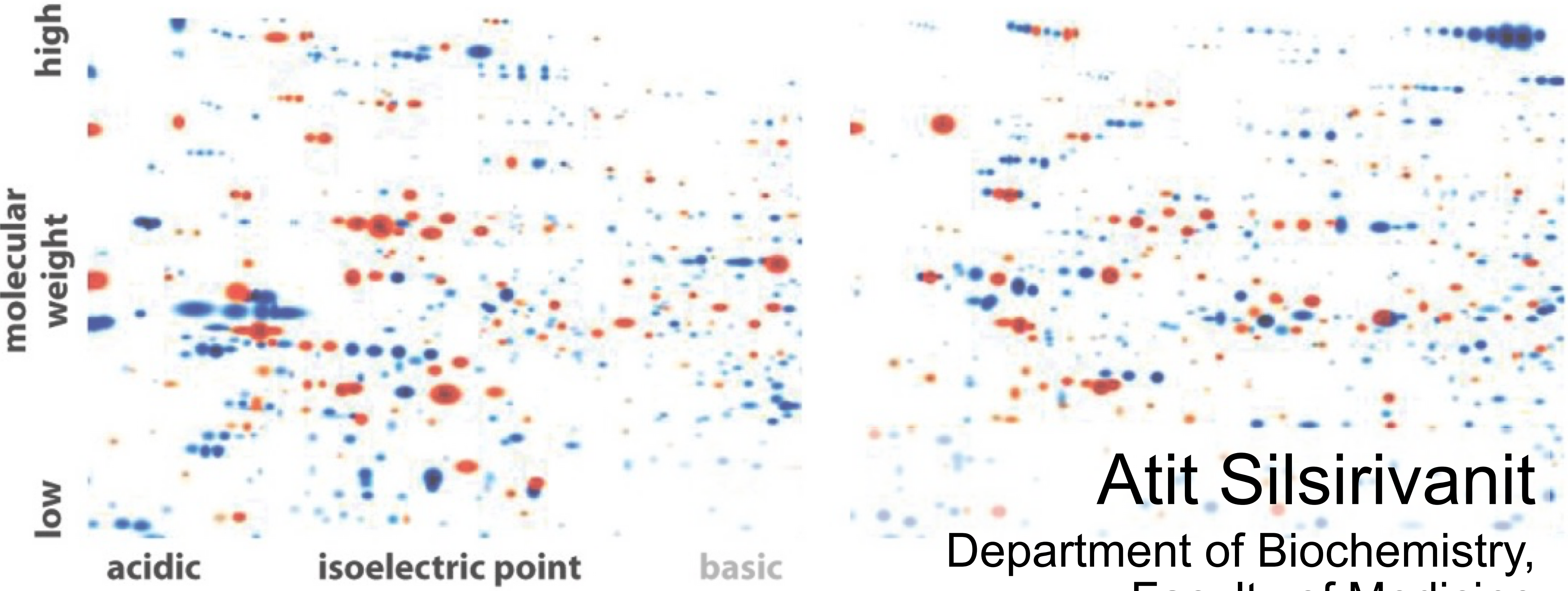
Regulation of Gene expression



Atit Silsirivanit

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Faculty of Medicine
email: atitsil@kku.ac.th

Figure 7-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Different cells:

- Similar DNA sequence
- Different protein expression
- Different function

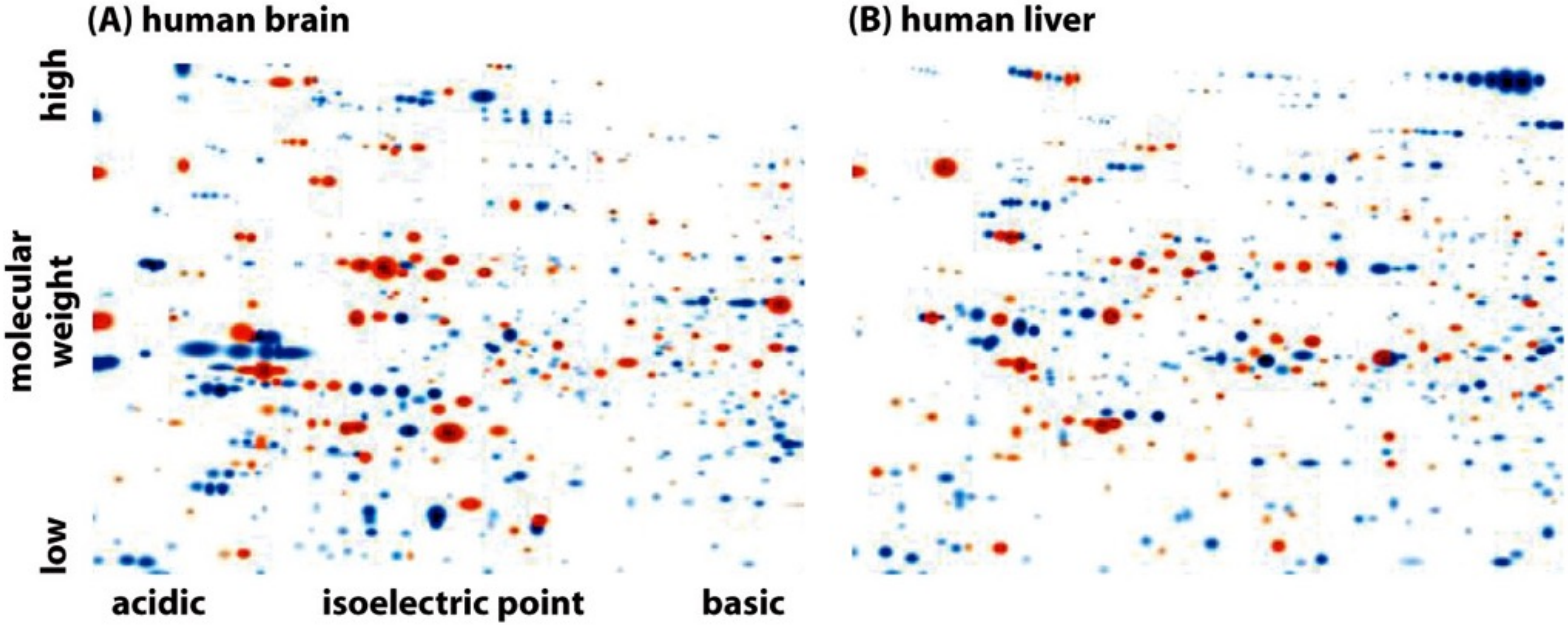
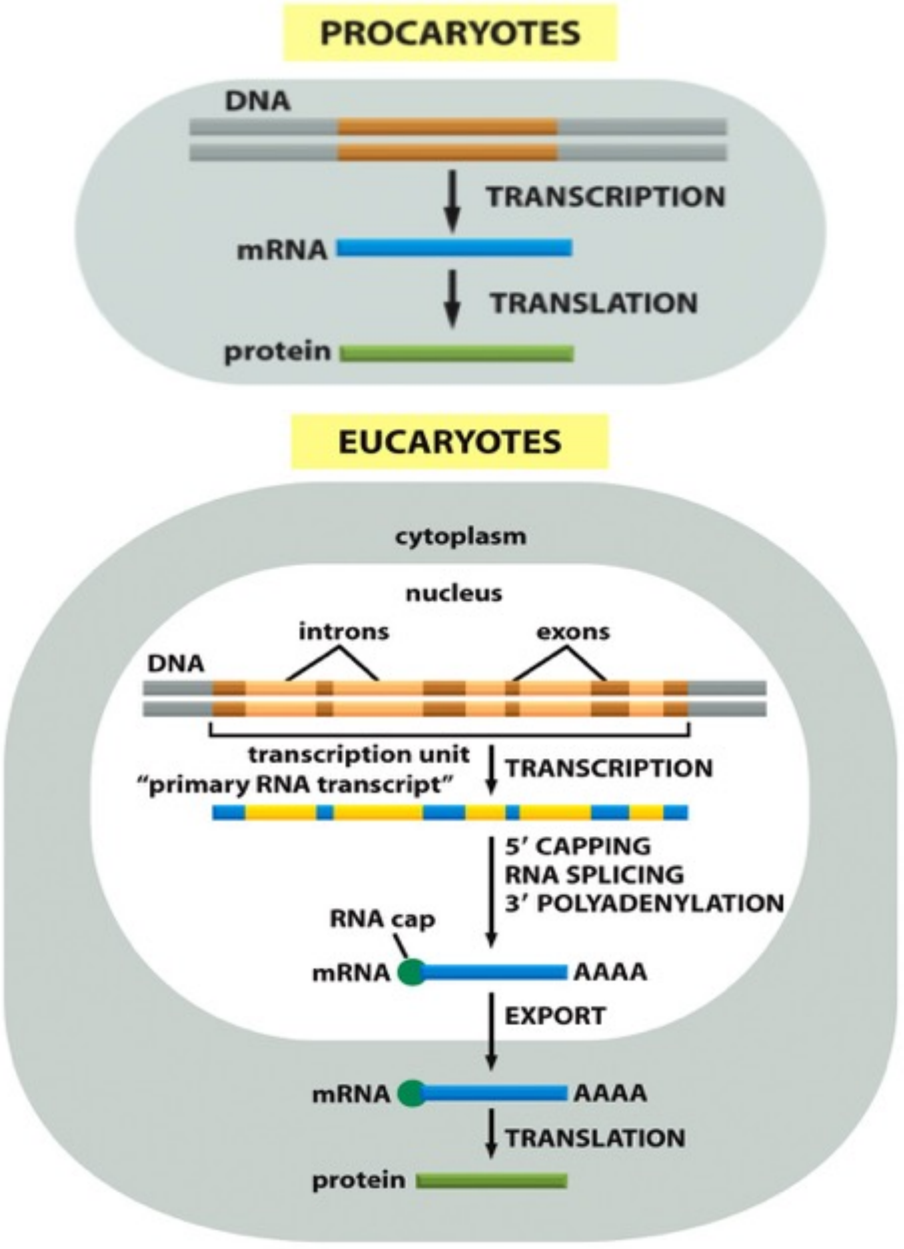


Figure 7-4 *Molecular Biology of the Cell* (© Garland Science 2008)

Regulation of Gene expression



Prokaryotic

-Transcriptional control

-Translational control

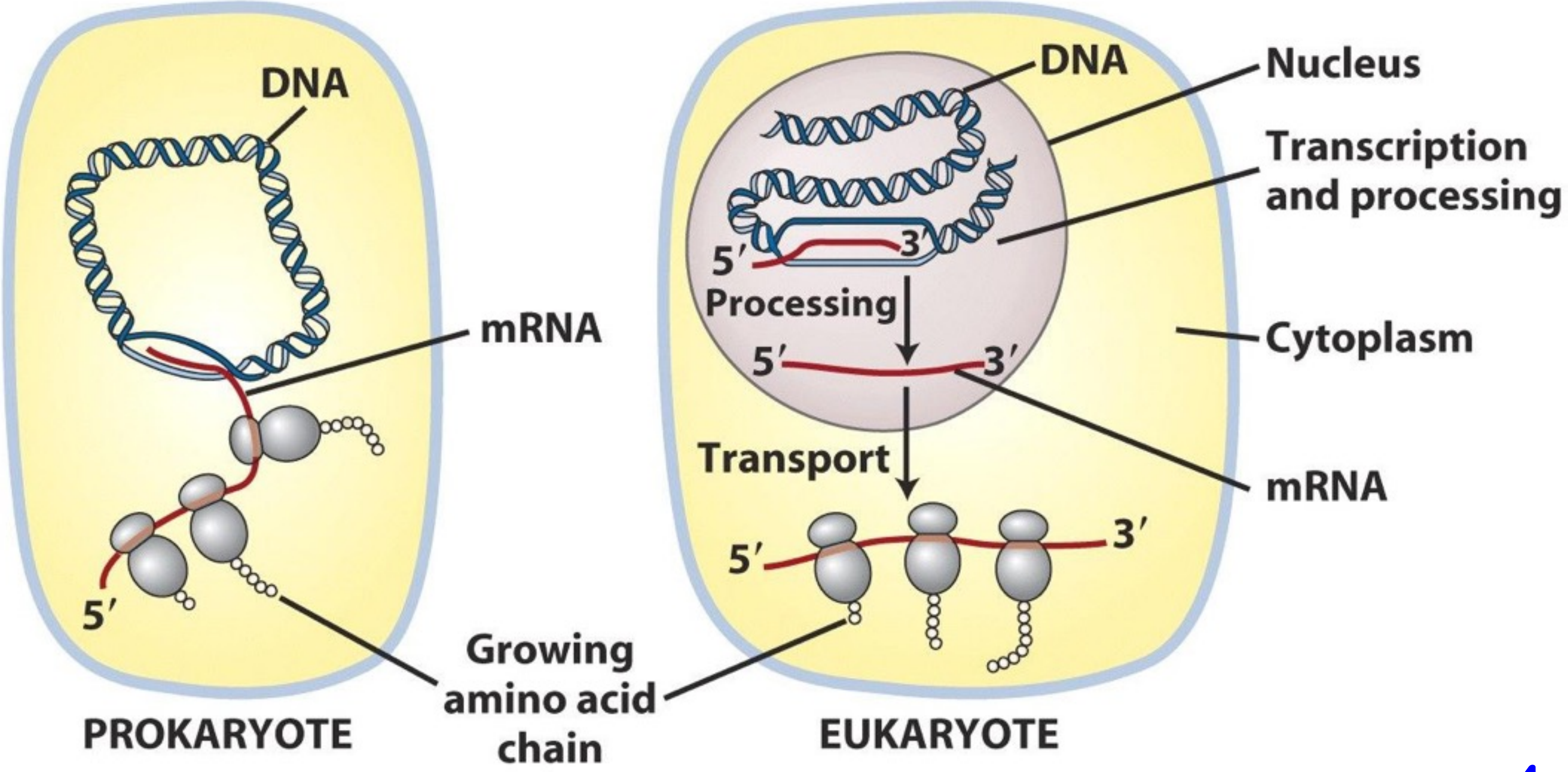
Eukaryotic

-Transcriptional control

- Post-transcriptional control
- Translational control
- Post-translational control

Gene expression

Prokaryotes *vs* Eukaryotes



http://apbio-werle.wikispaces.com/file/view/figure-08-01.JPG/68532827/figure-08-01.JPG

Regulatory proteins are important for gene regulation.

Regulatory proteins: Proteins that control the expression of genes

- **Repressors:** Negative regulators
- **Activators:** Positive regulators

Protein-DNA interaction

Bind specific sequence of DNA and facilitate gene expression.

