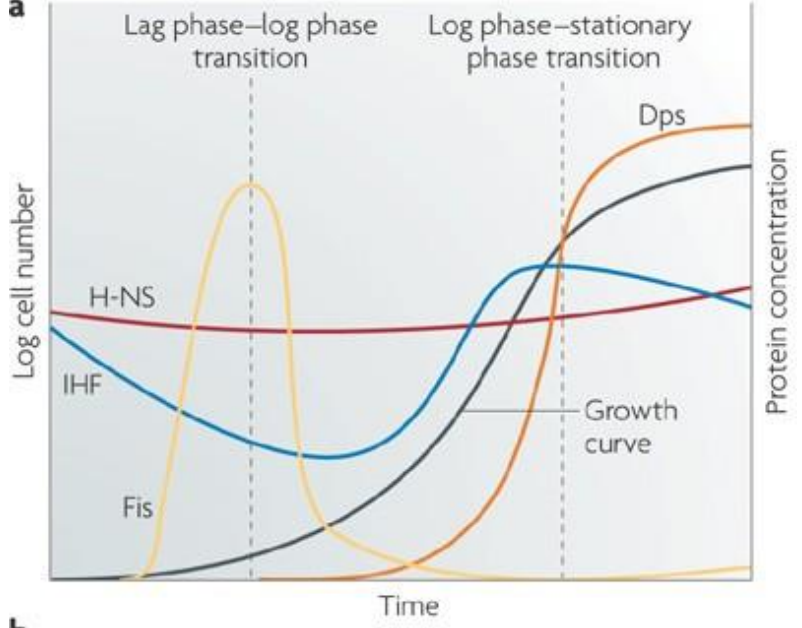


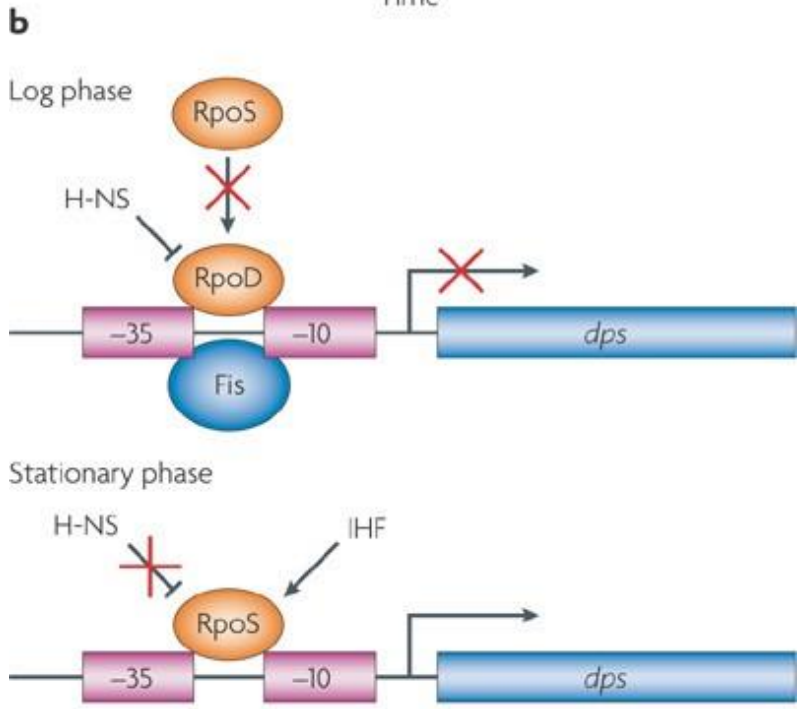


Nucleoidi nei batteri



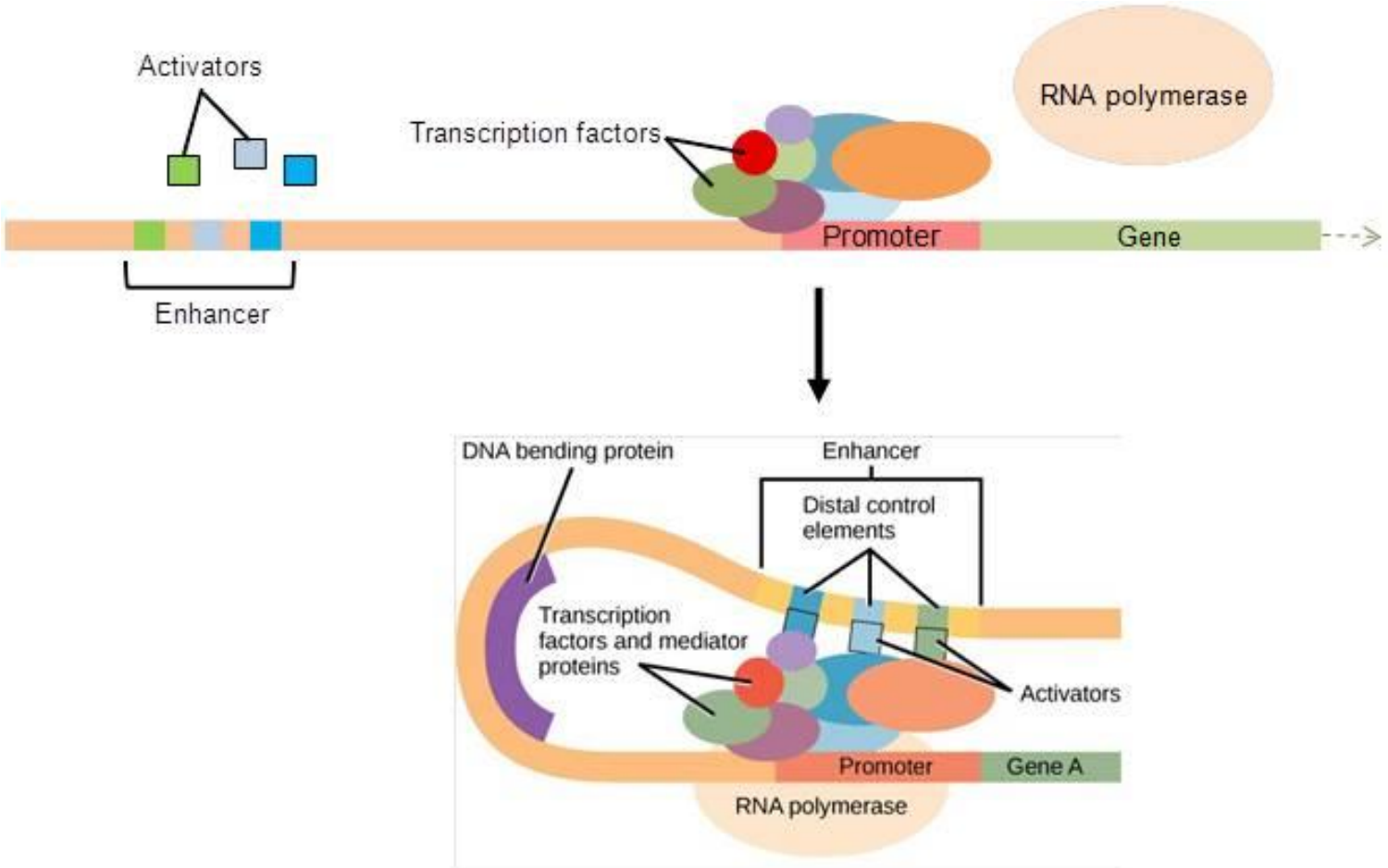
Regolazione dell'espressione genica nei batteri da proteine associate ai nucleoidi





a | The expression patterns of the four nucleoid-associated proteins (NAPs), DNA protection from starvation protein (Dps), factor for inversion stimulation (Fis), histone-like nucleoid-structuring protein (H-NS) and integration host factor (IHF). A typical bacterial growth curve is shown, with the lag phase–log phase and log phase–stationary phase transitions indicated. The expression curves of the four NAPs[102] are representational summaries. **b** | The regulatory inputs at the promoter of *dps*. During the early log phase of growth, Fis is abundant and binds to the *dps* promoter, creating a repression complex that traps RNA polymerase containing the RNA polymerase σ-factor RpoD[82]. H-NS has a negative influence at all stages of growth. RNA polymerase containing the σ-factor RpoS fails to gain access to the *dps* promoter as long as the Fis–RpoD–promoter repression complex is intact. This complex becomes less tenable as Fis concentrations fall throughout the log phase and reach almost undetectable levels with the onset of stationary phase. RNA polymerase containing RpoS can then overcome the repressive influence of H-NS; IHF then makes a positive contribution to *dps* promoter function. Levels of Dps therefore increase at the end of the exponential phase of growth.

