BI 8 LECTURE 9

COMPONENTS OF TRANSCRIPTIONAL REGULATORY MACHINES… & BACTERIAL MODES OF REGULATION, PART 1

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2 February 2016

Reading: Alberts 6th edition Ch. 7, pp. 369-383
Optional: on reserve, Watson et al, Ch. 16, 547-577

Decision to express most genes depends on combinations of circumstances

- Cell type okay?
- Nutritional status okay?
- Timing okay?
- Signals from environment induce or repress?

These decisions are made through the mobilization of different sequence-specific transcription factors in right combinations

Some crucial tools

- Define cis-regulatory regions ***functionally***
  - Reporter constructs (Chloramphenicol acetyltransferase, luciferase, GFP, β-galactosidase [lacZ]) with/without candidate cis-reg region
  - Mutagenesis methods
  - Transfection of reporter construct into cells that can exert regulation
  - Quick quantitative readouts in transfection systems

- Define source of transcription factor proteins
  - Biological specificity (cell type comparison)
  - Physiological specificity (conditional activation)

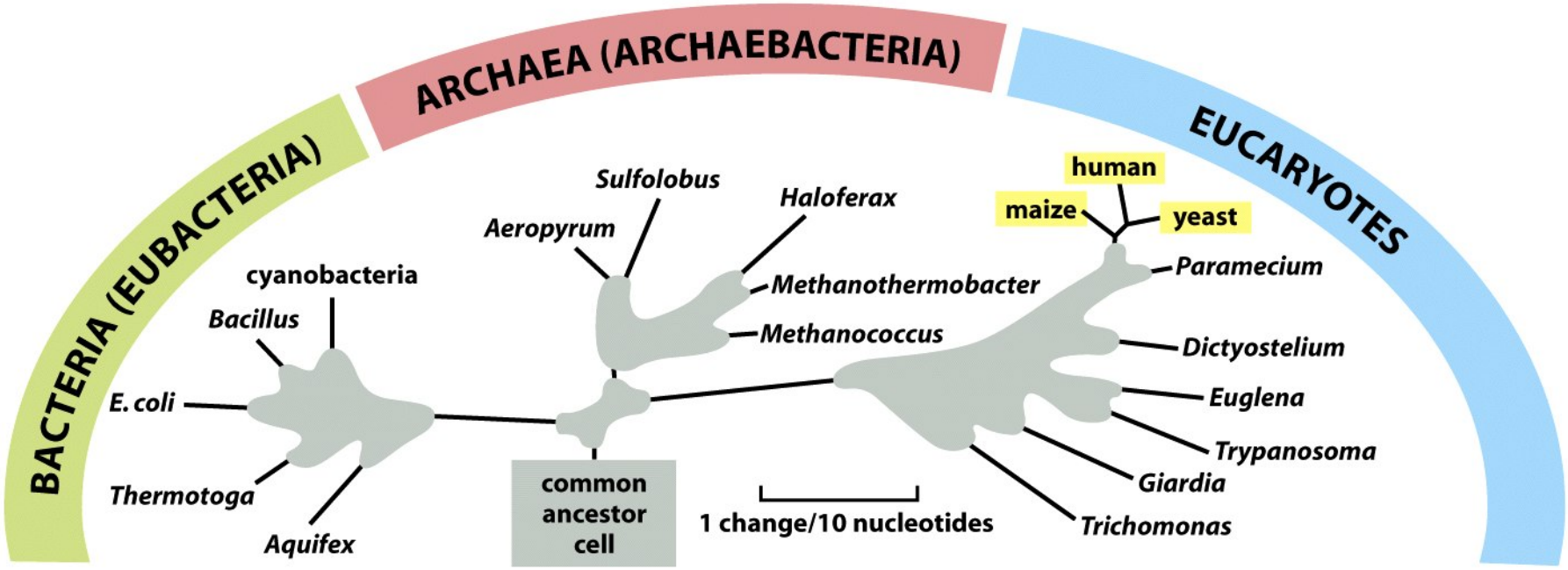
- ***Role of genetics to compare transcription factor positive and transcription factor negative versions of same cells***

Simple cases from E. coli provided intellectual framework for whole field

- Lac operon – controlling a group of enzymes needed to metabolize lactose
  - Use as carbon source when lactose is present
  - Don't waste energy to make these when a better sugar is present, like glucose

- Lambda phage repression/ activation
  - Two modes of existence: lytic and lysogenic
  - Lytic: use up cell's resources making more phage, destroy cell
  - Lysogenic: stay quietly incorporated into host bacterial DNA and let host do the replication
  - Switch from lysogenic to lytic mode under stress: "I'm splitting"

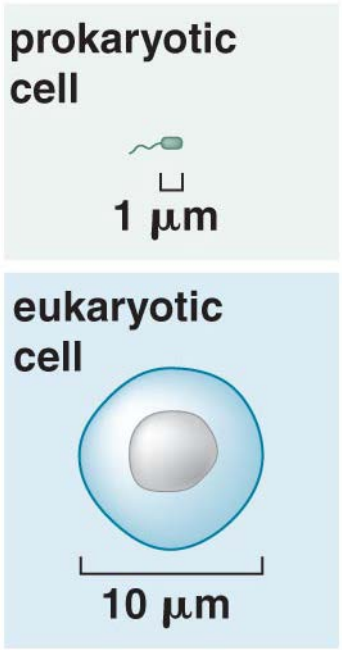
Huge divergence between the microbial and eukaryotic worlds…



And yet our own mitochondria and plant chloroplasts have their own organelle genomes, which are *prokaryotic* genomes within our cells

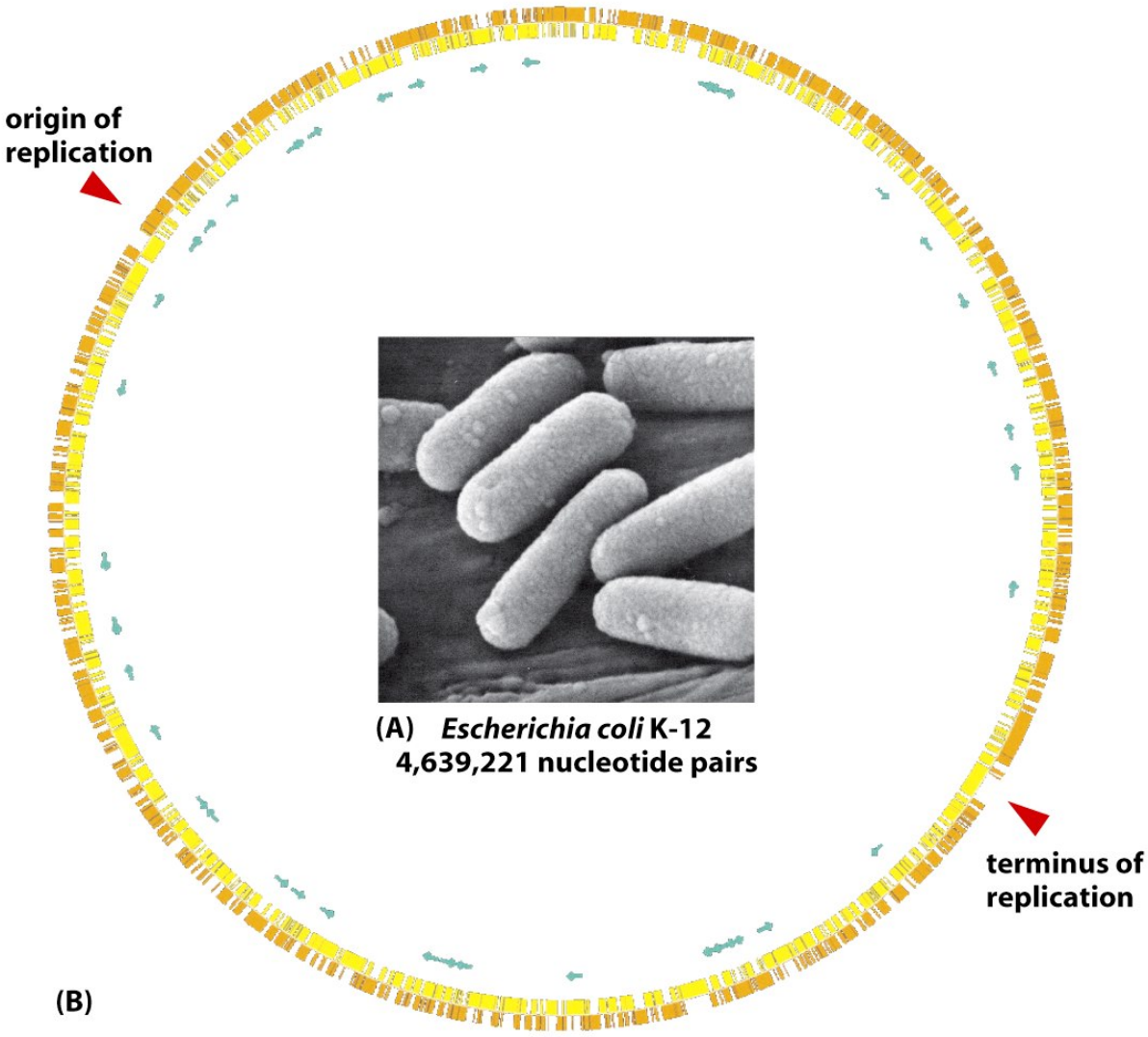
Figure 1-21 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