Protein-Protein interaction

Bind other proteins and activate transcription

Protein-RNA pol. interaction

Bind RNA polymerase and activate transcription

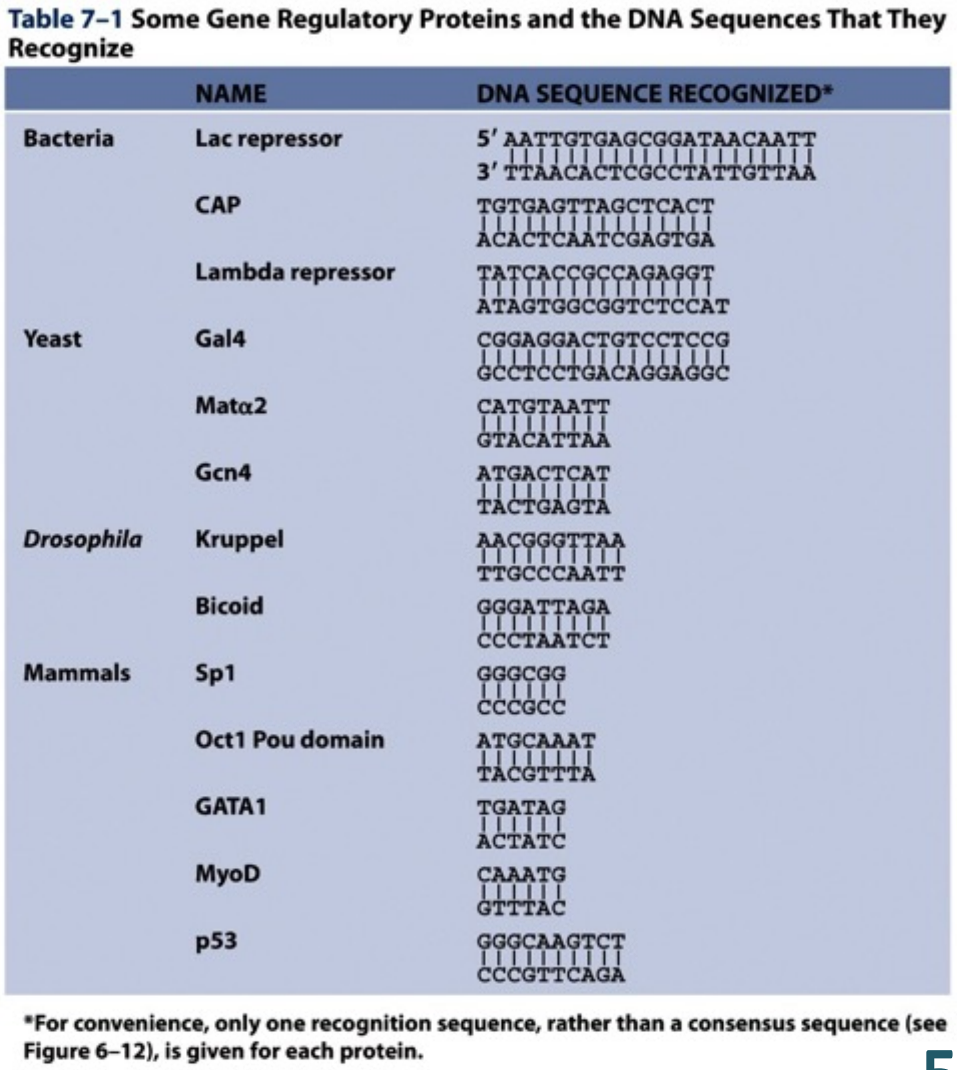


Table 7–1 Some Gene Regulatory Proteins and the DNA Sequences That They Recognize

	NAME	DNA SEQUENCE RECOGNIZED*
Bacteria	**Lac repressor**	5′ AATTGTGAGCGGATAACAATT 3′ TTAACACTCGCCTATTGTTAA
	CAP	TGTGAGTTAGCTCACT ACACTCAATCGAGTGA
	Lambda repressor	TATCACCGCCAGAGGT ATAGTGGCGGTCTCCAT
Yeast	**Gal4**	CGGAGGACTGTCCTCCG GCCTCCTGACAGGAGGC
	Matα2	CATGTAATT GTACATTAA
	Gcn4	ATGACTCAT TACTGAGTA
Drosophila	**Kruppel**	AACGGGTTAA TTGCCCAATT
	Bicoid	GGGATTAGA CCCTAATCT
Mammals	**Sp1**	GGGCGG CCCGCC
	Oct1 Pou domain	ATGCAAAT TACGTTTA
	GATA1	TGATAG ACTATC
	MyoD	CAAATG GTTTAC
	p53	GGGCAAGTCT CCCGTTCAGA

*For convenience, only one recognition sequence, rather than a consensus sequence (see Figure 6–12), is given for each protein.

Table 7-1 *Molecular Biology of the Cell* (© Garland Science 2008)

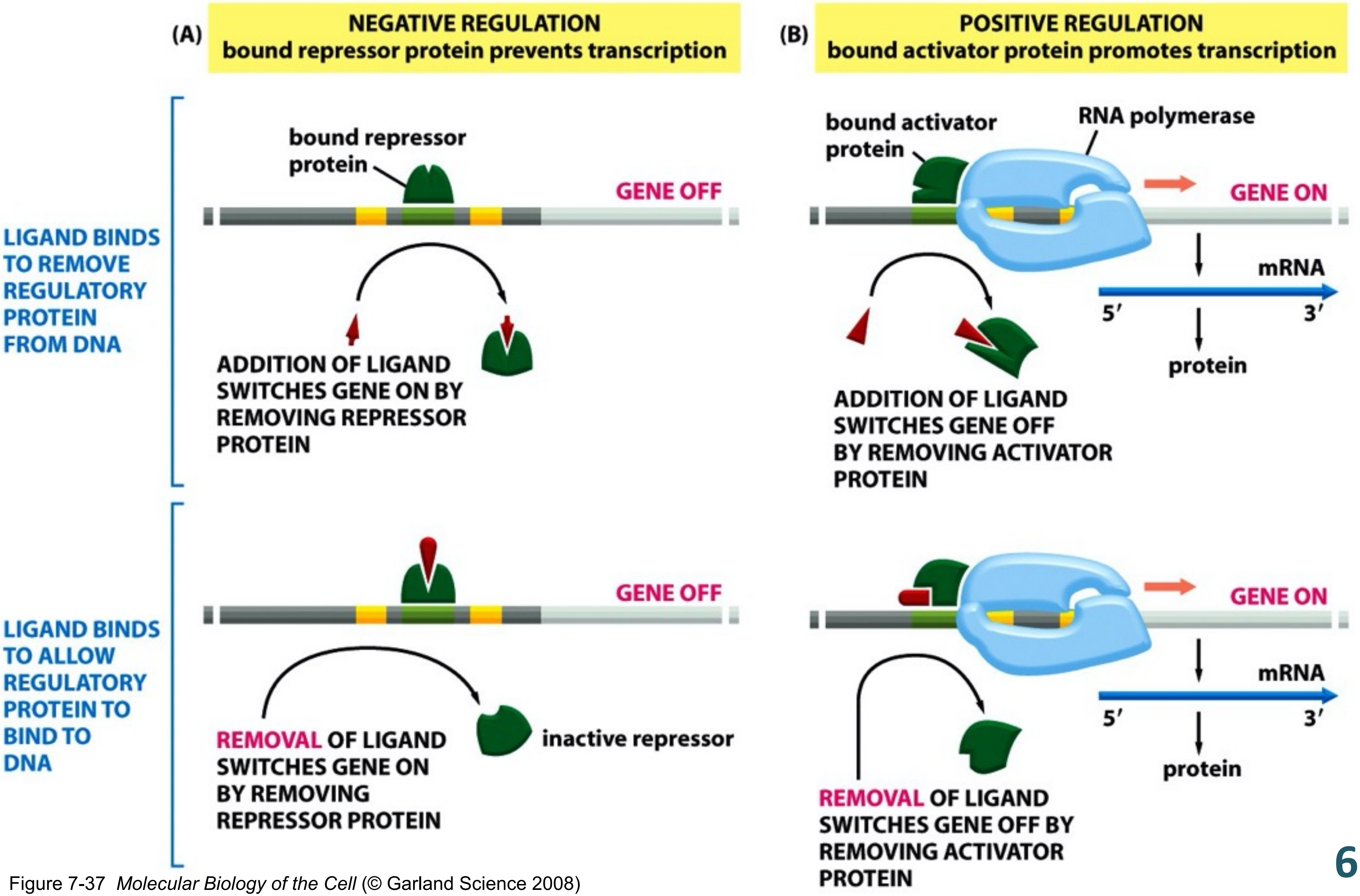


Figure 7-37 *Molecular Biology of the Cell* (© Garland Science 2008)

Regulation of Gene expression in Prokaryotes

Operon: group of genes that need the same set of regulatory machineries

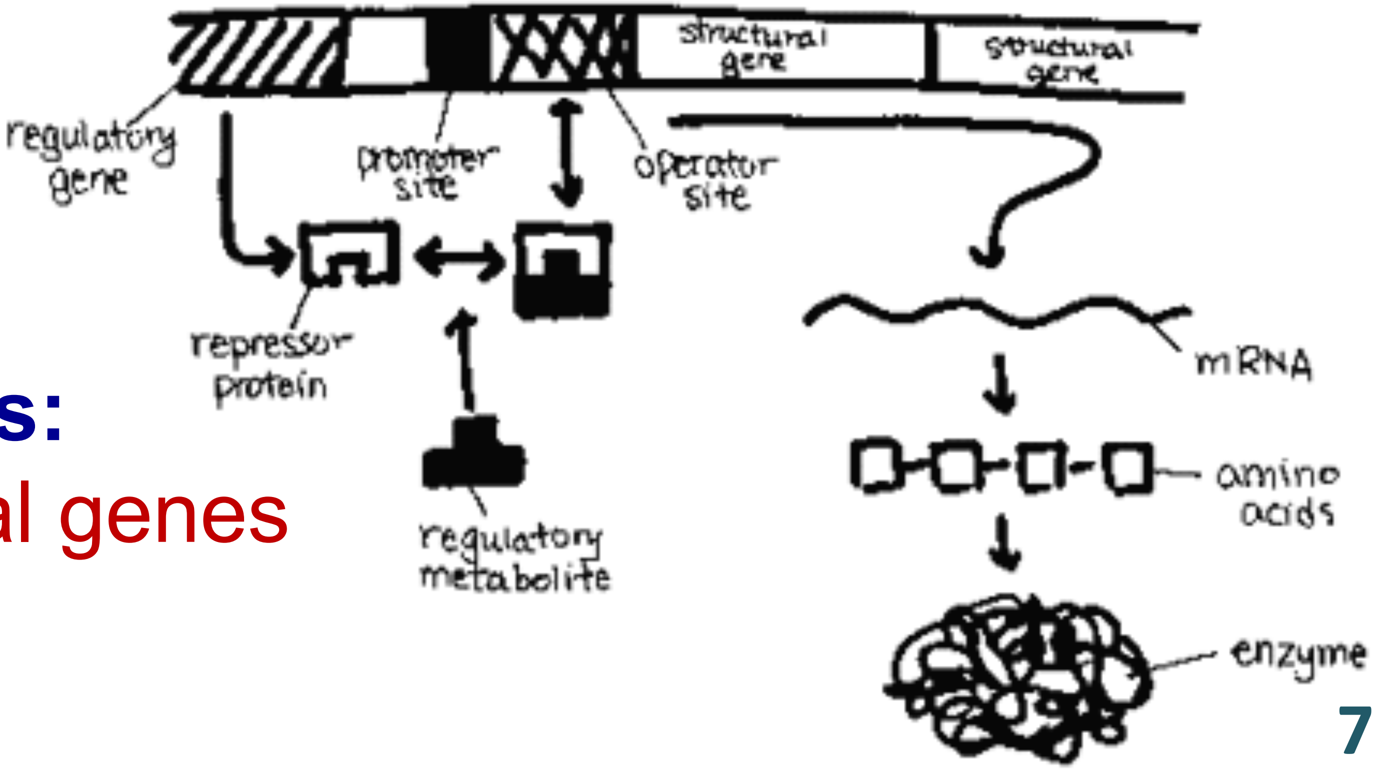
***lac* operon**
***trp* operon**

- Regulatory region:

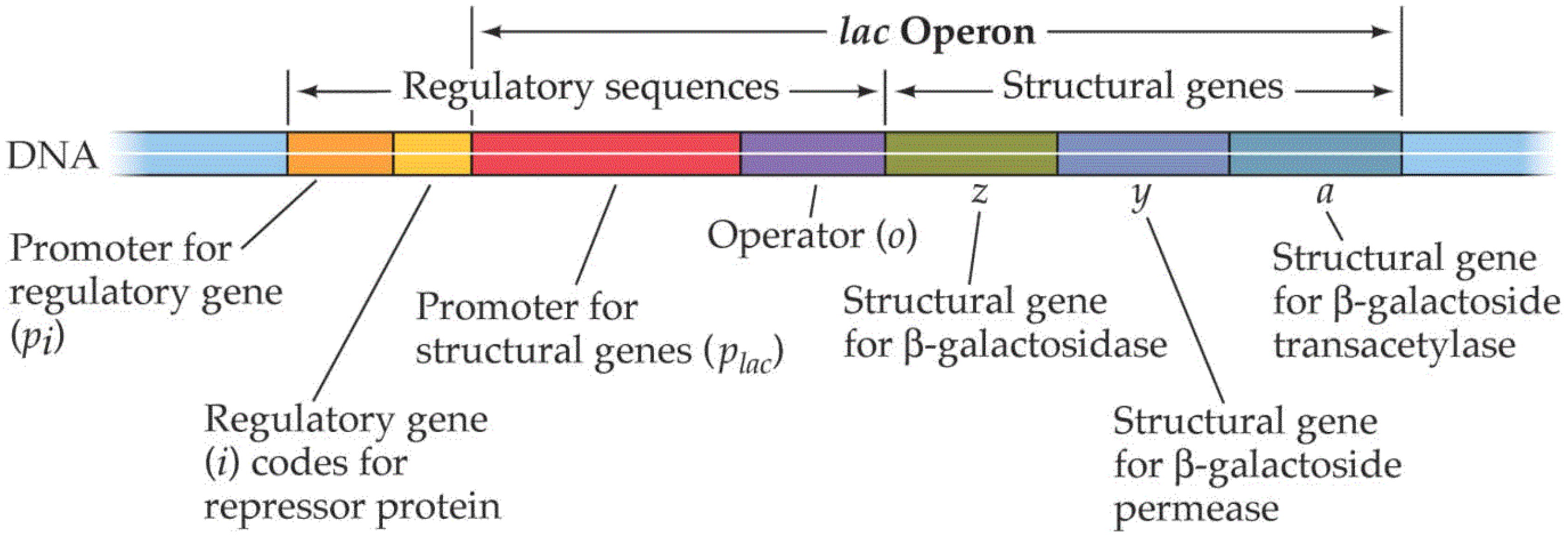
(1) Regulatory gene: Repressor
(2) Promoter
(3) Operator

- Structural genes:

Group of functional genes



lac operon



LIFE: THE SCIENCE OF BIOLOGY, *Seventh Edition*, **Figure 13.16 The *lac* Operon of *E. coli***

Regulatory protein:

lac repressor

Structural genes:

(1) β-galactosidase
(2) permease
(3) transacetylase

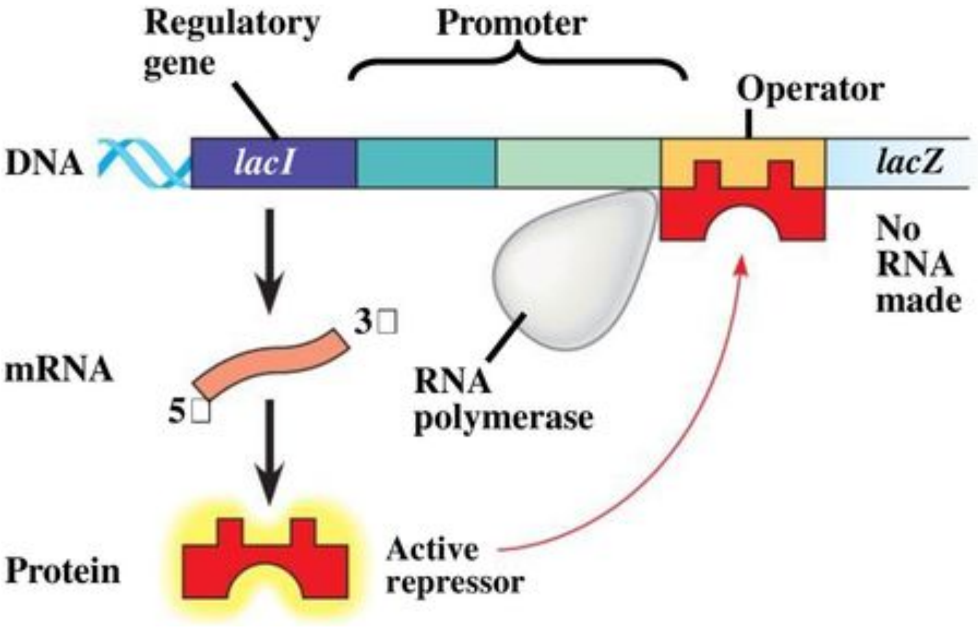
https://www.google.com/url?sa=i&url=https%3A%2F%2Fslideplayer.com%2Fslide%2F13333425%2F&psig=AOvVaw3jv8l_tPX-xuZj7H-N4dsa&ust=1583458895016000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCKj6gaeagugCFQAAAAAdAAAAABAT