Elementi prossimali e distali (enhancers) regolano l’espressione genica eucariotica



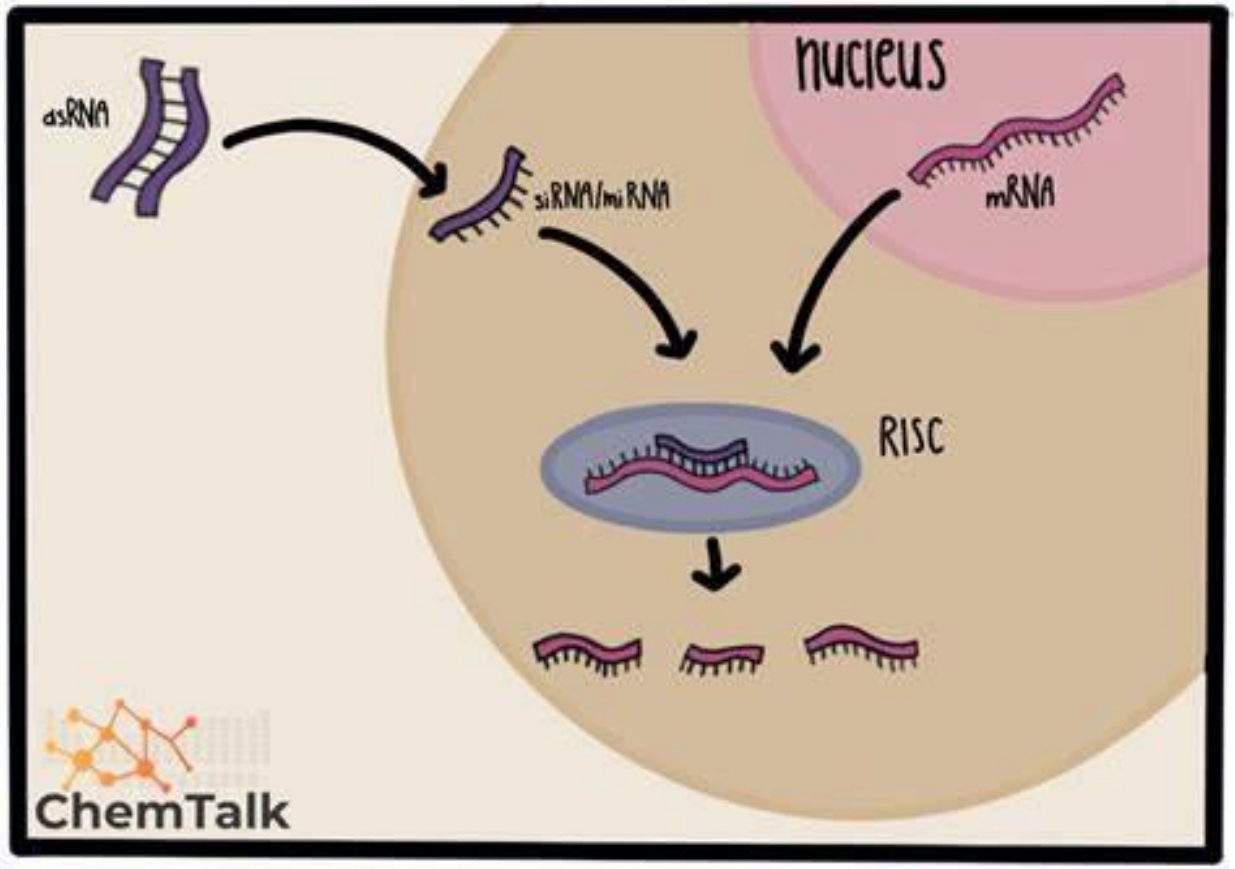
RNA regolatori di vari tipi regolano l’espressione genica con modalita’ differenti