The bacterial way: streamline everything



From a bacterially oriented perspective, higher eukaryotic biology is full of “junk”

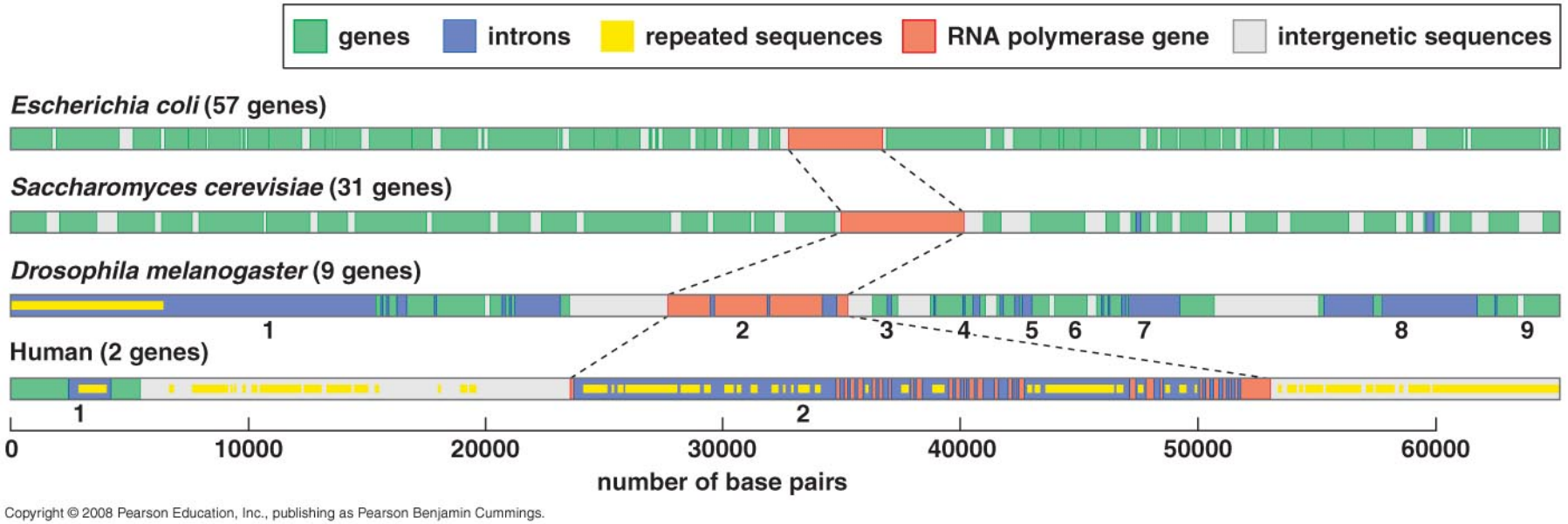
A single origin of replication, one replication initiation event per “cell cycle” (and a simple cell cycle)



**Consequently, selective pressure can favor cell that can replicate its genome faster than its neighbors… because the replication process has a shorter distance to cover

Figure 1-29 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

E. coli have a protein-coding buff's dream of a perfect genome…



No "waste", minimal intergenic regions, a genome of almost "all exon"

(also minimal regulatory requirements: just one cell type and little need for one cell to coordinate activity with another)

No wonder bacterial geneticists were offended by eukaryotic "junk DNA"

And for prokaryotic viruses (and some eukaryotic ones), you may need to double-use genetic information

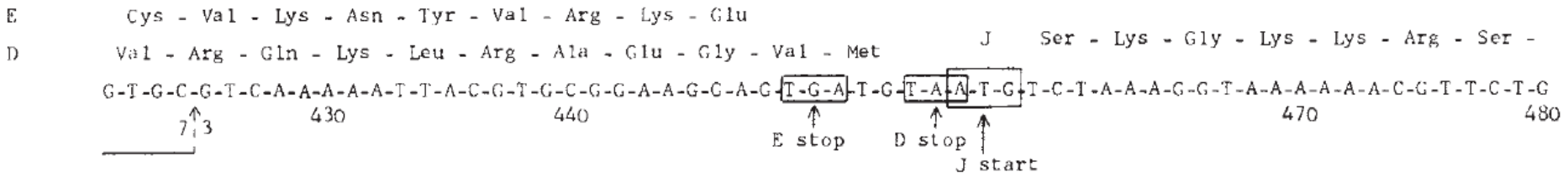
Barrell, Air & Hutchison: overlapping coding regions in phage ϕx174

Nature Vol. 264 November 4 1976

Table 1 φX174 coding capacity

Gene	Protein molecular weight from SDS gels*	Number of nucleotides: From protein molecular weight estimates	Number of nucleotides: From nucleotide sequence	Protein molecular weight from sequence information†
A	62,000	1,701		
(A′)	35,000	(960)	⩾1,851	67,400
B	19,000–25,000	⩾522		
C	7,000	192		
D	14,500	399	456	16,811‡
E	10,000–17,500	⩾273	(273)	9,940
J	5,000	138	114	4,097‡
F	48,000	1,317	1,287	46,700
G	19,000	522	525	19,053‡
H	37,000	1,014	981	35,500
Total Length of φX174 DNA		⩾6,078 ~5,370	5,374§	

Too little DNA to code for all proteins!



mRNAs in prokaryotes and eukaryotes are structurally different

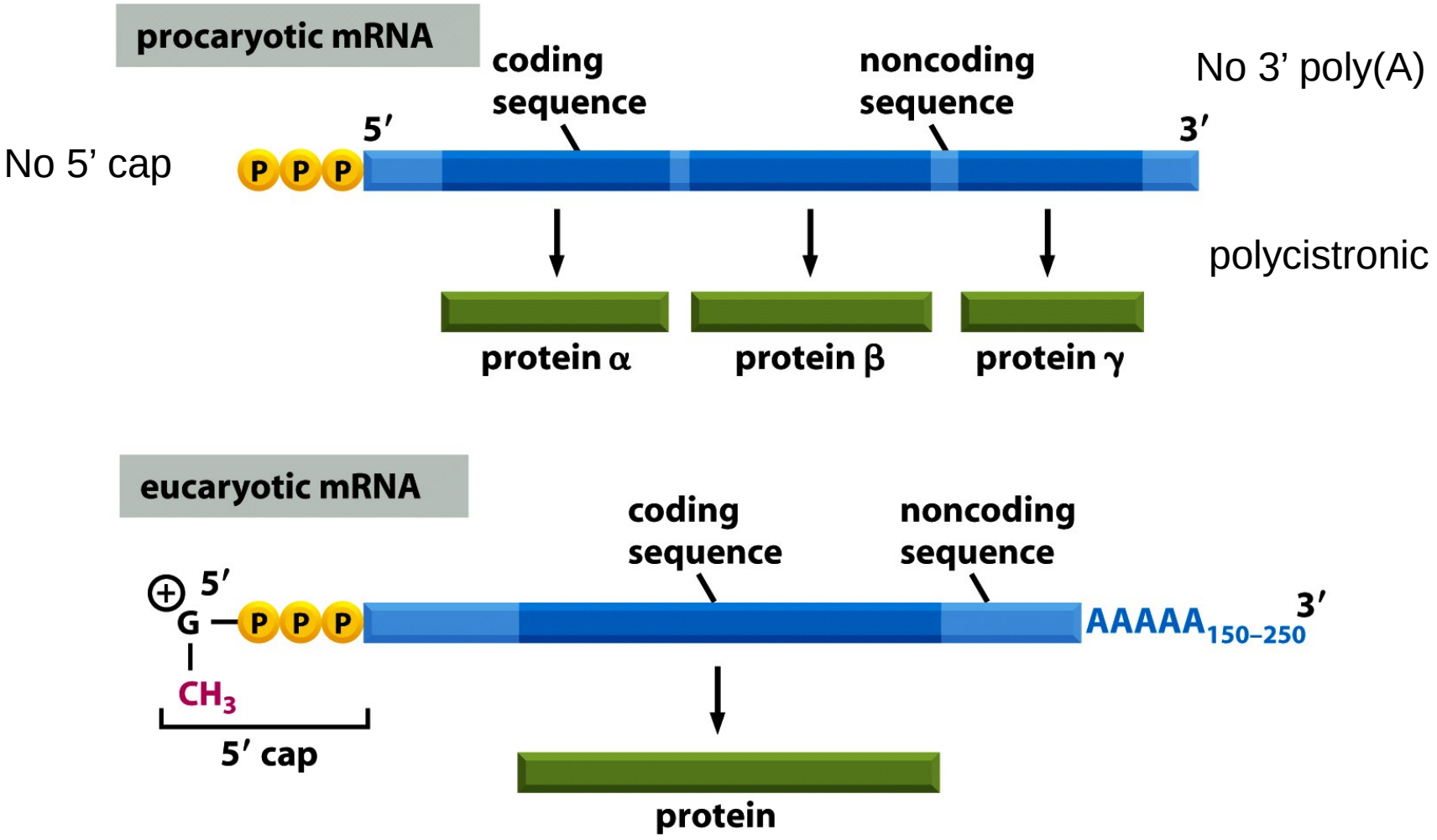
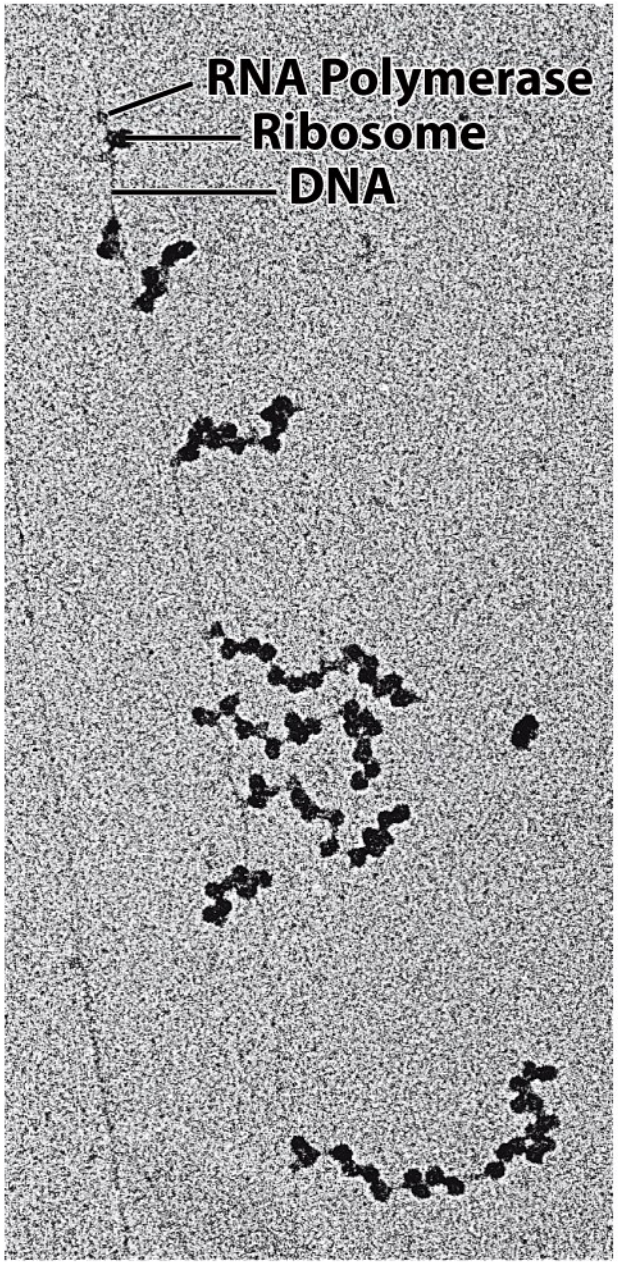