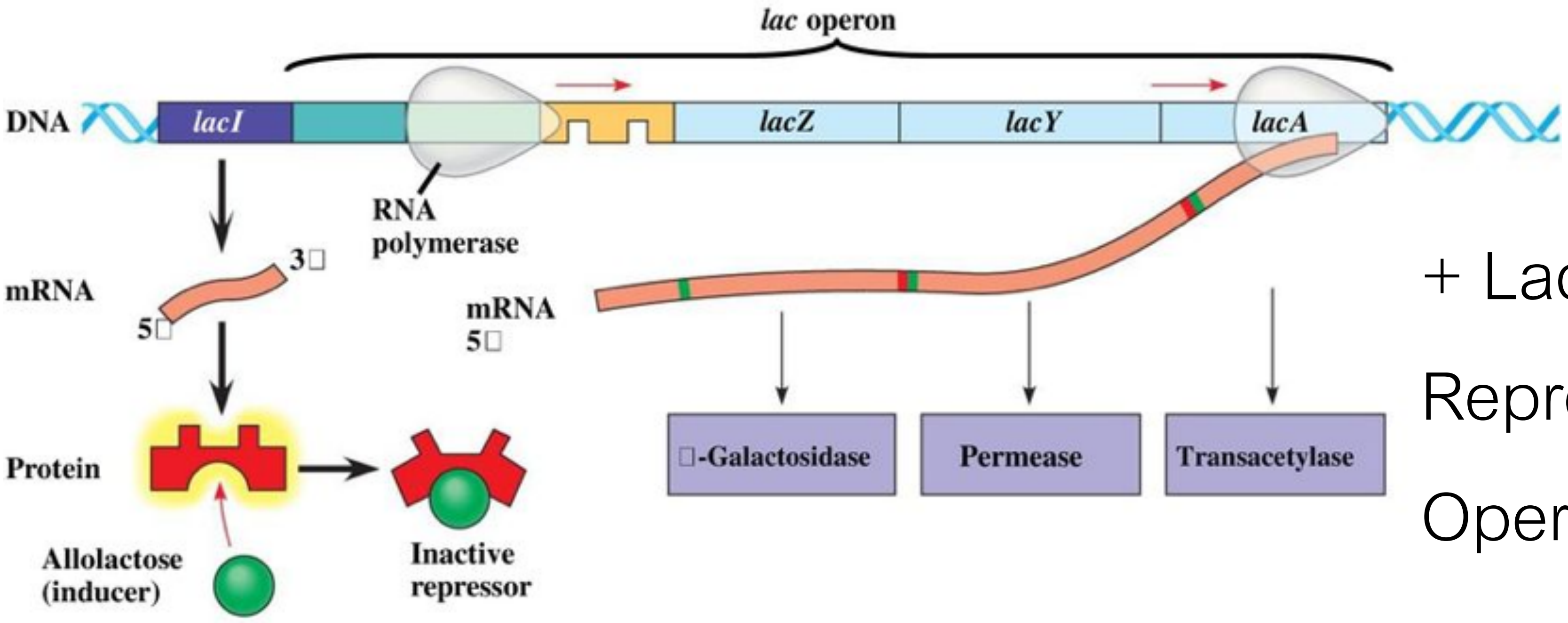
lac operon

No lactose

Repressor active

Operon off





(b) Lactose present, repressor inactive, operon on

+ Lactose

Repressor inactive

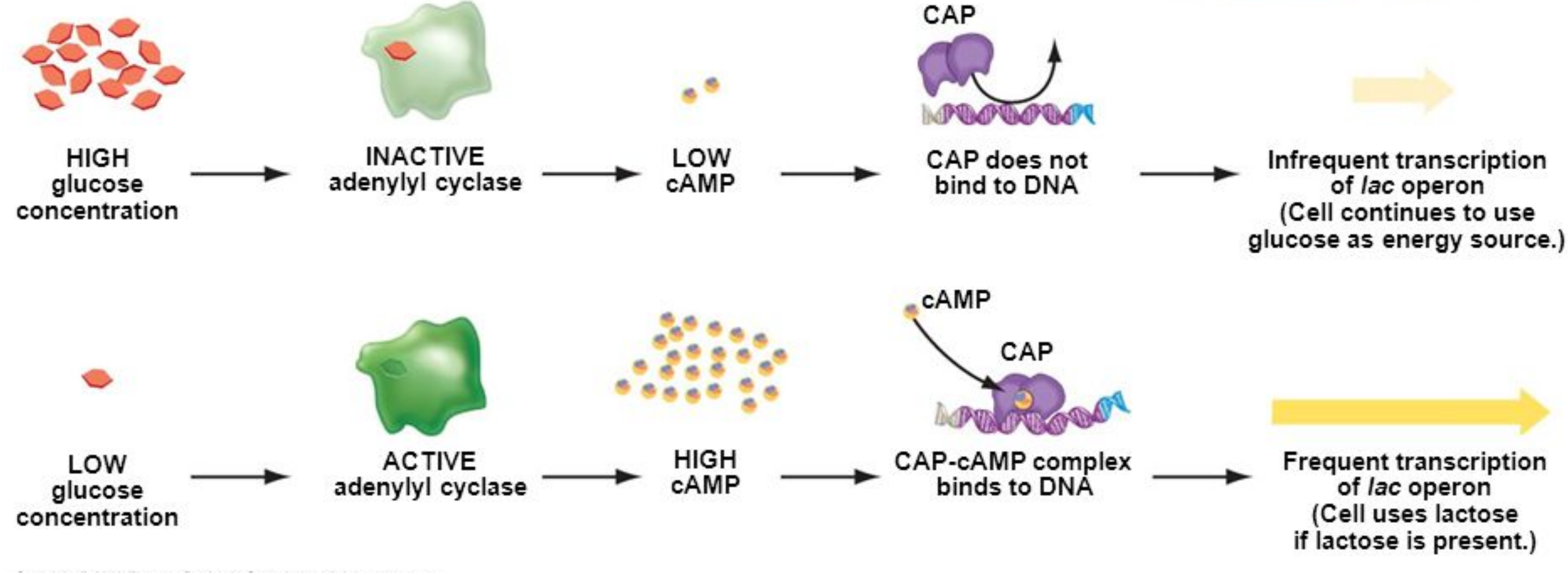
Operon on

https://www.google.com/url?sa=i&url=https%3A%2F%2Fslideplayer.com%2Fslide%2F13333425%2F&psig=AOvVaw3jv8I_tPX-xuZj7H-N4dsa&ust=1583458895016000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCKj6gaeagugCFQAAAAAdAAAAABAT

lac operon

CAP (Catabolite gene activator protein)

activator



lac operon

CAP -
Lac Repressor -

CAP -
Lac Repressor +

CAP +
Lac Repressor +

CAP +
Lac Repressor -

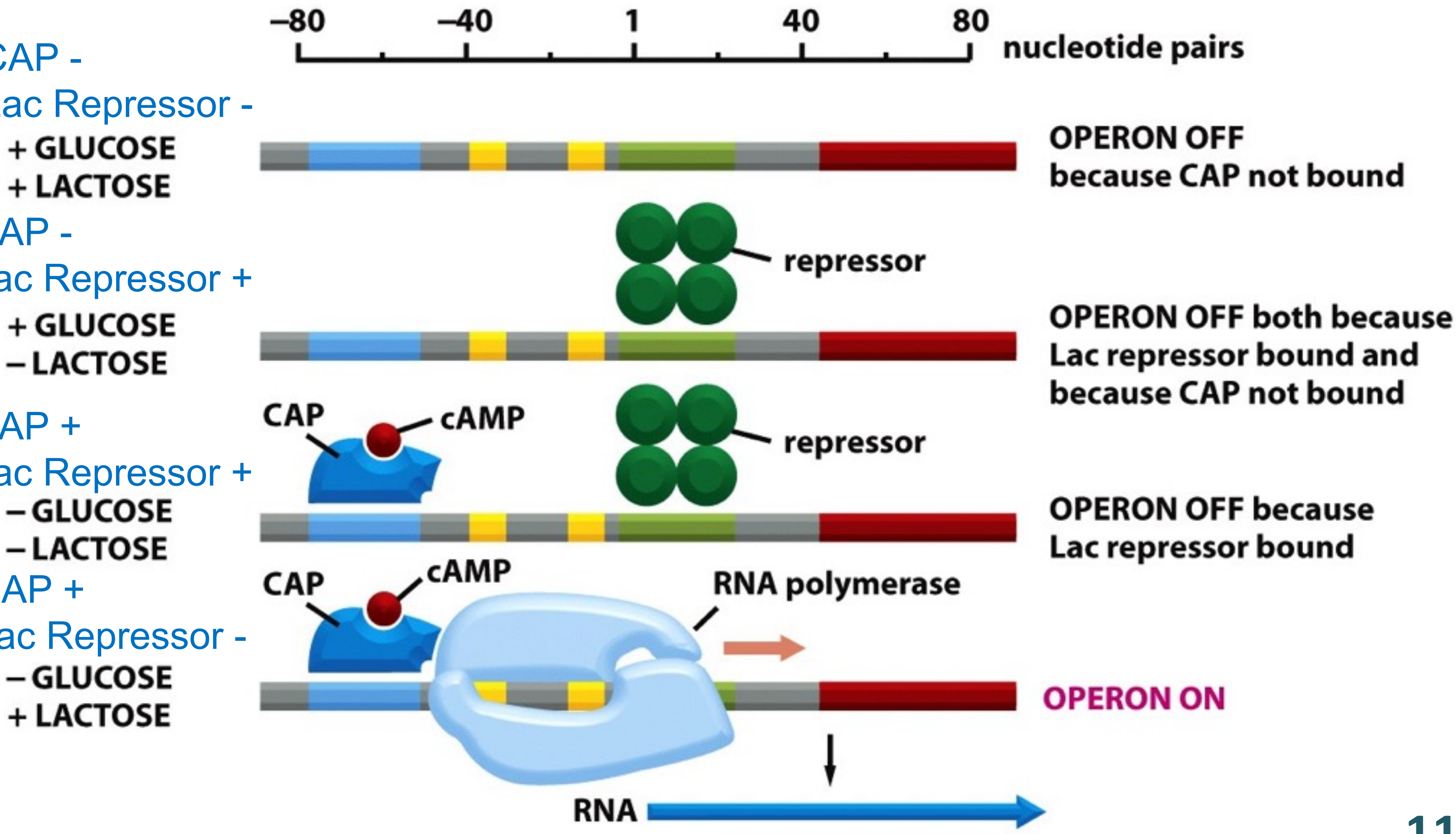


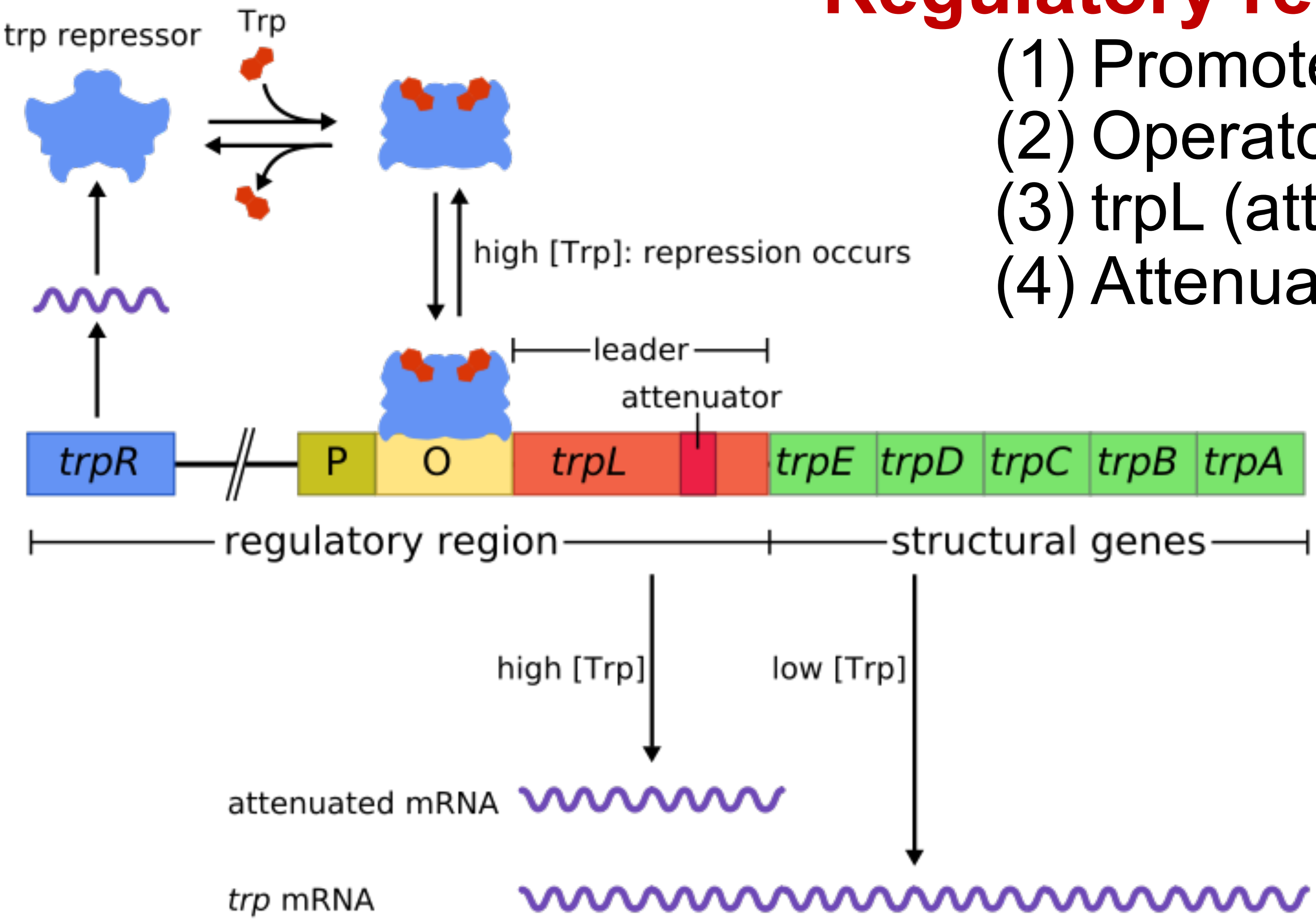
Figure 7-39 *Molecular Biology of the Cell* (© Garland Science 2008)

trp operon

Regulatory gene: trp repressor

Regulatory region:

(1) Promoter
(2) Operator
(3) trpL (attenuated mRNA)
(4) Attenuator



http://upload.wikimedia.org/wikipedia/commons/thumb/9/95/Trpoperon.svg/753px-Trpoperon.svg.png

Structural genes:

(1) trpE
(2) trpD
(3) trpC
(4) trpB
(5) trpA

trp operon

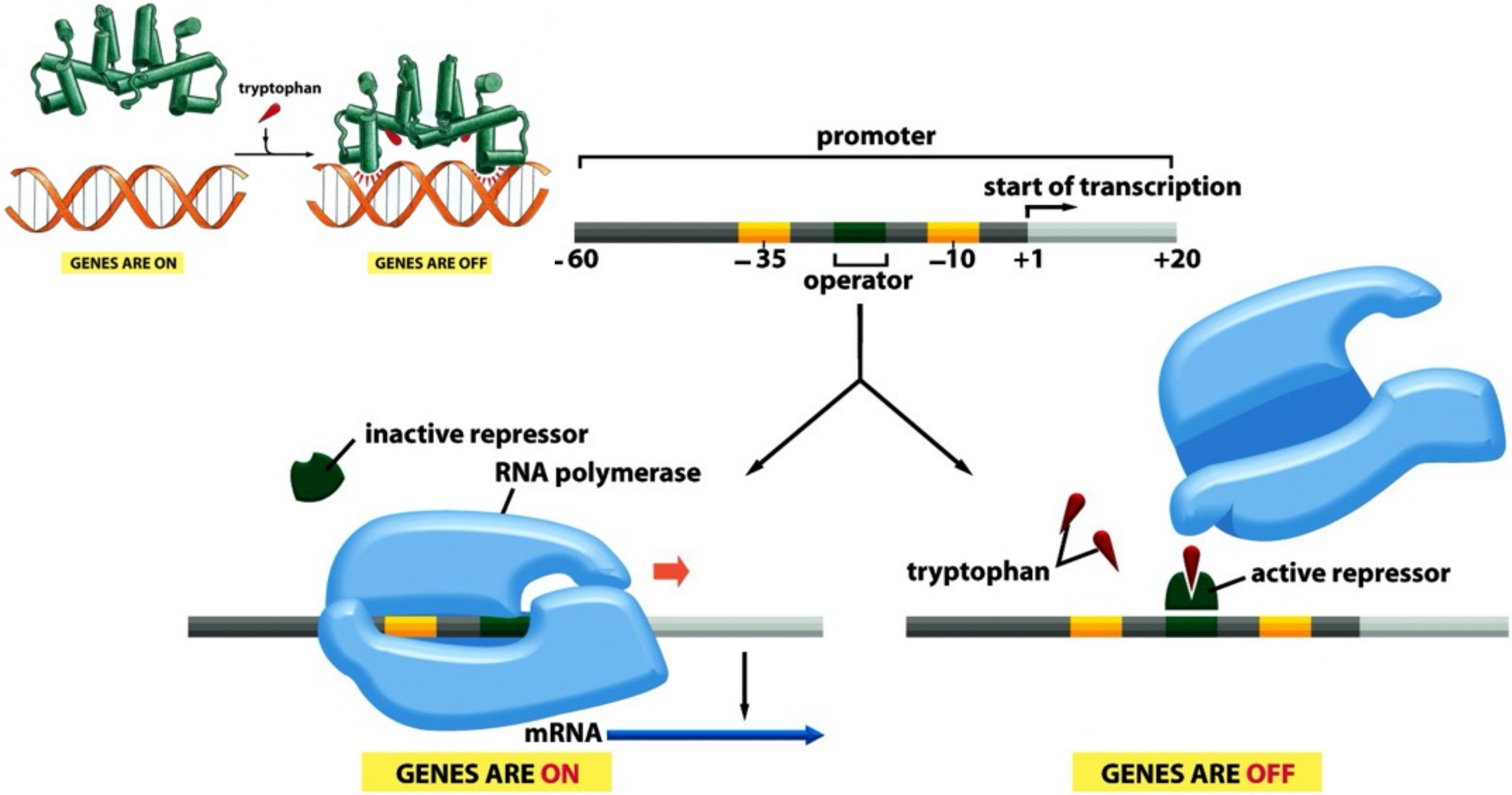
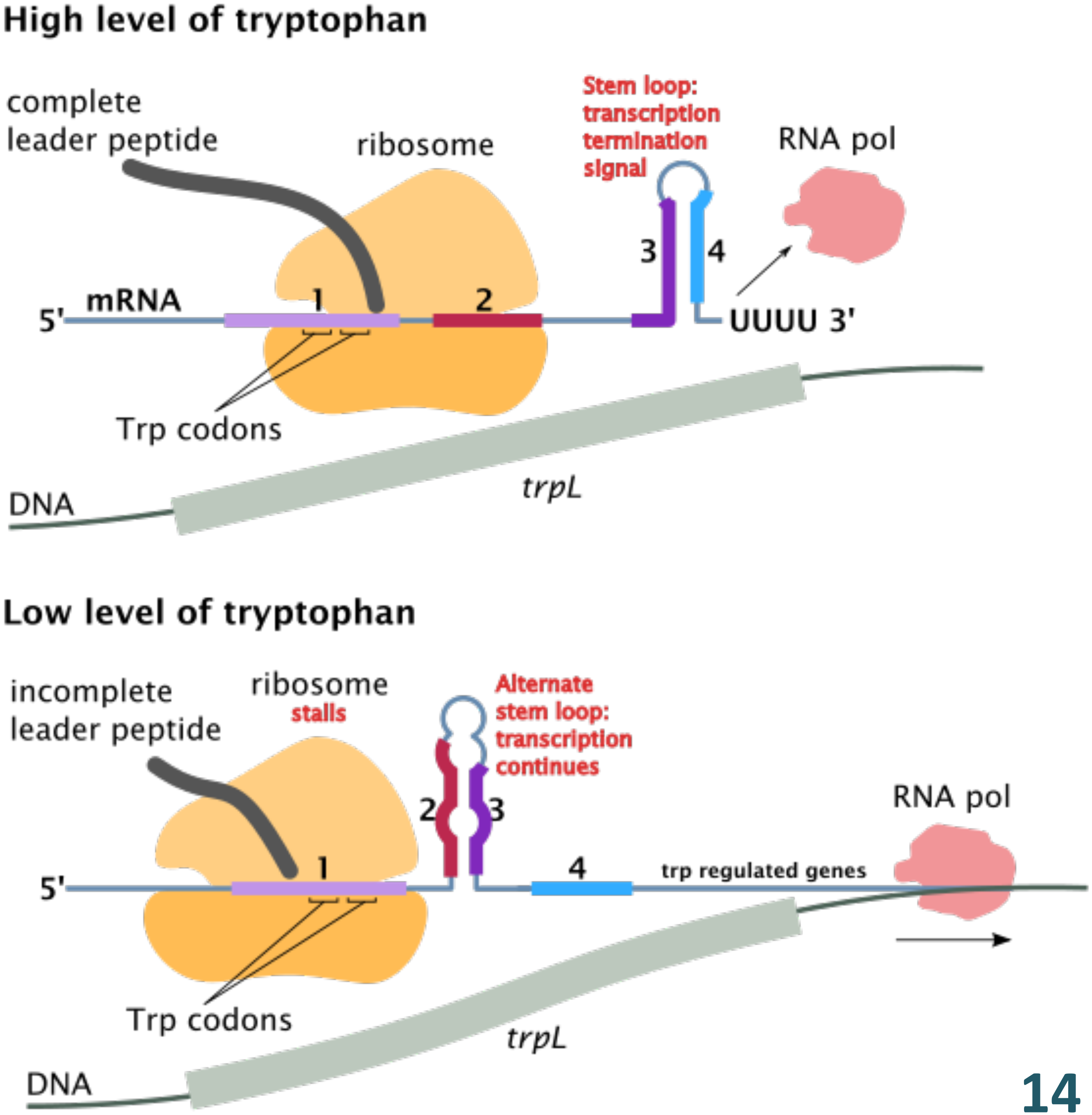


Figure 7-35, 36 *Molecular Biology of the Cell* (© Garland Science 2008)

trp operon

Attenuation



http://upload.wikimedia.org/wikipedia/commons/thumb/2/22/Trp_operon_attenuation.svg/588px-Trp_operon_attenuation.svg.png

Regulation of Gene expression in Eukaryotes

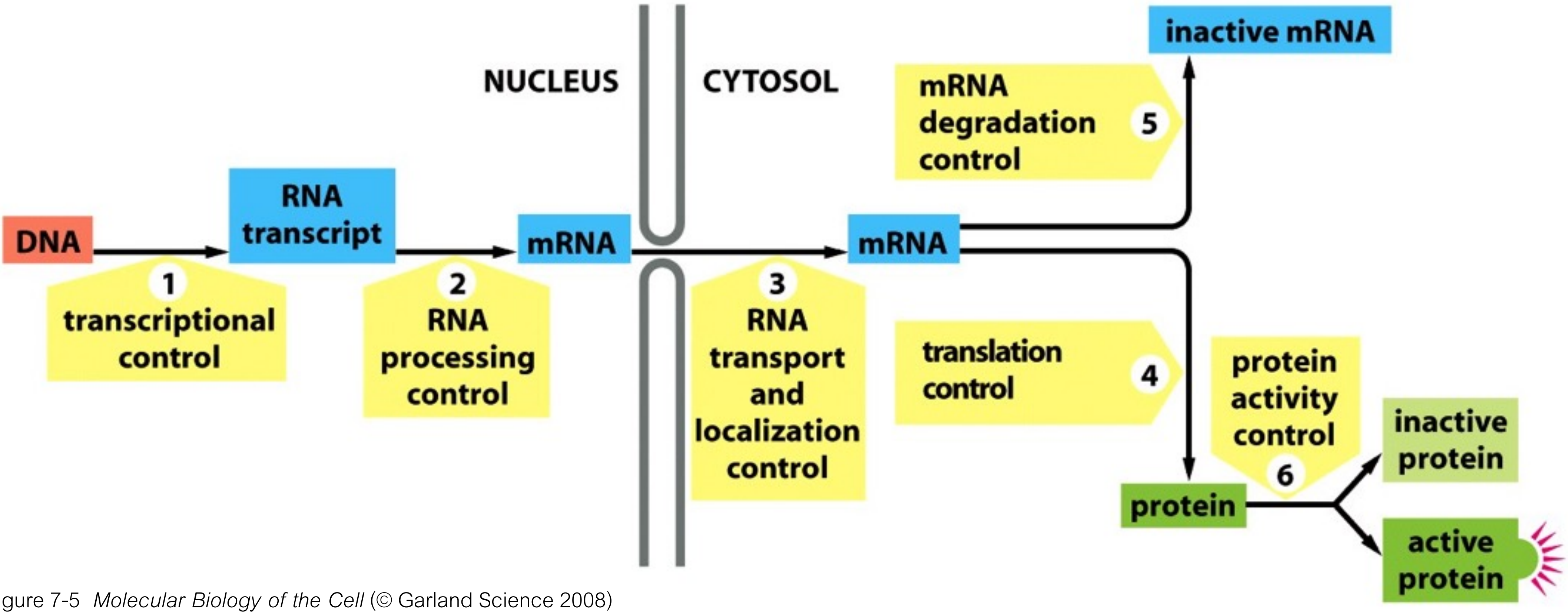
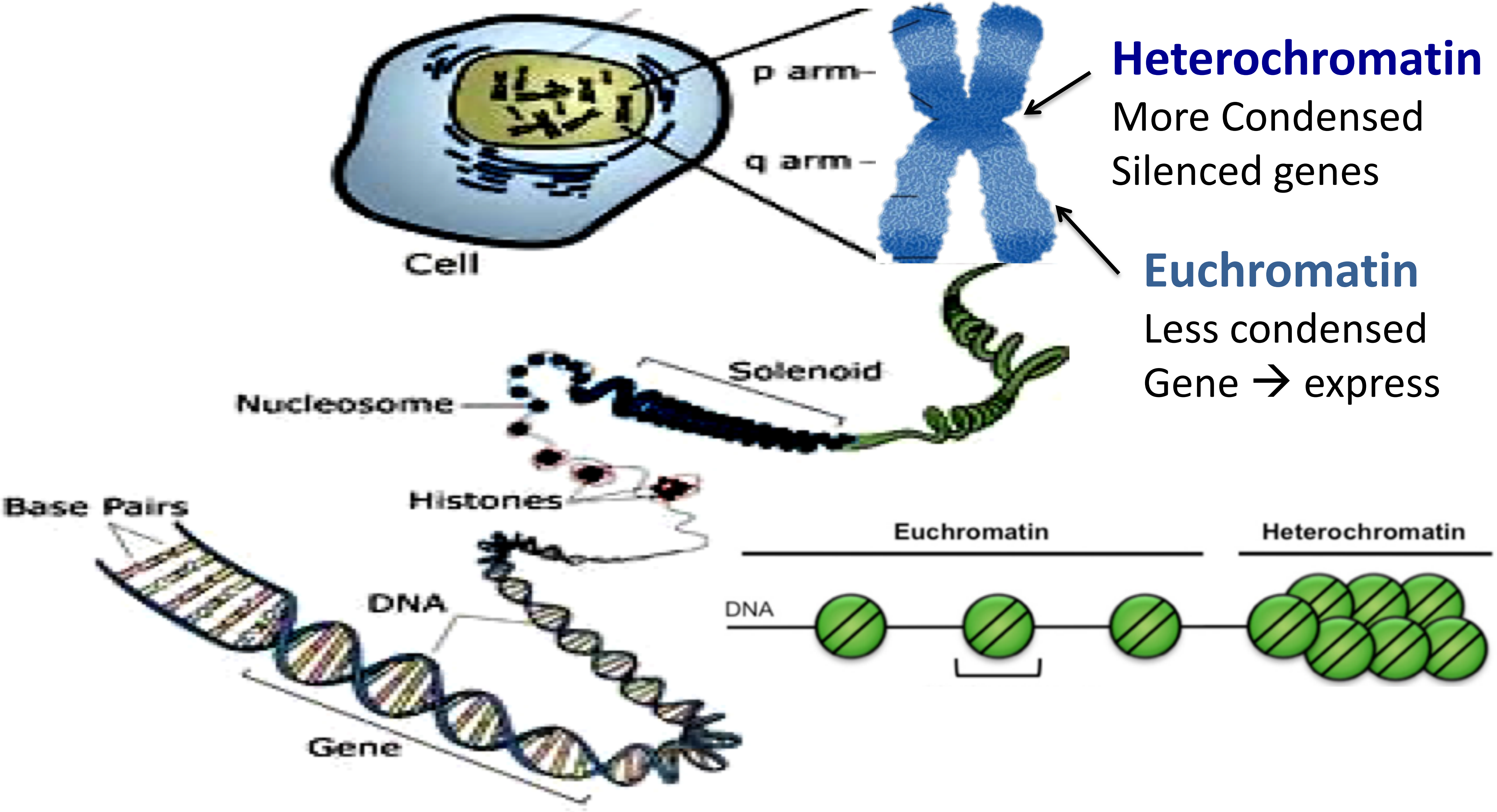


Figure 7-5 *Molecular Biology of the Cell* (© Garland Science 2008)

Chromosome structure of Eukaryotic cells is complicated.