- microRNA (or miRNA)
- RNA interference (RNAi)
- lncRNA (long non coding RNA)

RNAi e miRNA

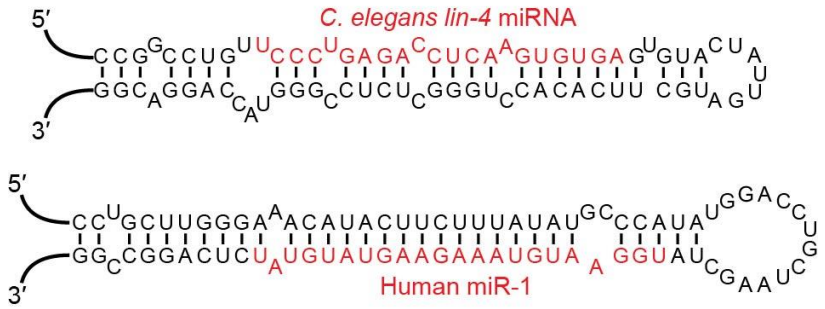
1. Un enzima chiamato *Dicer* processa una sequenza di dsRNA che è tagliata in frammenti di lunghezza minore (19-21 paia di basi).
2. Il frammento di dsRNA (chiamato *short interfering RNA*, o siRNA) si associa ad un complesso enzimatico denominato *complesso silenziatore indotto dal Rna*, o RISC.
3. L'RNA a doppio filamento viene *aperto*, probabilmente da elicasi: solo il filamento di RNA antisenso rimane associato a RISC, mentre il filamento *senso* viene degradato.
4. La RISC è ora attiva: è in grado di trovare un mRNA complementare al frammento di RNA antisenso associato al complesso stesso.
5. Se l'appaiamento tra siRNA e mRNA è perfetto (o quasi perfetto), una componente della RISC (detta *argonaute protein* o *Argo*) è in grado di operare un taglio sull'mRNA. I due frammenti di mRNA risultanti, privo di cappuccio al 5' uno e di coda di poliA al 3' l'altro, vengono così rapidamente degradati dalle RNAsi della cellula stessa. Negli animali, avviene piu spesso un inibizione a livello di traduzione, cosa che avviene anche quando l'appaiamento e' meno perfetto.