Figure 6-22a *Molecular Biology of the Cell* (© Garland Science 2008)



No physical separation between transcription compartment and translation compartment: translation can (& does) start on nascent RNA transcripts

Obviously cannot require intact 3' end for translational QC, either!

Overview of transcription of bacterial mRNA: by default, one promoter and one polymerase-loading factor (σ)

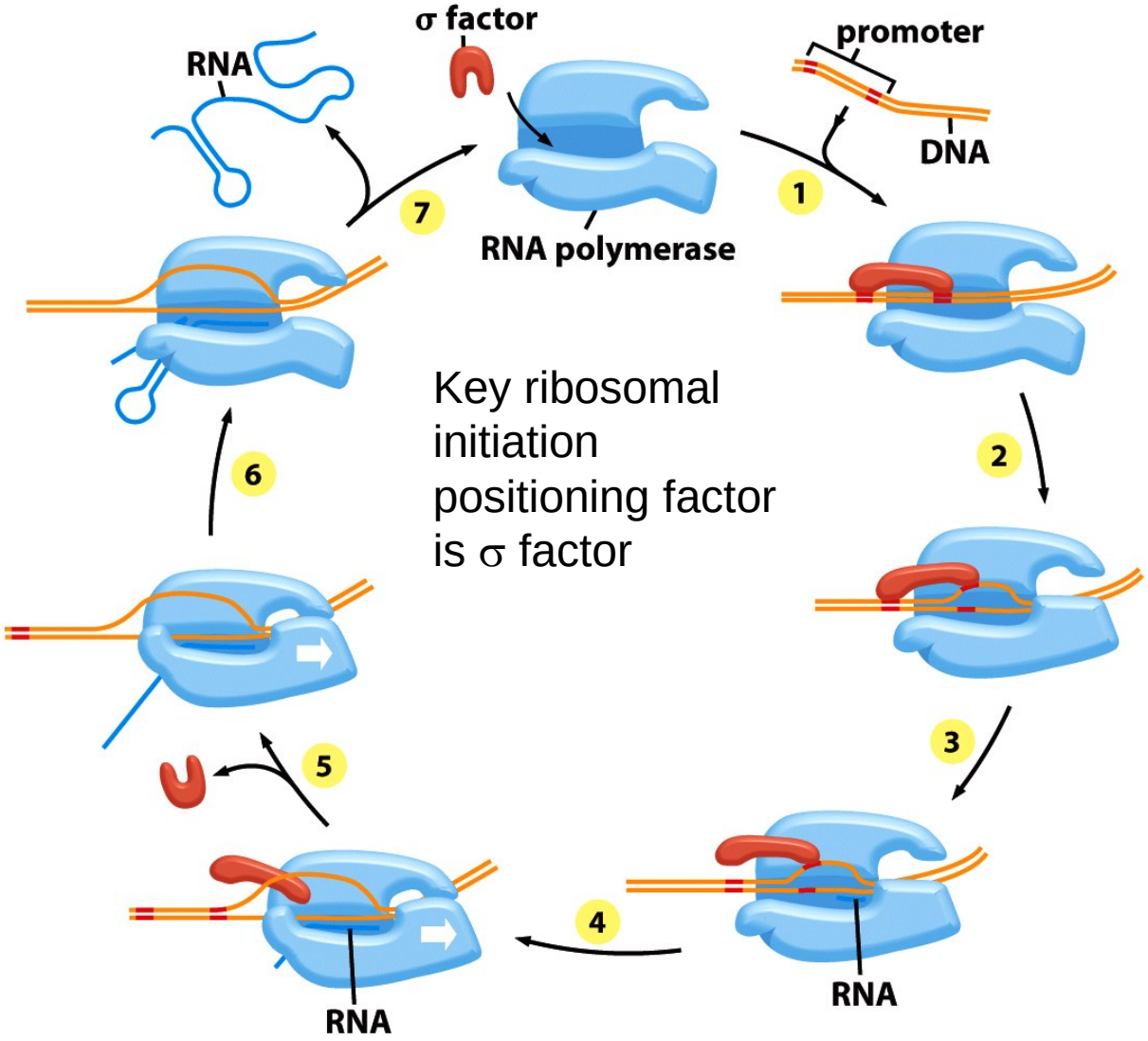
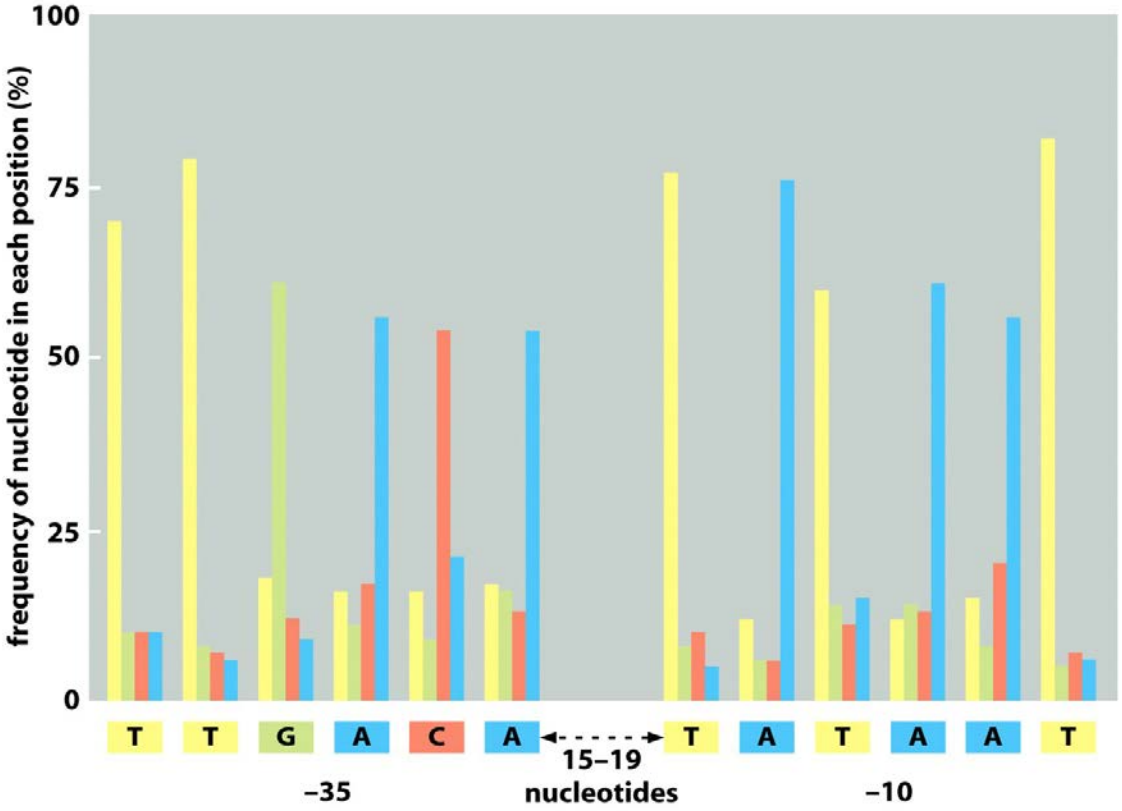