Heterochromatin

More Condensed

Silenced genes

Euchromatin

Less condensed

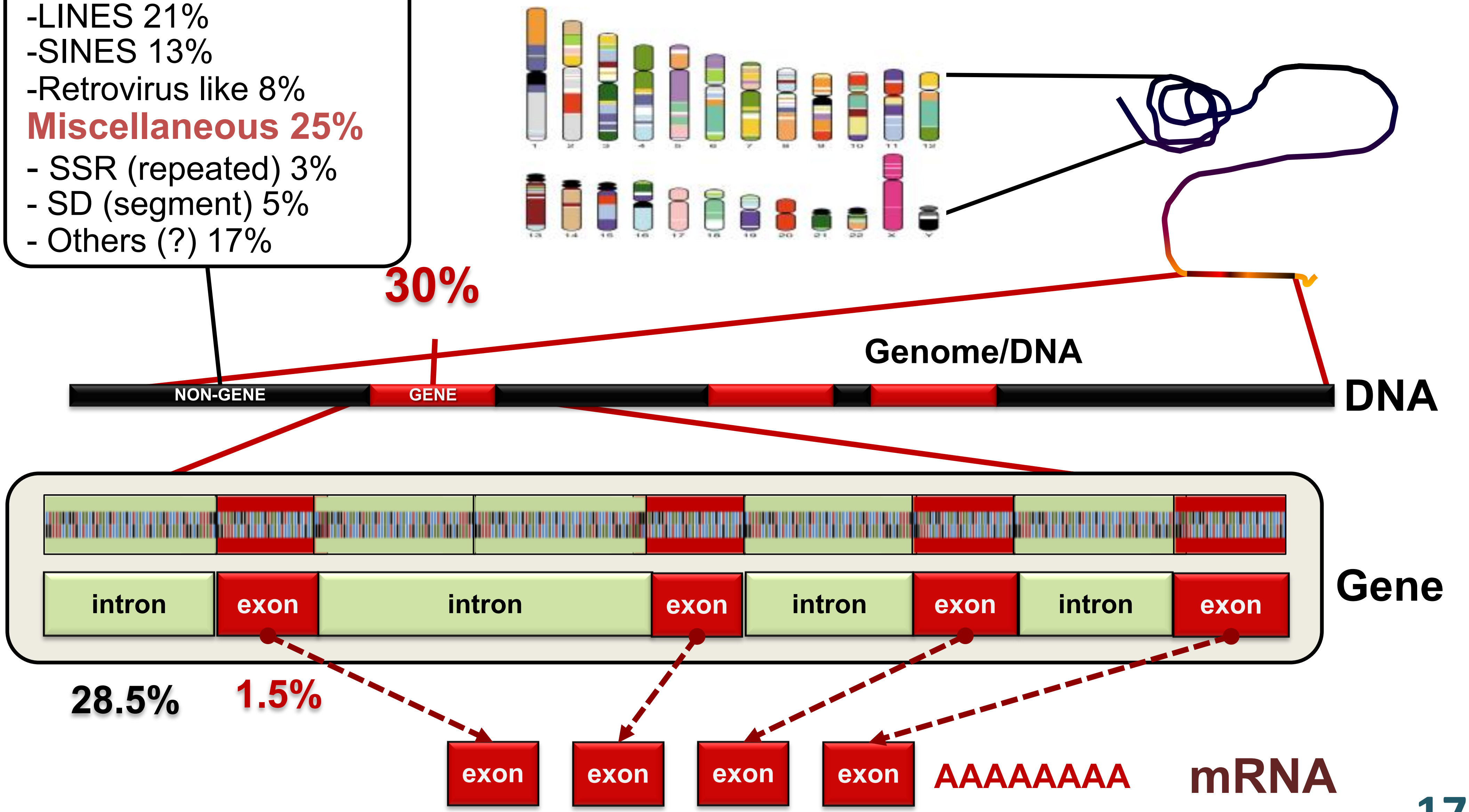
Gene → express

https://images2.clinicaltools.com/images/gene/chromosomebreakdown.jpg

From Chromosome to DNA to RNA

Human DNA = 3.2 X 10^9 bp → ~35,000 genes

Transposon 45%
-LINES 21%
-SINES 13%
-Retrovirus like 8%
Miscellaneous 25%
- SSR (repeated) 3%
- SD (segment) 5%
- Others (?) 17%



Epigenetics

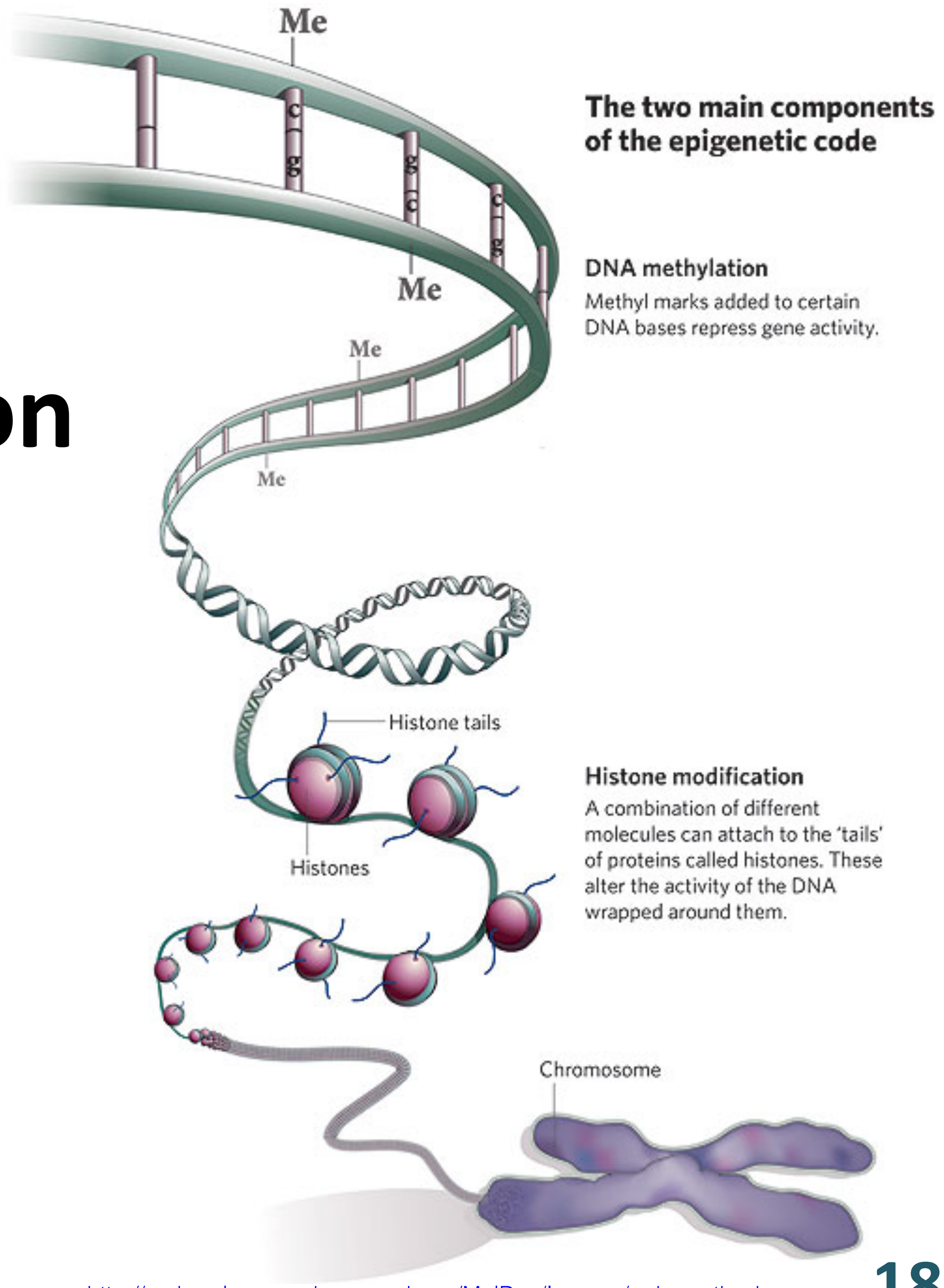
DNA-methylation

Histone modification

- Acetylation
- Methylation
- Ubiquitination
- Phosphorylation

Polycomb/Trithorax

Transcription control



The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

http://embryology.med.unsw.edu.au/MolDev/Images/epigenetics.jpg

DNA methylation

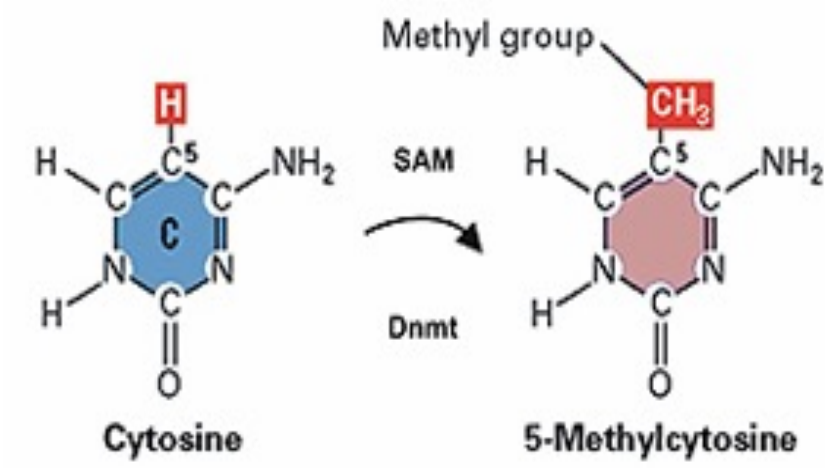
- Methyl group (CH3)
- Cytosine (C) at “**C**pG”
- Promoter : CpG island
- Gene suppression

- DNA methytransferases (DNMT)