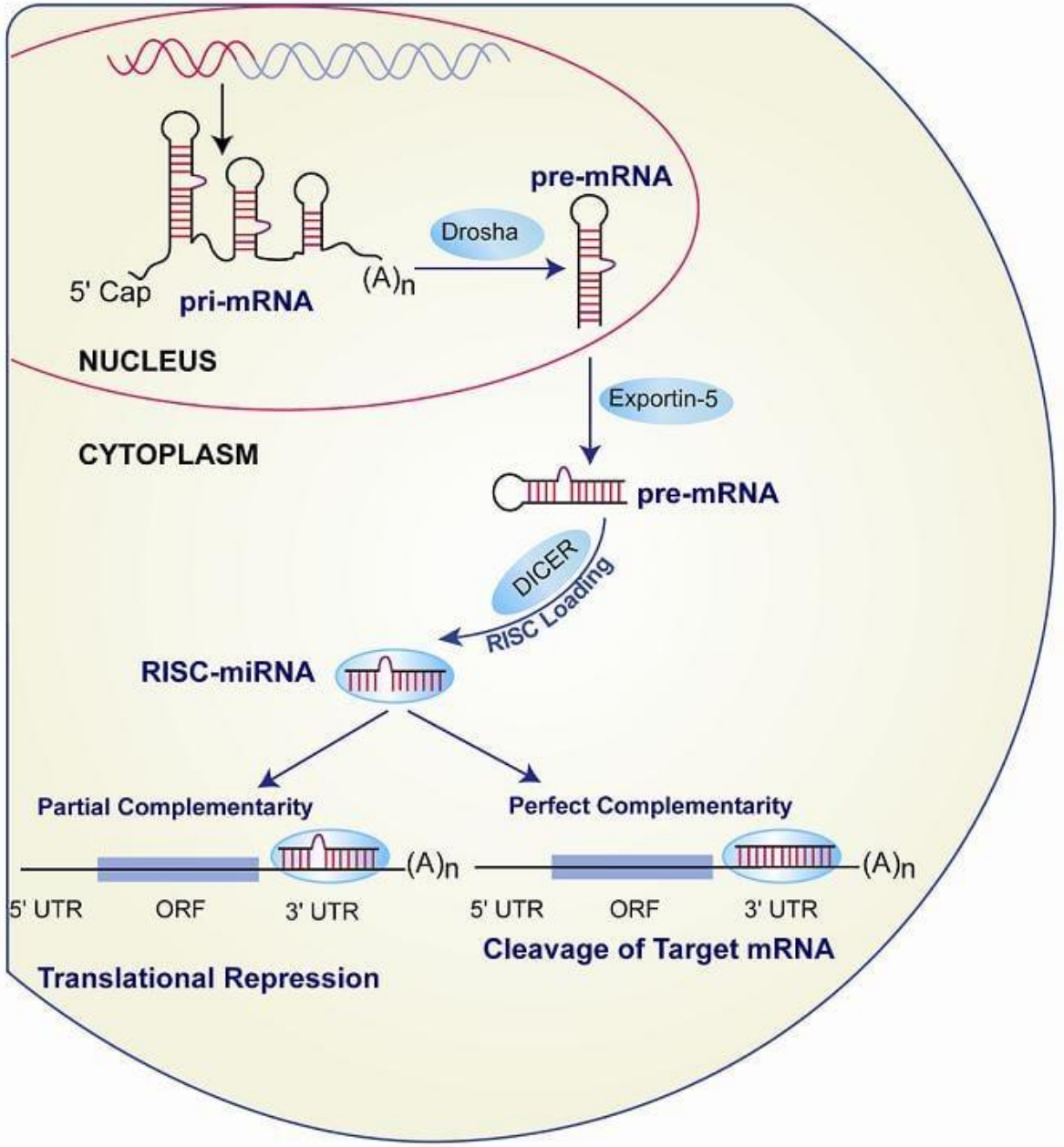
RNAi



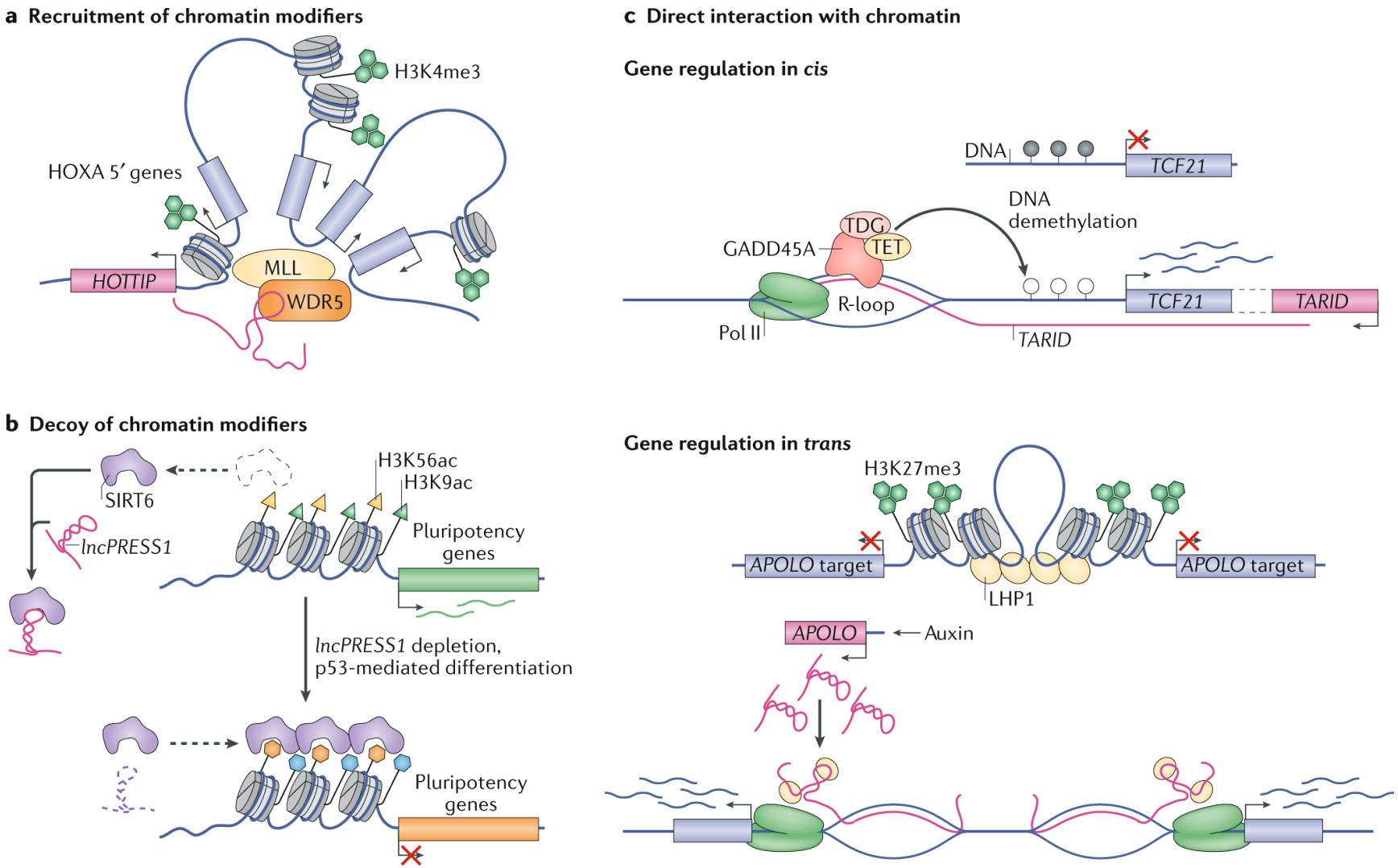
miRNA

>2500 miRNA accertati nel genoma umano, coinvolti in vari processi (sviluppo del cuore, del muscolo liscio, neuronale, etc.)