Figure 6-11 *Molecular Biology of the Cell* (© Garland Science 2008)



Most common σ factor (σ^{70}) binds to a simple pair of promoter-proximal elements

(Like eukaryotic TATA box but at -10 instead of -30)

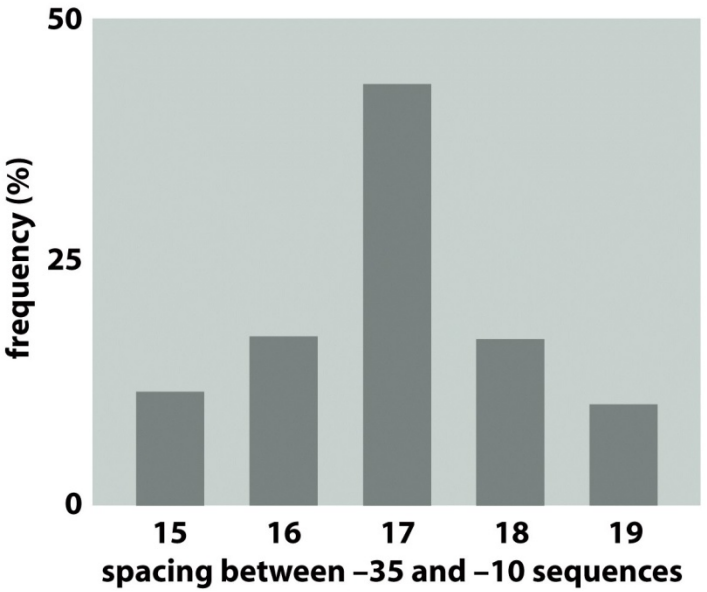
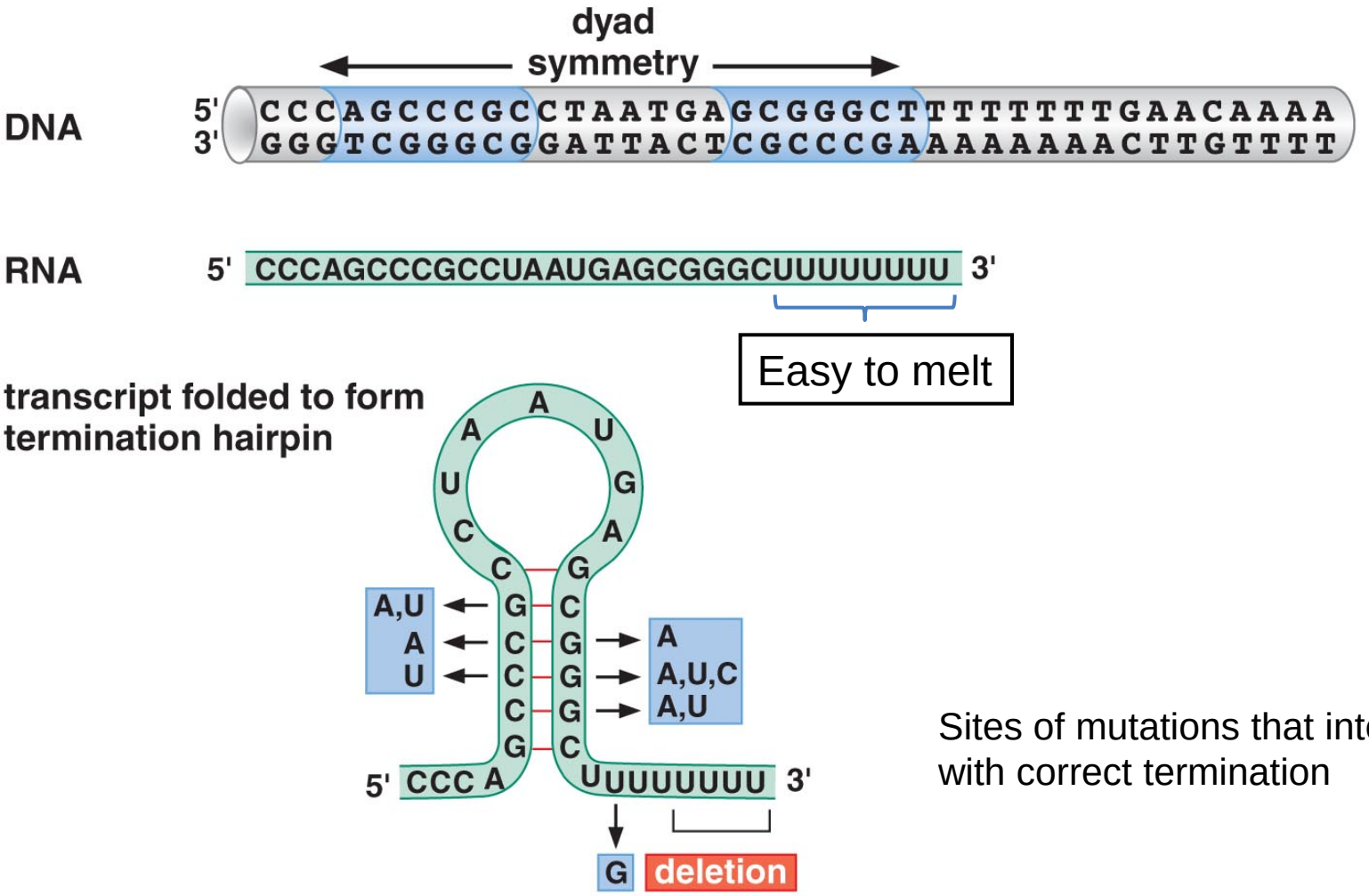


Figure 6-12 *Molecular Biology of the Cell* (© Garland Science 2008)

Bacterial transcription termination: a distinct signal

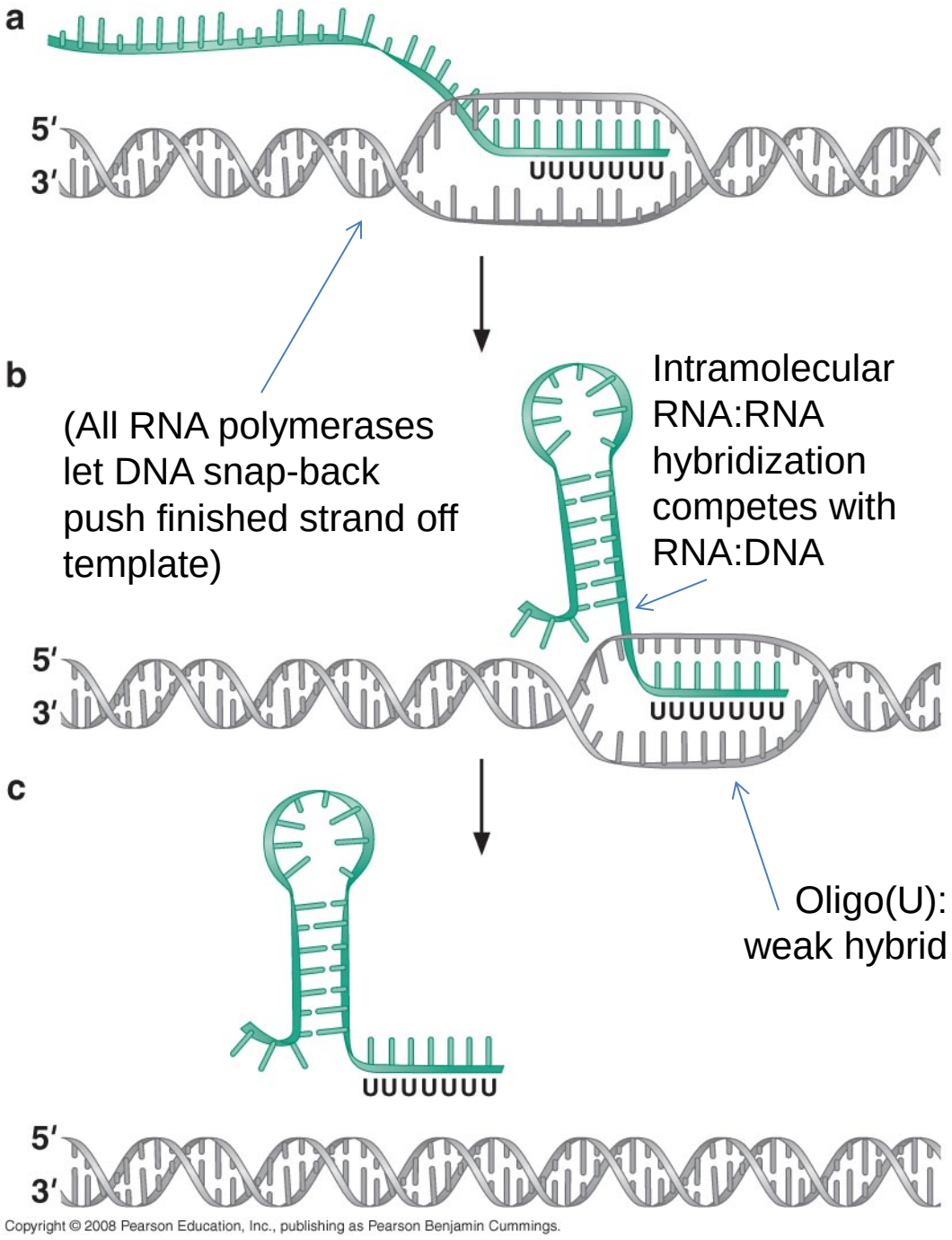
unlike eukaryotes, no role for cleavage & poly(A) addition