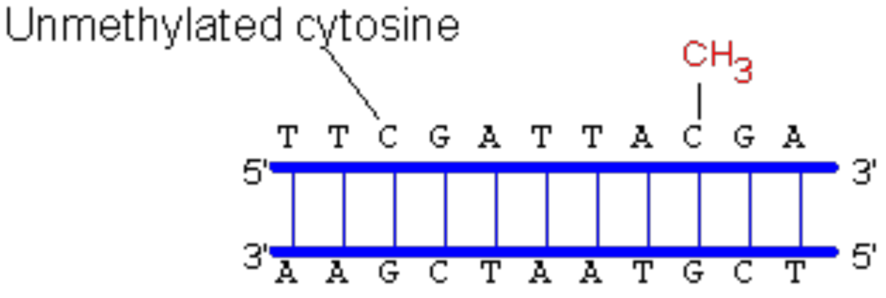
-DNMT1

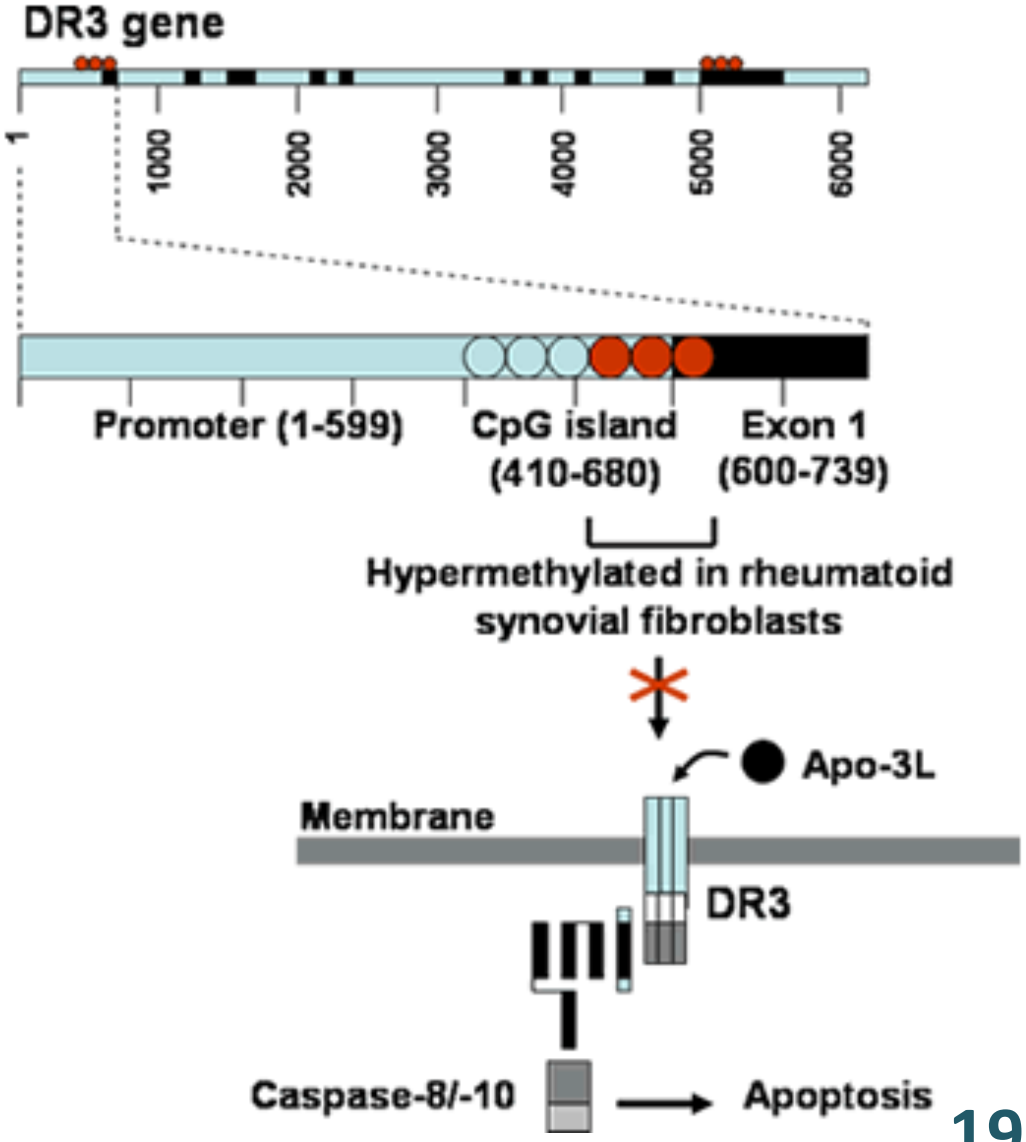
-DNMT2

-DNMT3

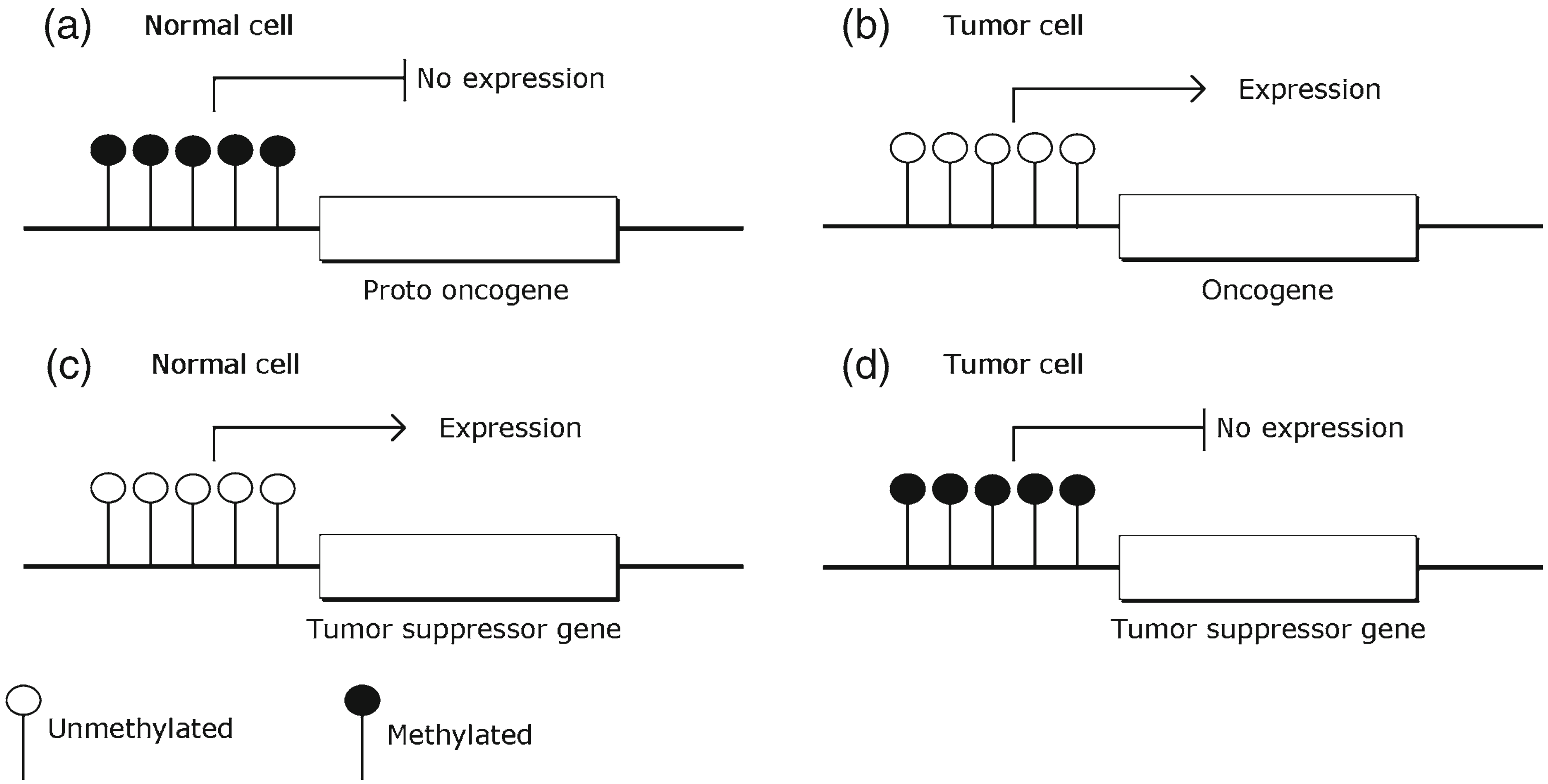


http://www.newsperuvian.com/wp-content/uploads/2011/05/dna-methylation-4.jpg



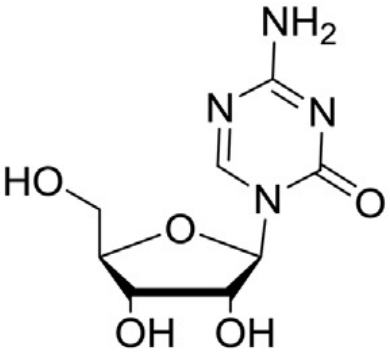


DNA methylation

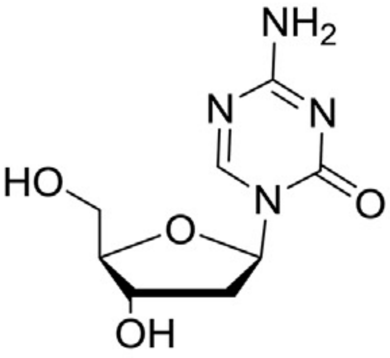


Aithal et al, J of Genetics, 2013

DNA methylation targeting for cancer treatment

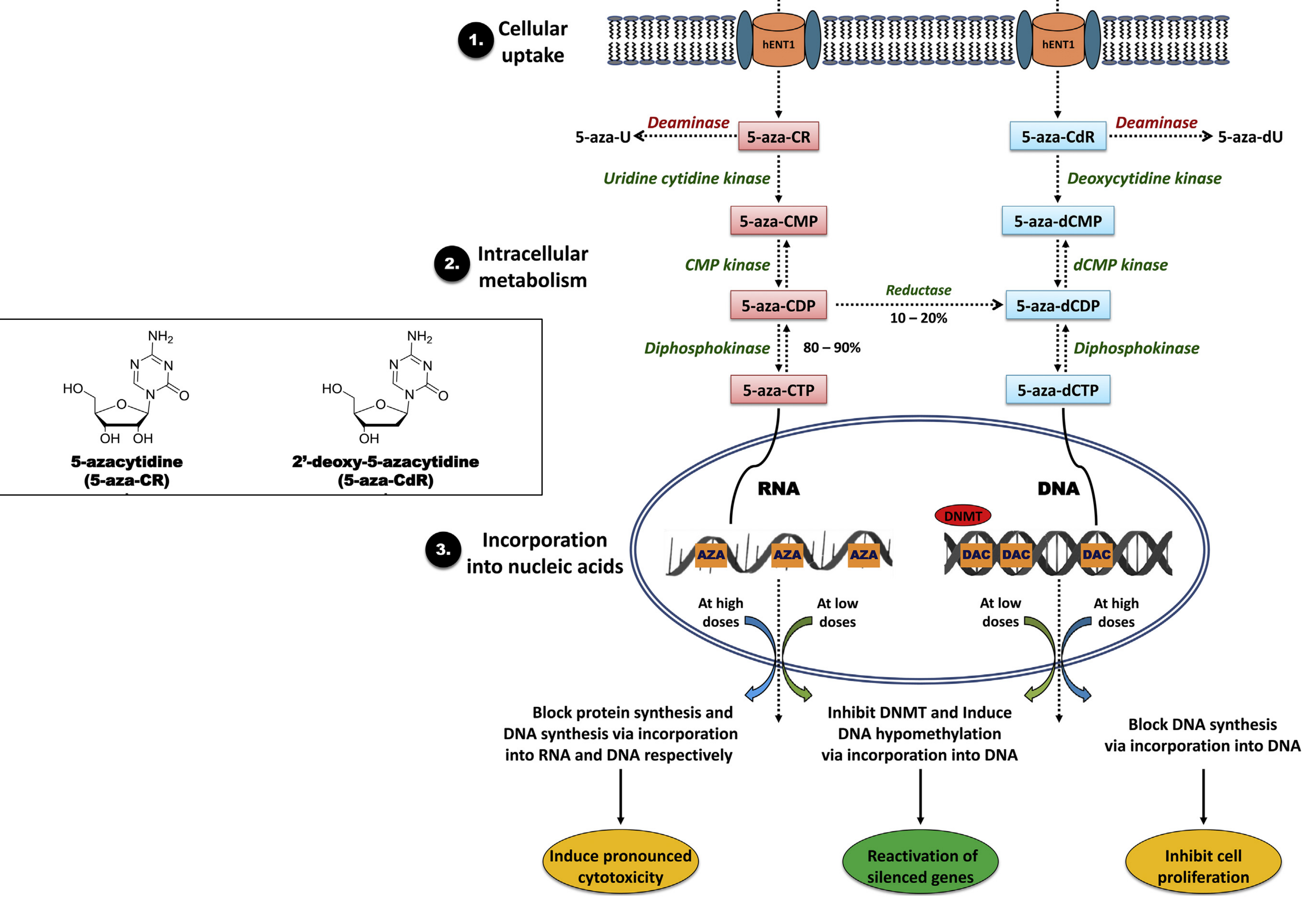


5-azacytidine
(5-aza-CR)

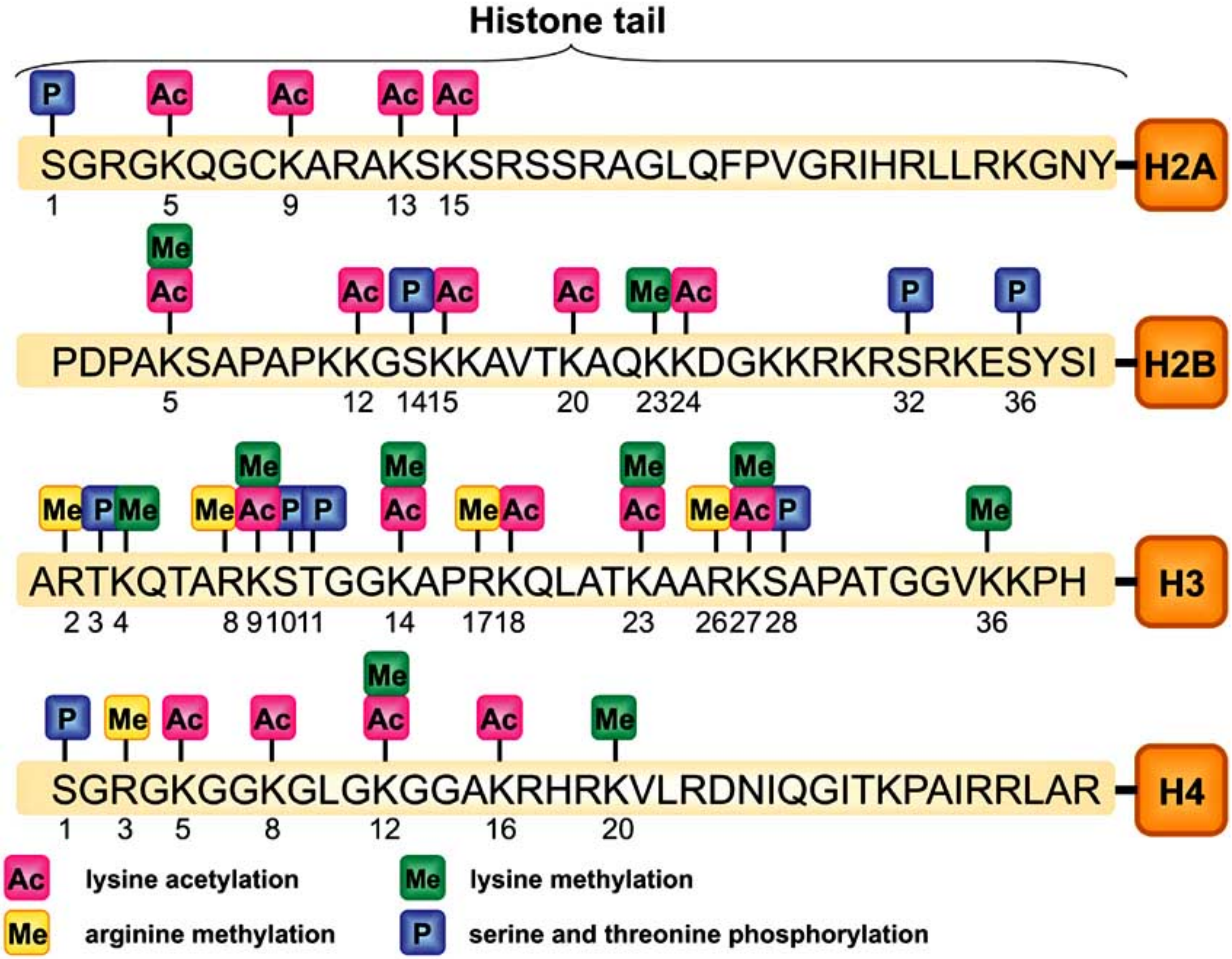


2’-deoxy-5-azacytidine
(5-aza-CdR)

1. Cellular uptake
2. Cellular metabolism
3. Incorporate into DNA/RNA

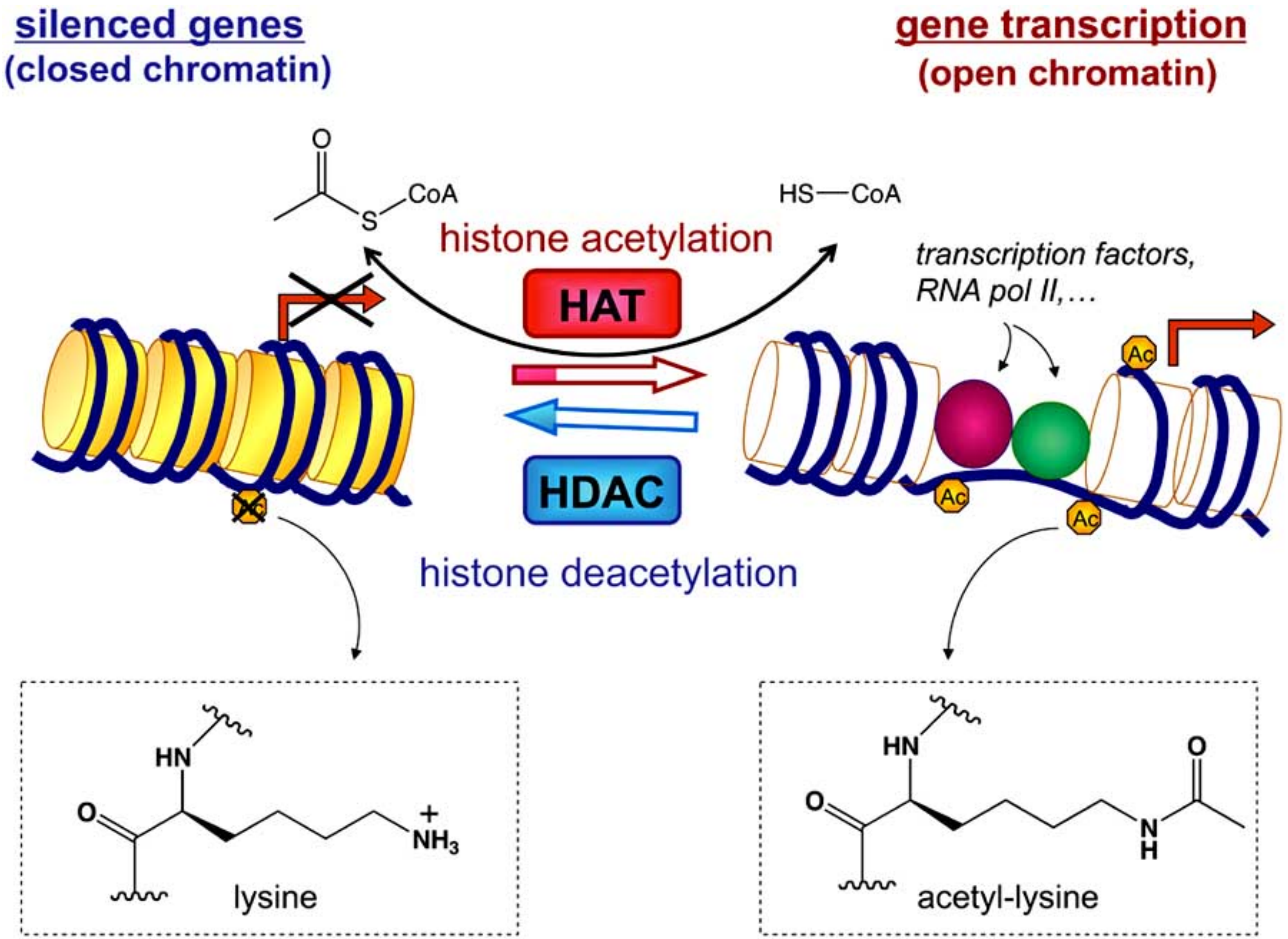

1.
Cellular uptake
hENT1
hENT1
Deaminase
5-aza-U
5-aza-CR
5-aza-CdR
Deaminase
5-aza-dU
Uridine cytidine kinase
Deoxycytidine kinase
5-aza-CMP
5-aza-dCMP
2.
Intracellular metabolism
CMP kinase
dCMP kinase
5-aza-CDP
Reductase
10 – 20%
5-aza-dCDP
Diphosphokinase
80 – 90%
Diphosphokinase
5-aza-CTP
5-aza-dCTP
NH2
N
N
HO
N
O
O
OH
OH
5-azacytidine
(5-aza-CR)
NH2
N
N
HO
N
O
O
OH
2'-deoxy-5-azacytidine
(5-aza-CdR)
RNA
DNA
DNMT
3.
Incorporation into nucleic acids
AZA
AZA
AZA
DAC
DAC
DAC
At high doses
At low doses
At low doses
At high doses
Block protein synthesis and DNA synthesis via incorporation into RNA and DNA respectively
Inhibit DNMT and Induce DNA hypomethylation via incorporation into DNA
Block DNA synthesis via incorporation into DNA
Induce pronounced cytotoxicity
Reactivation of silenced genes
Inhibit cell proliferation