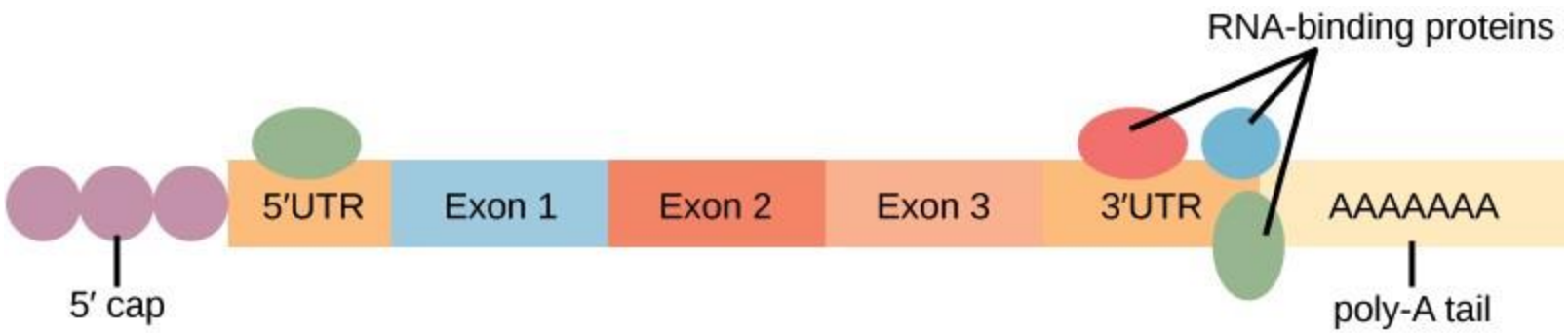




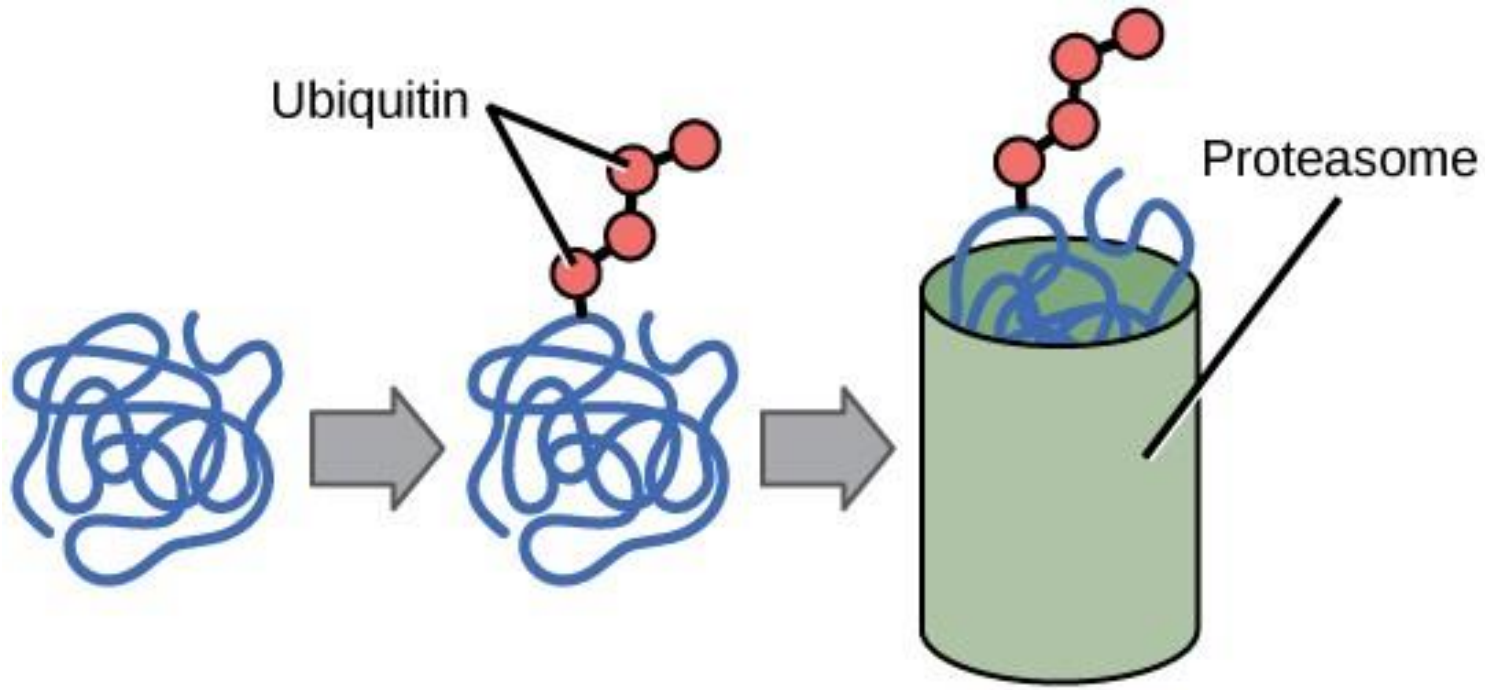
lncRNA possono regolare la cromatina

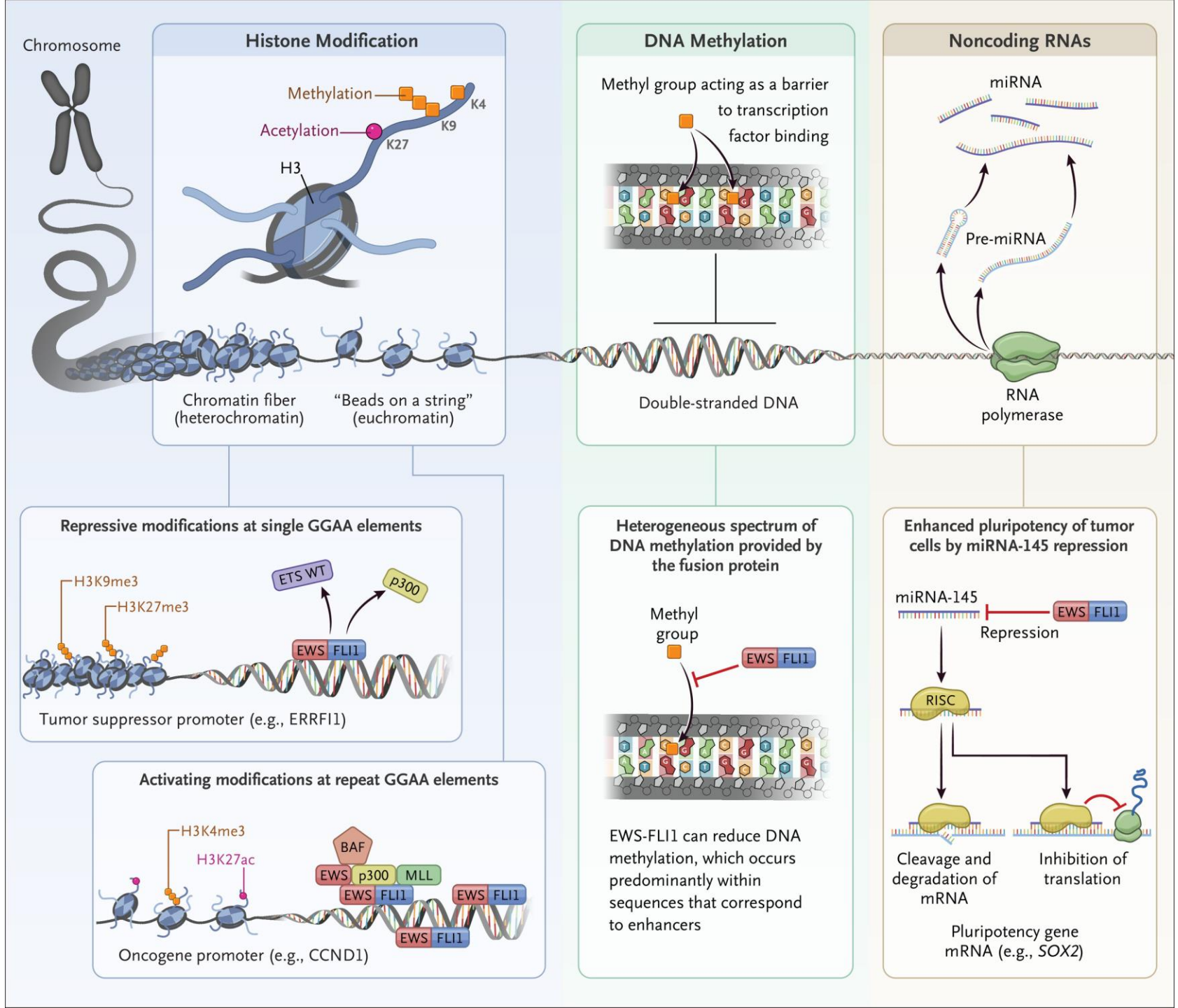


Proteine che legano l’RNA regolano la stabilita’ dell’RNA al 3’ e 5’



La regolazione post-traduzionale contribuisce a determinare l’abbondanza della proteina finita




Chromosome
Histone Modification
Methylation
K4
K9
Acetylation
K27
H3
Chromatin fiber (heterochromatin)
"Beads on a string" (euchromatin)
DNA Methylation
Methyl group acting as a barrier to transcription factor binding
Double-stranded DNA
Noncoding RNAs
miRNA
Pre-miRNA
RNA polymerase
Repressive modifications at single GGAA elements
H3K9me3
H3K27me3
ETS WT
p300
EWS FLI1
Tumor suppressor promoter (e.g., ERRFI1)
Activating modifications at repeat GGAA elements
H3K4me3
H3K27ac
BAF
EWS p300 MLL
EWS FLI1
EWS FLI1
EWS FLI1
Oncogene promoter (e.g., CCND1)
Heterogeneous spectrum of DNA methylation provided by the fusion protein
Methyl group
EWS FLI1
EWS-FLI1 can reduce DNA methylation, which occurs predominantly within sequences that correspond to enhancers
Enhanced pluripotency of tumor cells by miRNA-145 repression
miRNA-145
EWS FLI1
Repression
RISC
Cleavage and degradation of mRNA
Inhibition of translation
Pluripotency gene mRNA (e.g., SOX2)