Sites of mutations that interfere with correct termination

As RNA polymerase (only one) reaches end of coding region…

Secondary structure folding and melting off the DNA template help to release RNA



Greatly reduced role in bacteria for coordinated transcription factor action at dispersed genomic sites

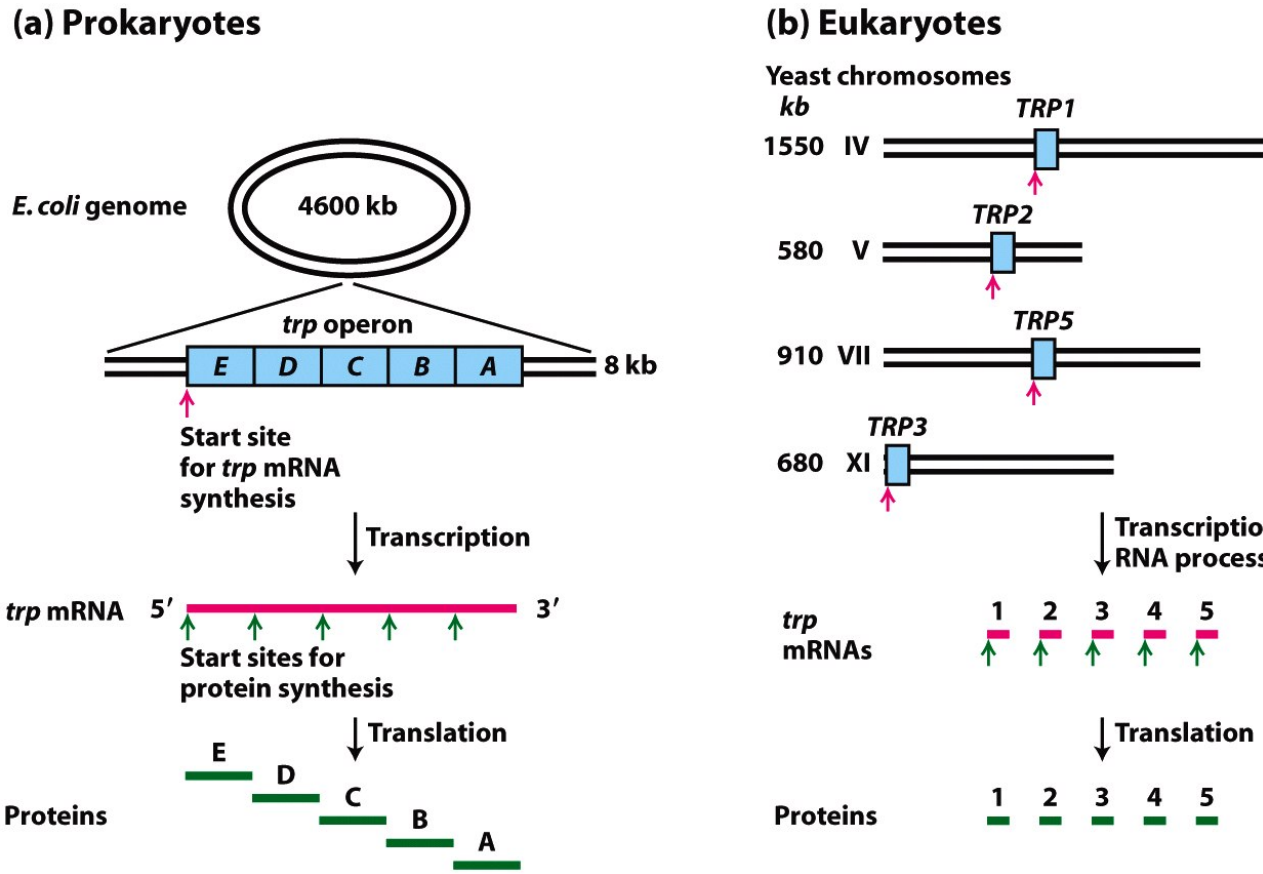
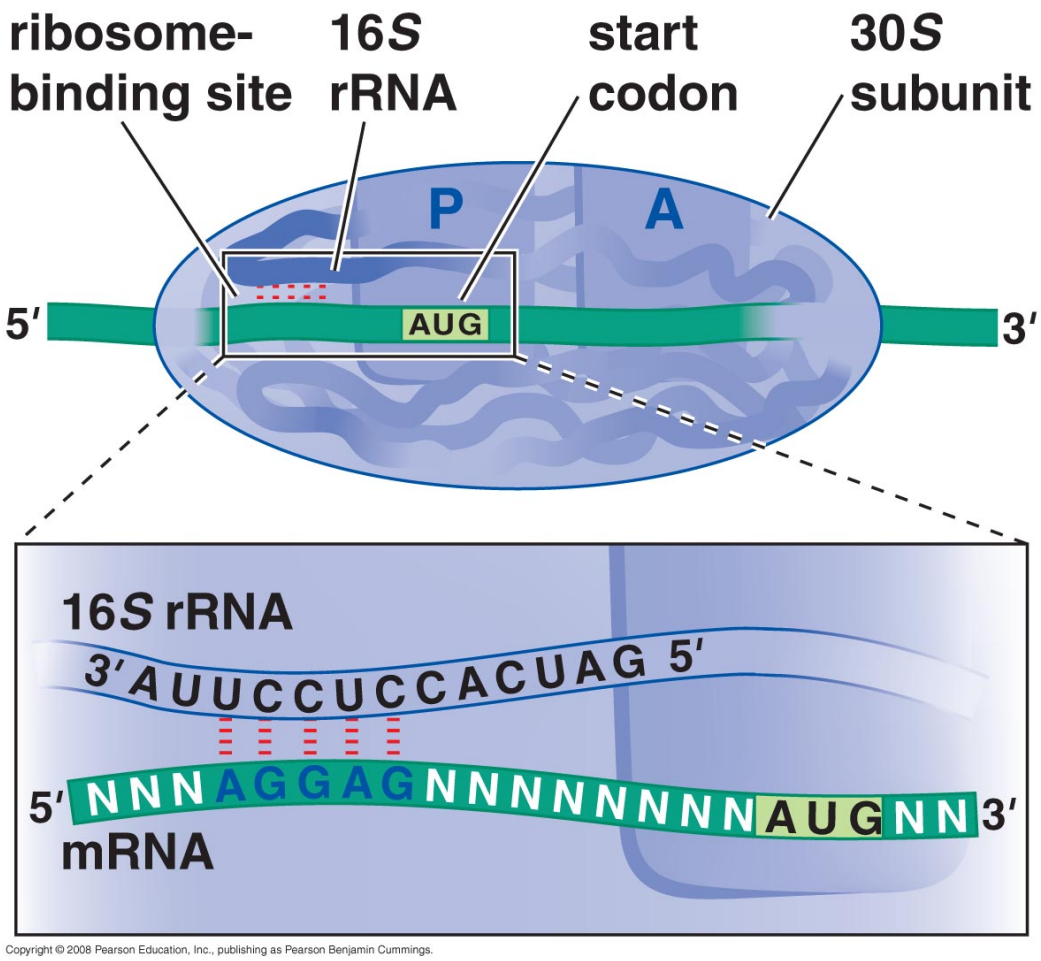


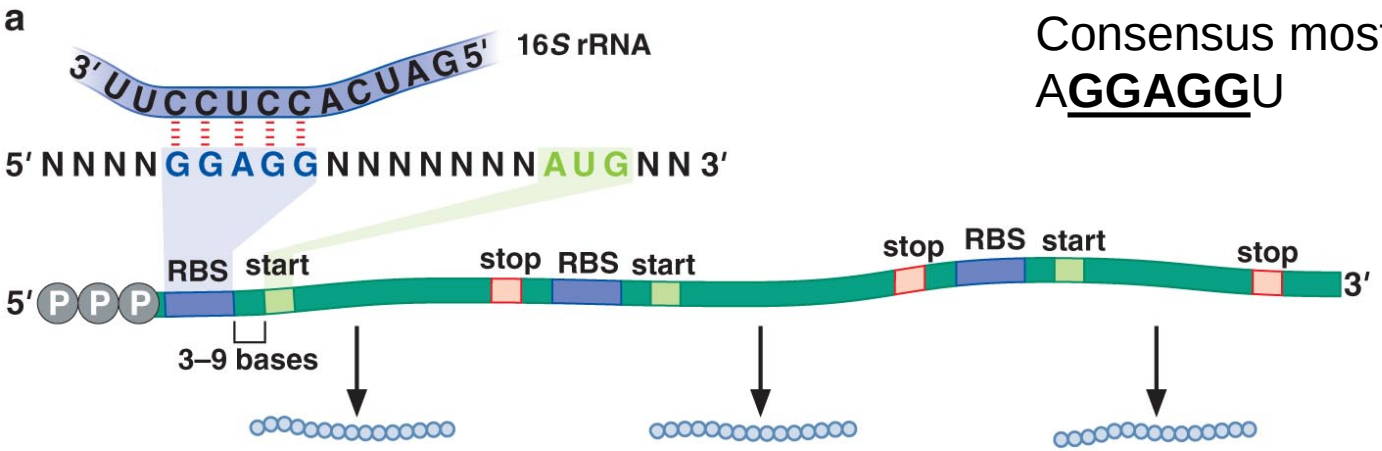
Figure 4-13
Molecular Cell Biology, Sixth Edition

But a new problem: how to control multiple starts of translation?