Histone modification

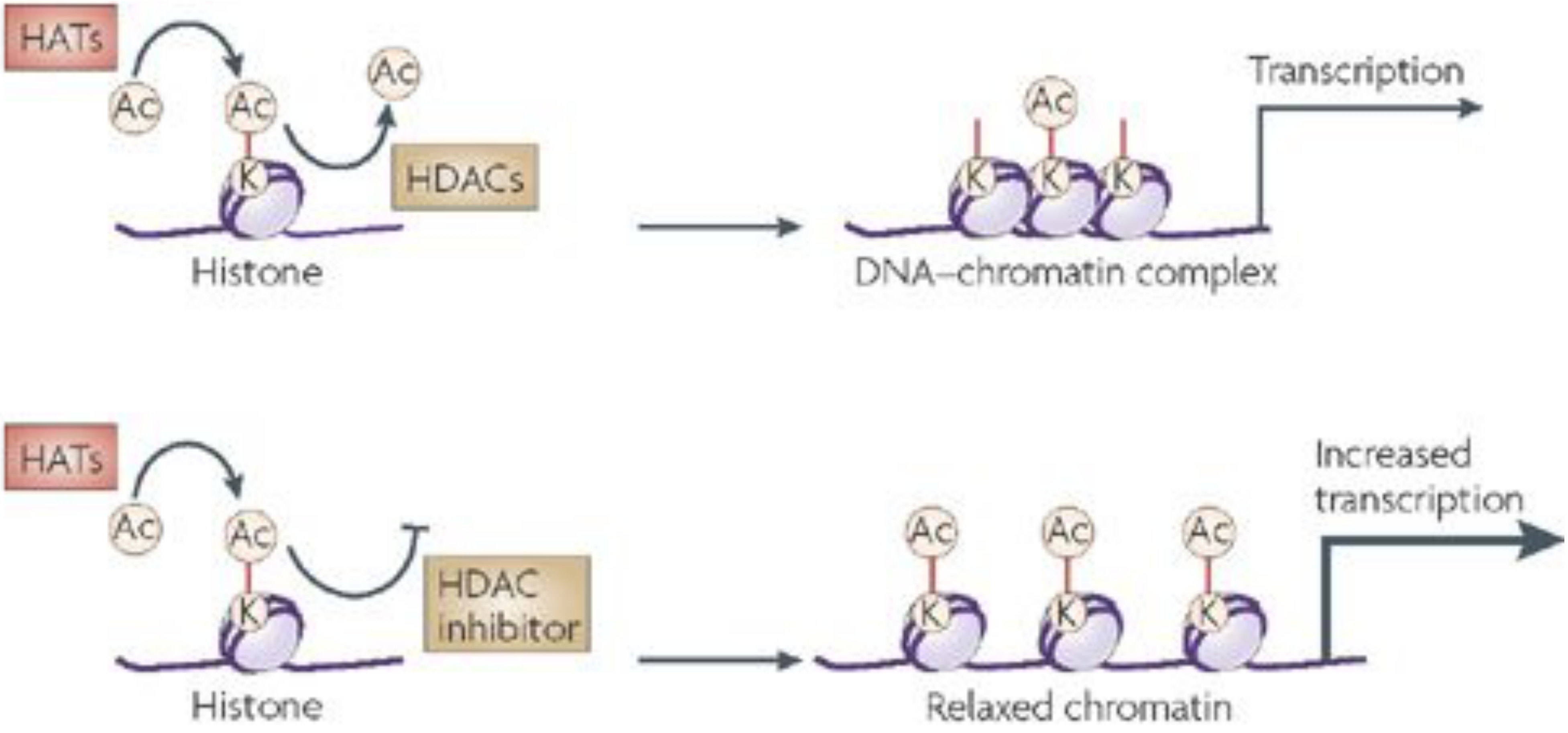


Folmer et al, *Current Nutrition & Food Science*, 2010

Histone acetylation and deacetylation



HDAC targeting for cancer treatment



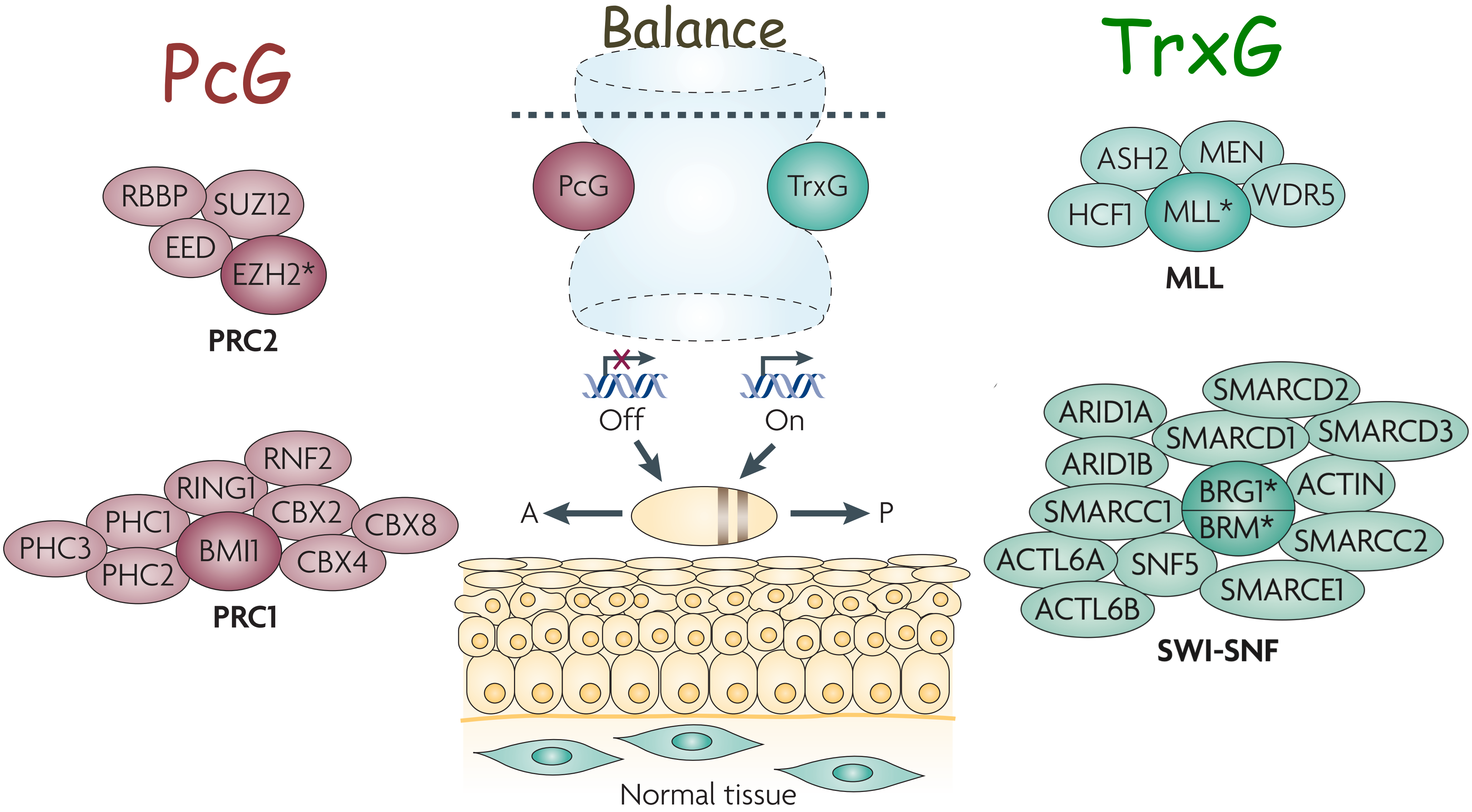
Nature Reviews | Drug Discovery

Polycomb (PcG) & Trithorax (trxG)

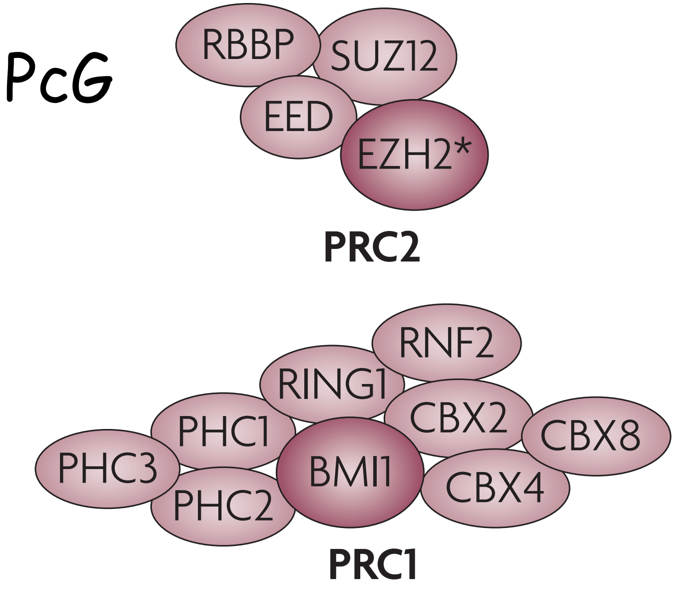
	Drosophila melanogaster	Human
PcG complexes		
PhoRC	dSfmbt	?
	Pho	?
PRC2	E(z)	EZH2
	Esc	EED
	Su(z)12	SUZ12
	N55	RpAp48
		RpAp46
PRC1	dRing	RING1A
	Pc	HPC1-3
	Ph	HPH1-3
	Psc	BMI1
	Scm	SCMH1-2
	TBP-associated factors	

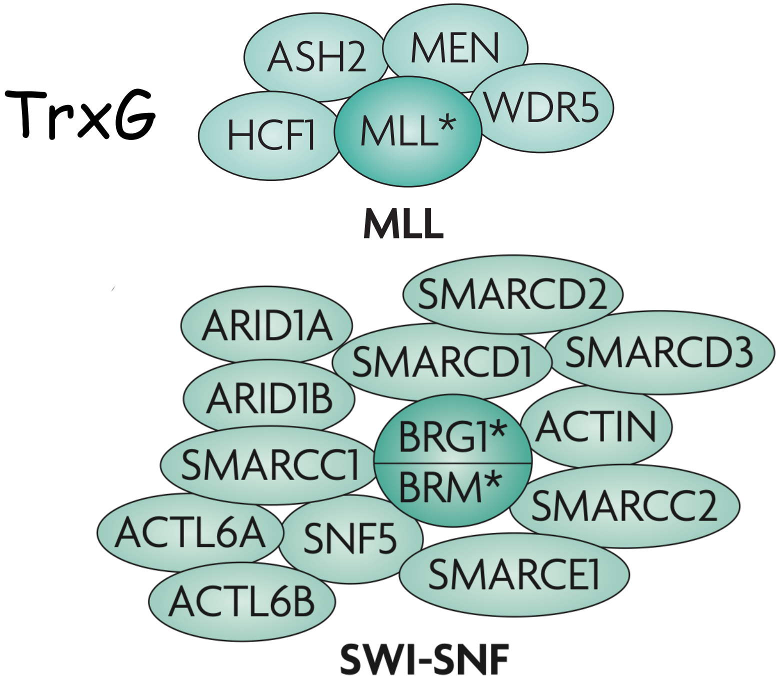
trxG complexes		
SWI/SNF	Brm	BRM
	Osa	BAF250
	Moira	BAF170
	Snr1	BAF47
NURF	Iswi	SNF2L
	N38	?
	N301	BPTF
	N55	RpAp46 RpAp48
TAC1	Trx[a]	
	dCBP	
	Sbf1	
Ash1	Ash1	
	dCBP	
MLL1-3		MLL1-3[a]
		WDR5
		ASH2L
		RbBP5
		CFP1

Polycomb (PcG) & Trithorax (trxG)



PcG & TrxG complexes regulate chromatin remodeling through histone modifications.

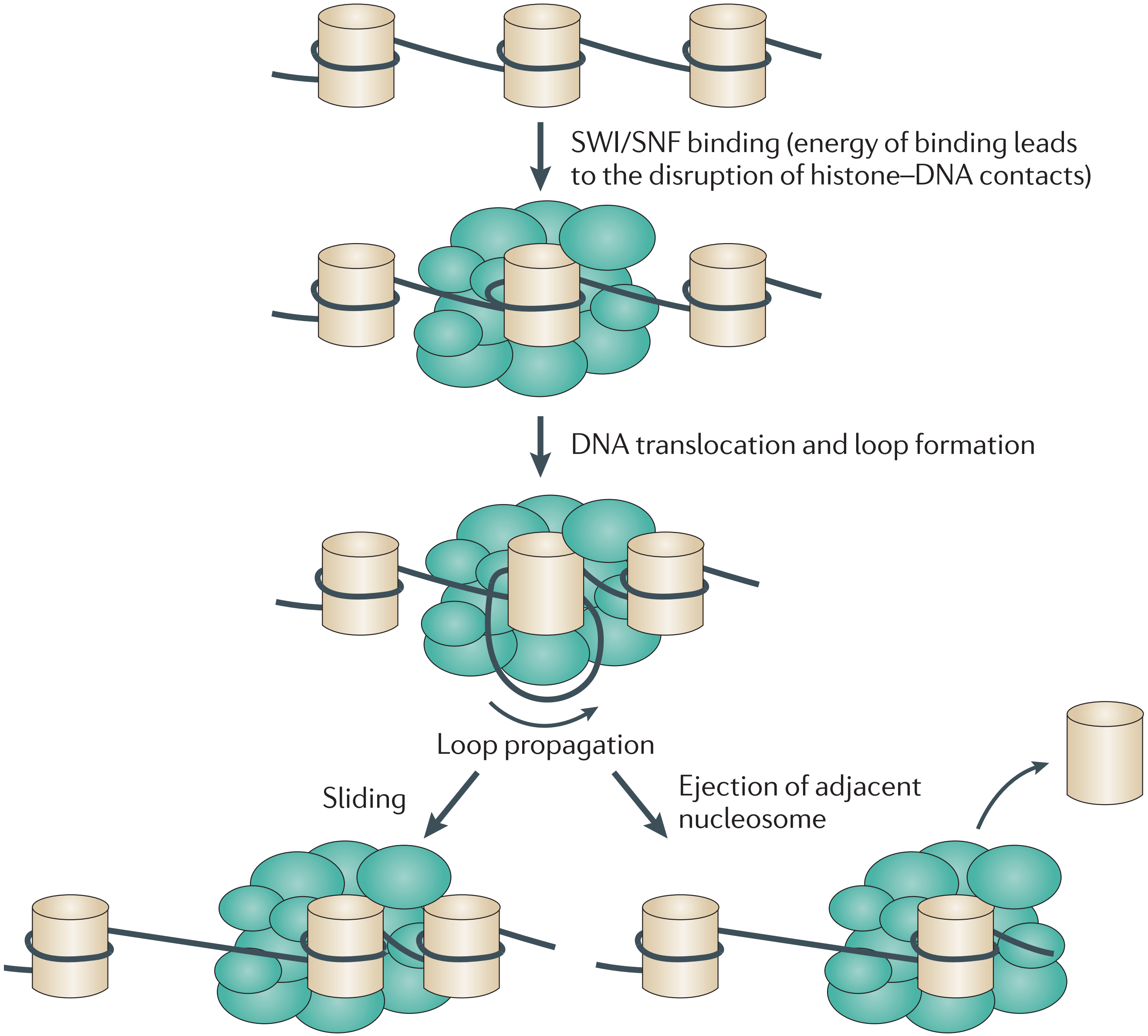




Polycomb group (PcG: PRC2 and PRC1) and trithorax (TrxG: mixed-lineage leukaemia (MLL) complex **directly methylate histone tails through the histone methyltransferase (HMT) activity of the subunit***. SWI–SNF, ATP-dependent chromatin remodeling complex, mobilize nucleosomes through the **ATPase activity of the subunit***.