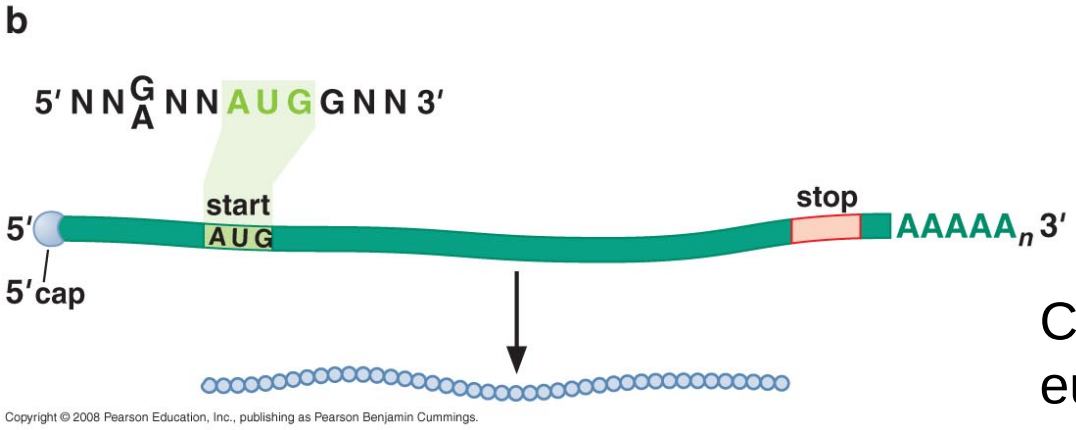
Bacterial translational initiation is oriented by “Shine Dalgarno box” complementary to 16S rRNA which can lie anywhere in mRNA



This enables bacterial ribosomal small subunit to scan for initiation sites anywhere in the mRNA, *any number of times,* without regard to 5' end



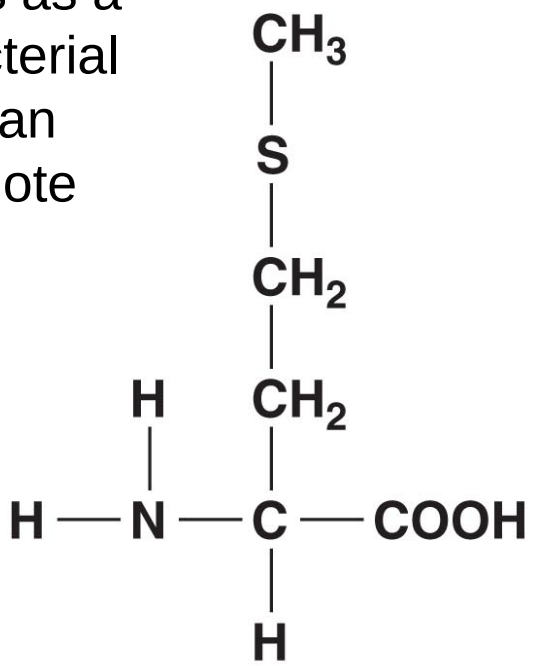
Consensus most often A**GGAGG**U



Contrast with 5' cap limited eukaryotic ribosomal scan

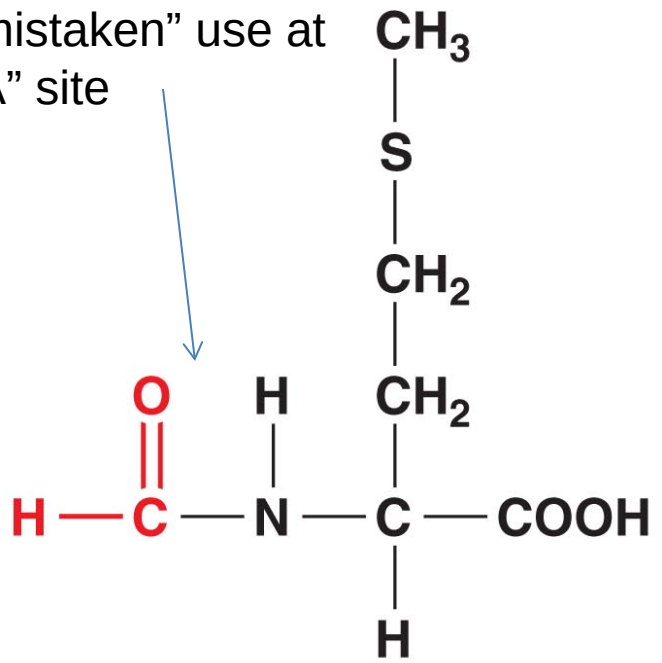
Bacteria use a specially modified Met to charge their initiator AUG tRNAs

(Metazoan immune cells recognize fMet-initiated peptides as a signature for bacterial presence… and an activator to promote attack!)



methionine

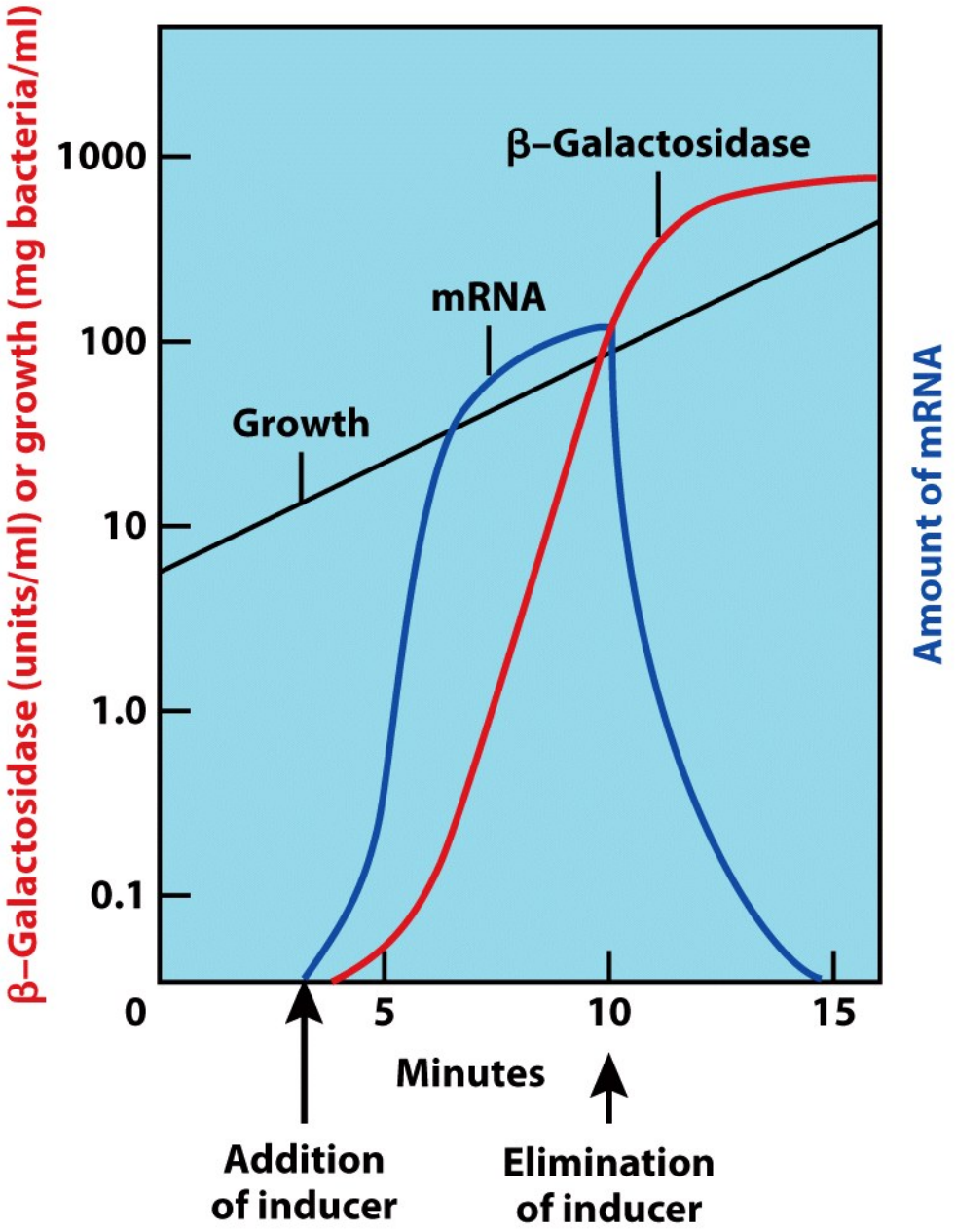
This amine group is preblocked against any "mistaken" use at "A" site



N-formyl methionine (fMet)