ACTL, actin-like; ARID1, AT-rich interactive domain; ASH2, absent small or homeotic 2; CBX, chromobox homologue; DNMT, DNA methyltransferase; EED, embryonic ectoderm development; EZH2, enhancer of zeste homologue 2; HCF1, host cell factor C1; PHC, polyhomeotic homologue; PHD, plant homeodomain; RBBP, retinoblastoma binding protein; RNF, ring finger protein; SUZ12, suppressor of zeste homologue 12; WDR5, WD repeat domain 5.

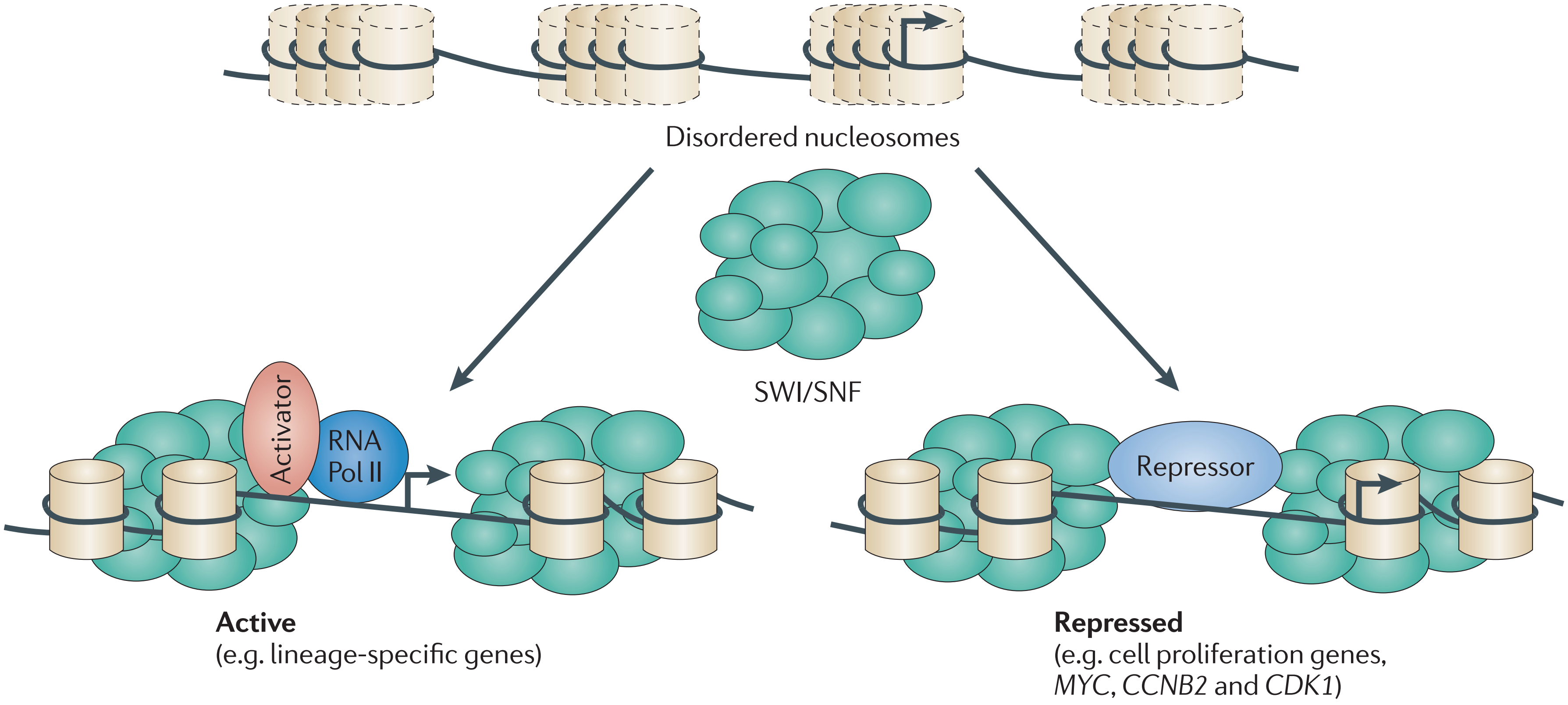
How BAF complex remodel of chromatin?



The steps of remodeling include

- SWI/SNF binding,
- Disruption of histone–DNA contacts,
- Creation of a loop of DNA that propagates around the nucleosome in a wave-like manner
- Repositioning of DNA with respect to the nucleosome (sliding). Sliding may also lead to the ejection of an adjacent nucleosome.

SWI/SNF (BAF) complexes facilitate factor binding.



At active genes that are rich in SWI/SNF binding the transcription start site is flanked by precisely positioned nucleosomes, thus providing unobstructed access to a nucleosome-depleted region that contains transcription factor binding sites. SWI/SNF complexes also contribute to the dynamic silencing of targets that are required for lineage-specific differentiation and that facilitate the binding of repressors

Eukaryotic cells require several factors to initiate transcription.

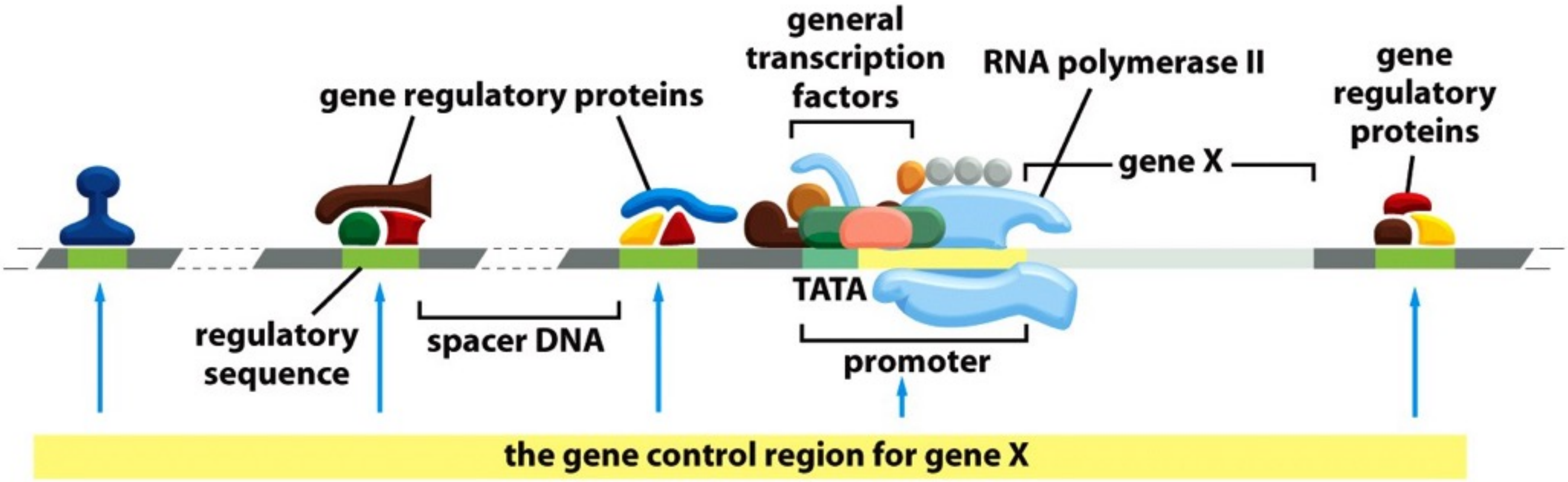


Figure 7-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Eukaryotic cells require several factors to initiate transcription.

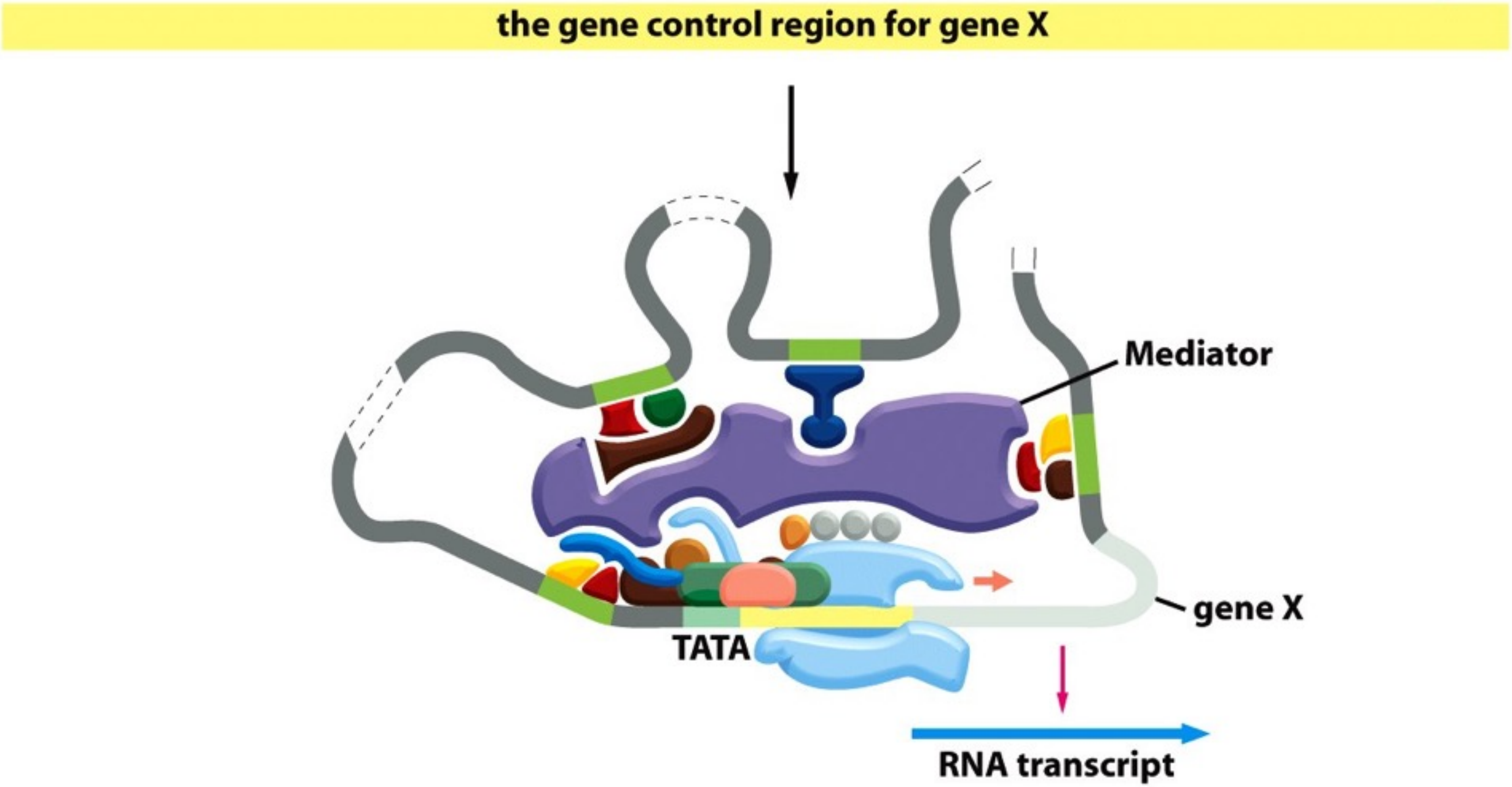


Figure 7-44 *Molecular Biology of the Cell* (© Garland Science 2008)

Post-transcriptional regulation

mRNA processing, transportation, and degradation

5’ capping

RNA splicing

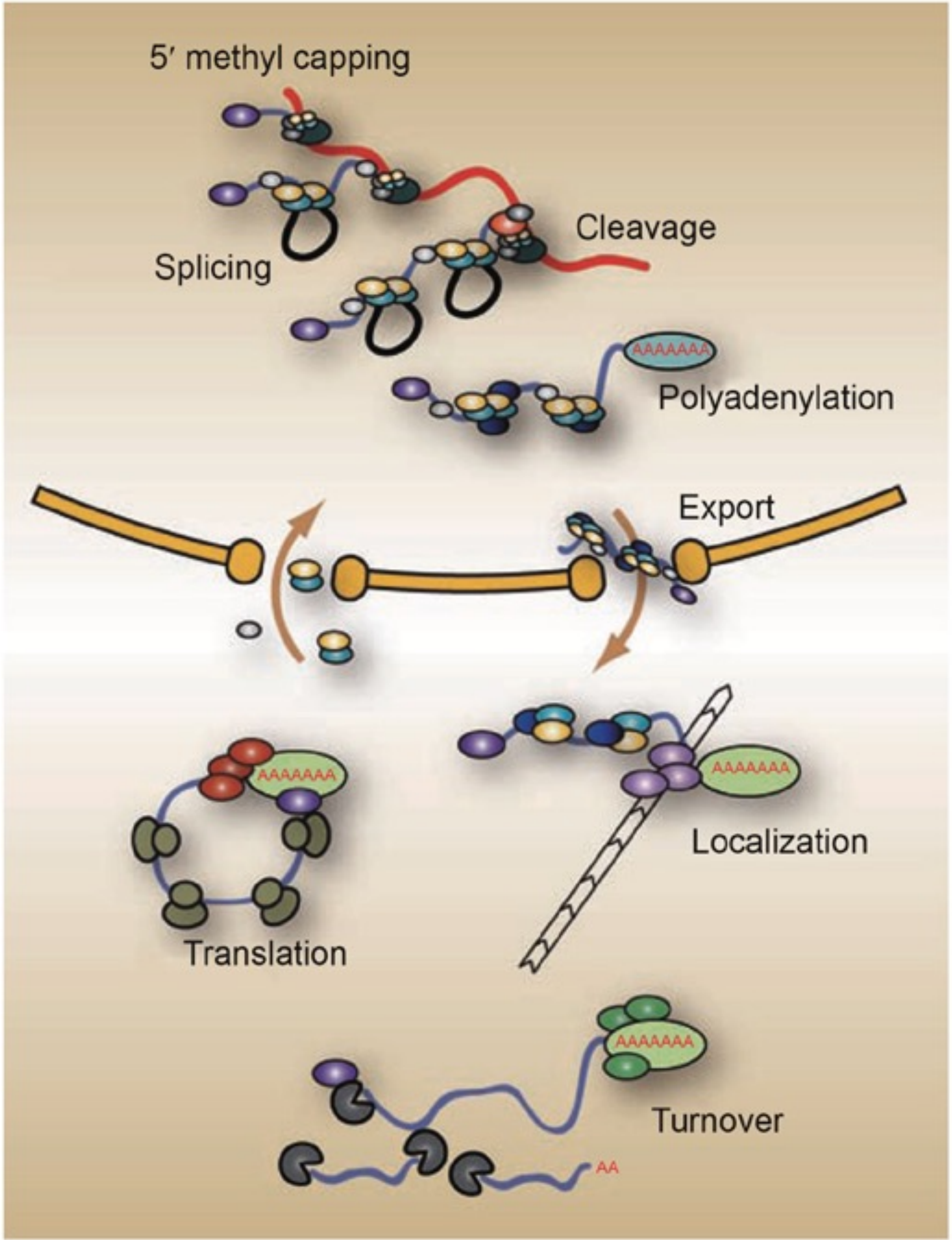
Polyadenylation

Export

Localization

Translation

Degradation



http://www.nature.com/cr/journal/v17/n7/images/cr200754f1.jpg