Bacteria do carry out precise and powerful gene expression regulation

Short mRNA half-life ensures massive dynamic range



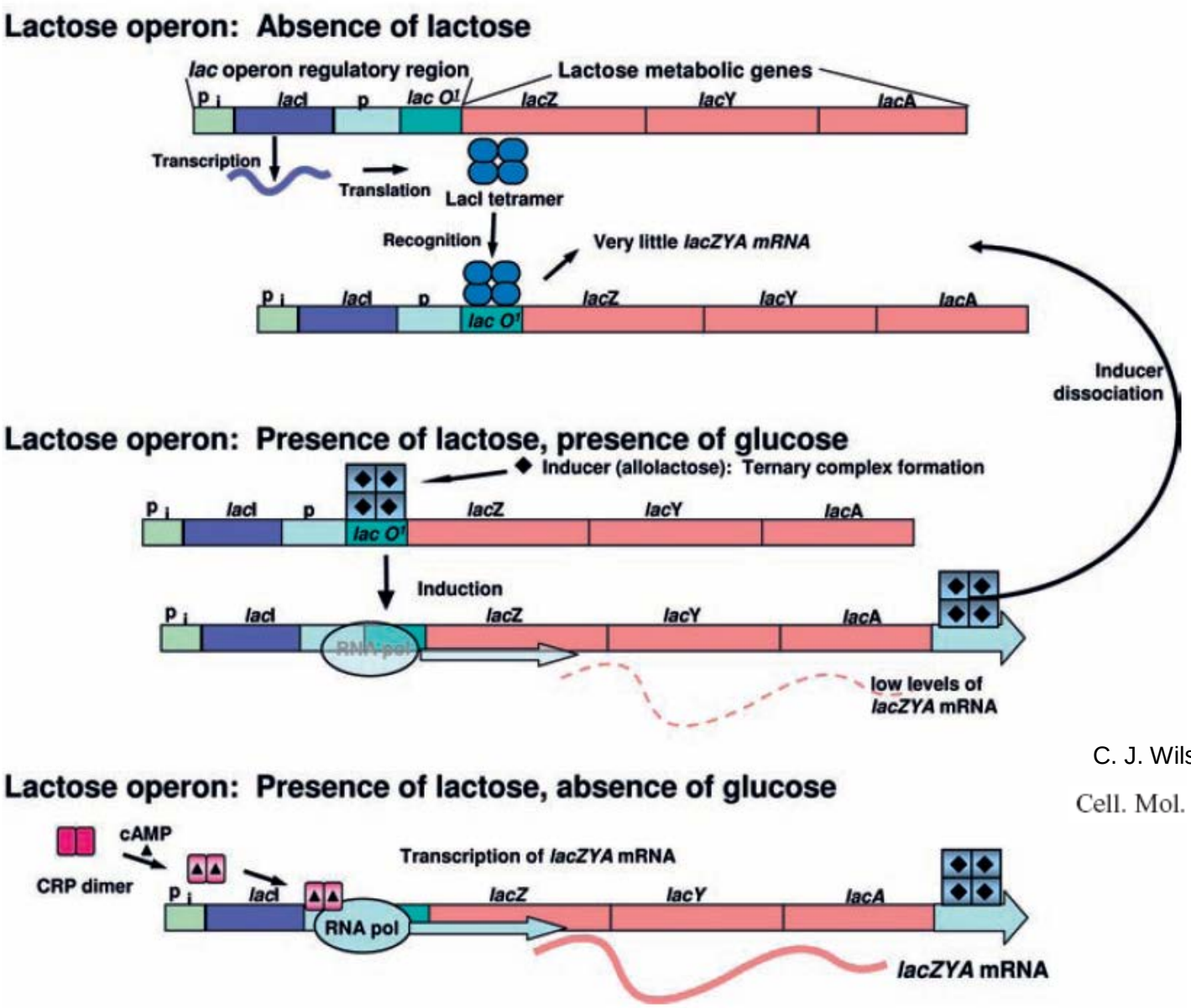
Lac operon

- Major milestone in concepts of gene regulation (Jacob & Monod)

- Combination of positive and negative regulation
  - Negative regulation when lactose is not present
  - Negative regulator: LacI = “Lac repressor”
  - Positive regulation when glucose is not present
  - Positive regulation via Catabolite Activator Protein (cAMP activated protein)
- Most important: METABOLIC RELIEF OF REPRESSED STATE

- Key to induction by lactose is allosteric change in LacI when it binds inducer, causing loss of DNA binding (Monod, Wyman, Changeux or “MWC theory”)

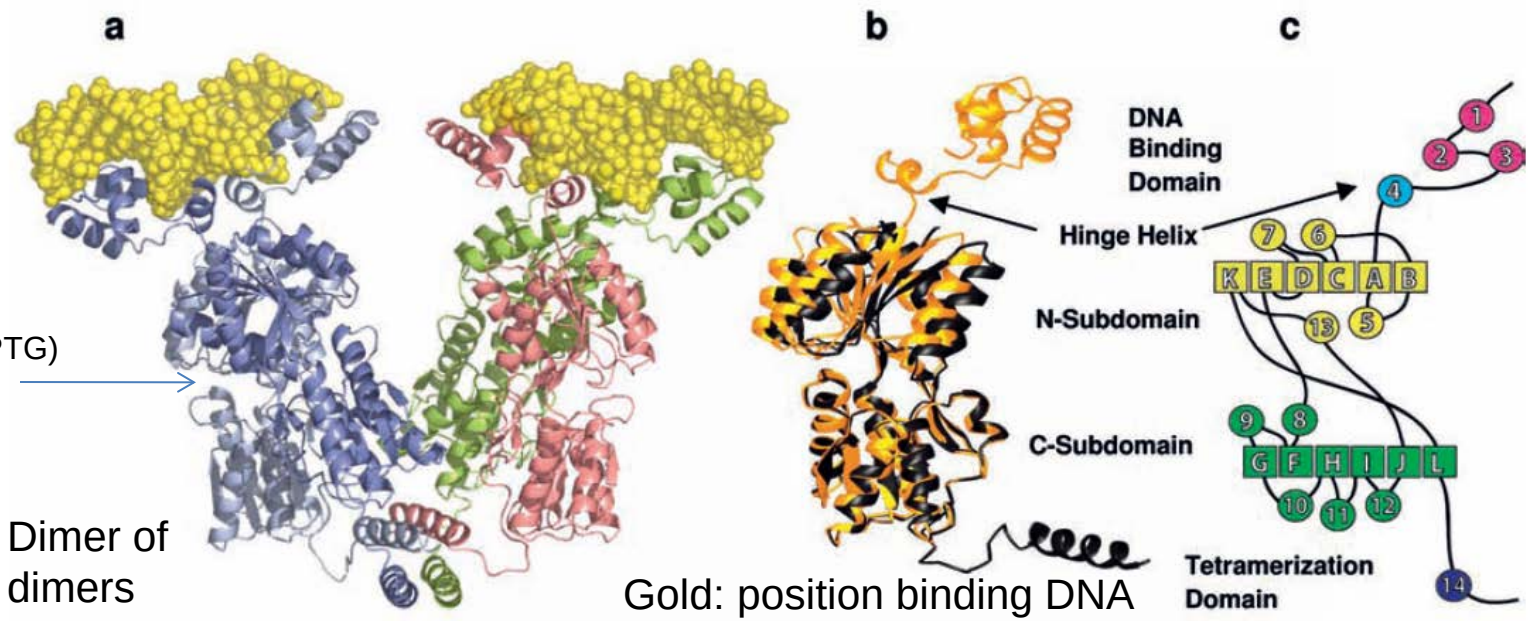
E. coli lac operon



C. J. Wilson … & K. S. Matthews

Cell. Mol. Life Sci. 64 (2007) 3–16

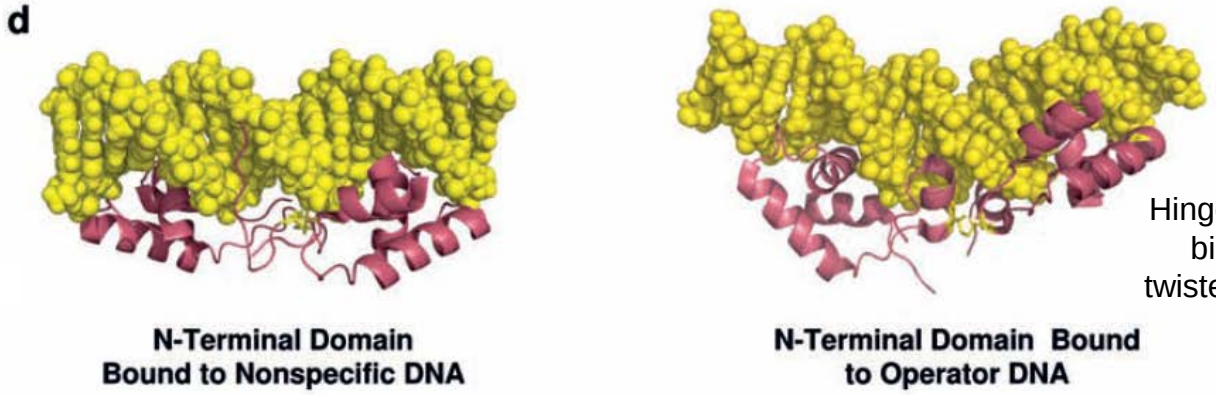
Lac Repressor (LacI protein): domains, ***allosteric*** change in response to inducer, affects DNA binding



Inducer (IPTG) binds here

Dimer of dimers

Gold: position binding DNA
Black: position when IPTG triggers release



Hinge helix helps binding: this is twisted away with inducer

C. J. Wilson … & K. S. Matthews

Cell. Mol. Life Sci. 64 (2007) 3–16

Ligand dependent ***release of repression*** at the Lac operon

plus activation dependent on a second ligand: a two-transcription factor system

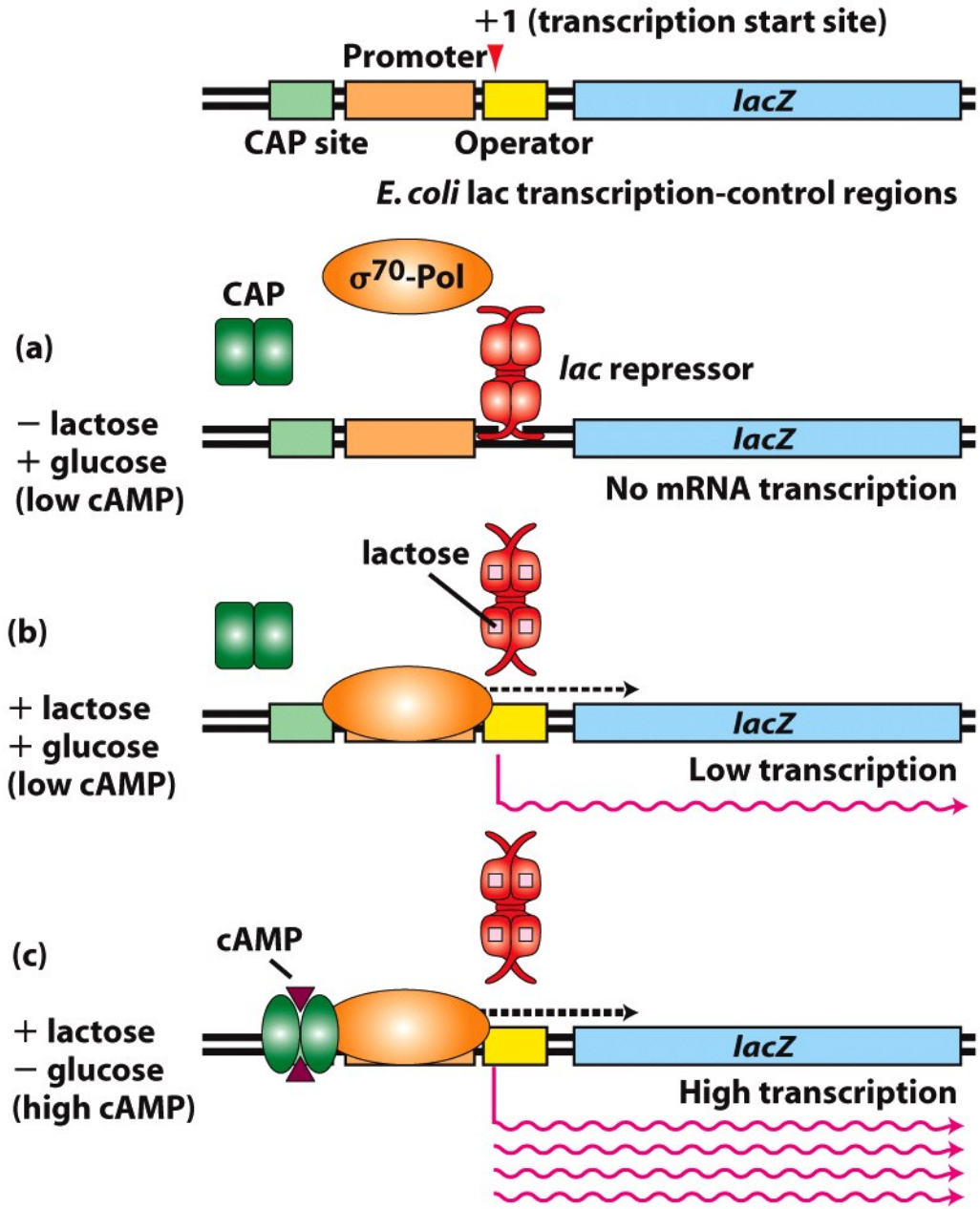
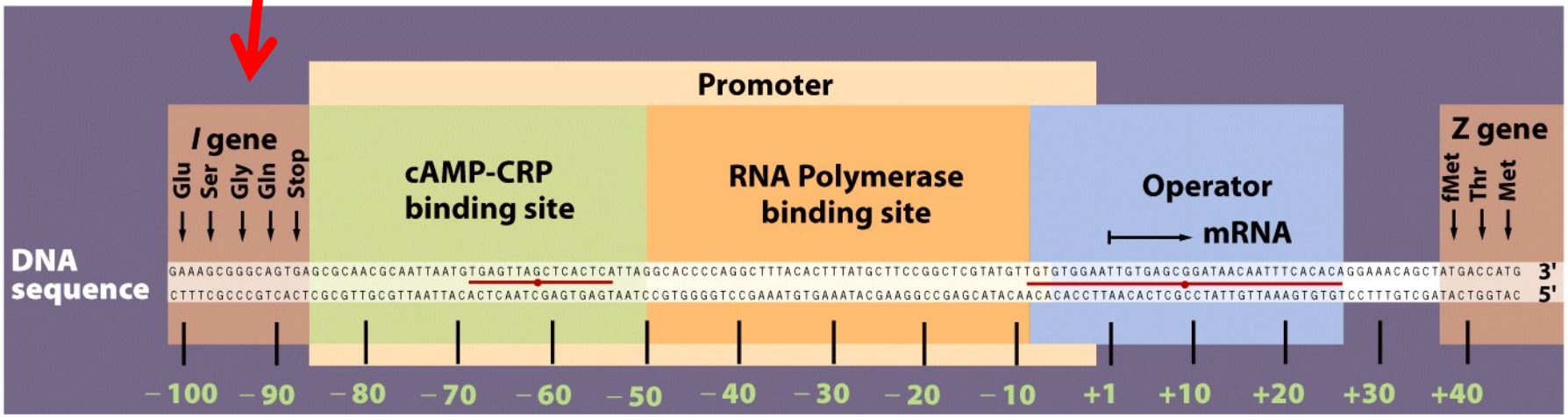


Figure 7-2
Molecular Cell Biology, Sixth Edition

Closeup of lac operator shows overlap in binding sites for RNA polymerase and repressor

Repressor gene itself is part of same gene cluster, but under different regulation



lacI

repressor

(via ribosomes…)

Repressed state is maintained by the single Lac repressor as tetramer, interacting with two neighboring sites as well as operator (+11)

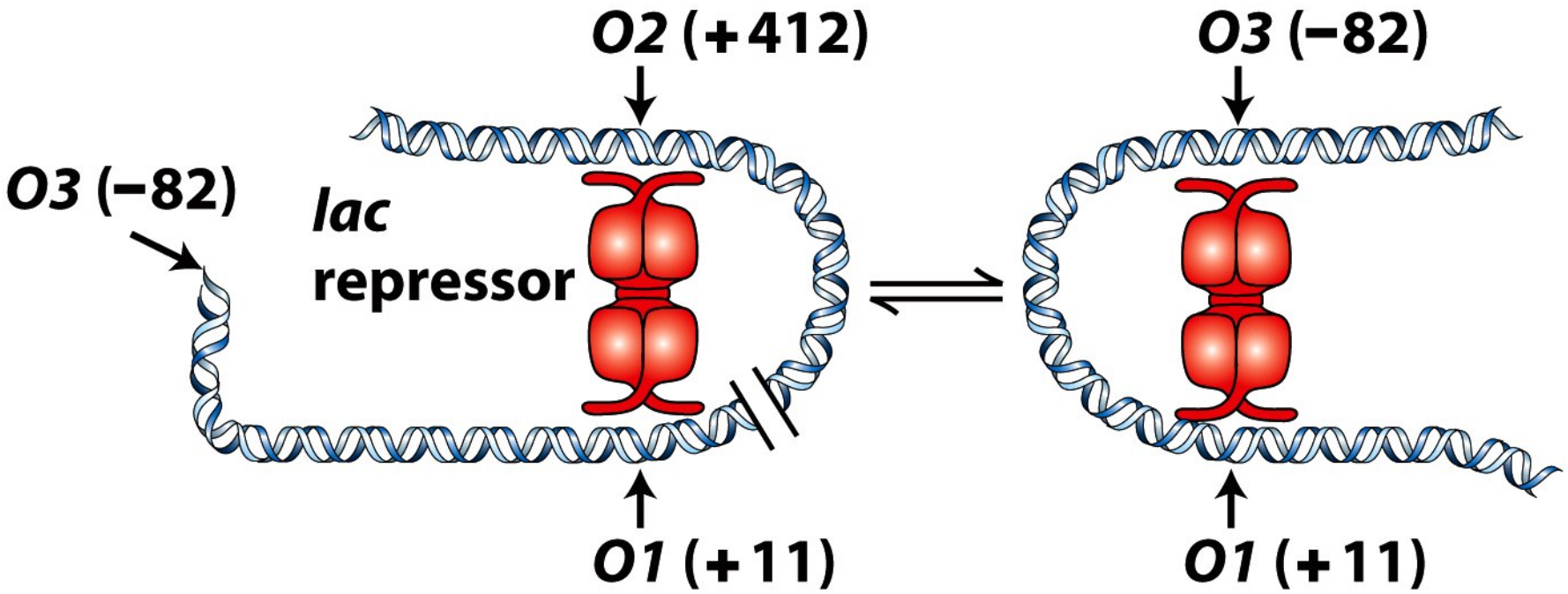
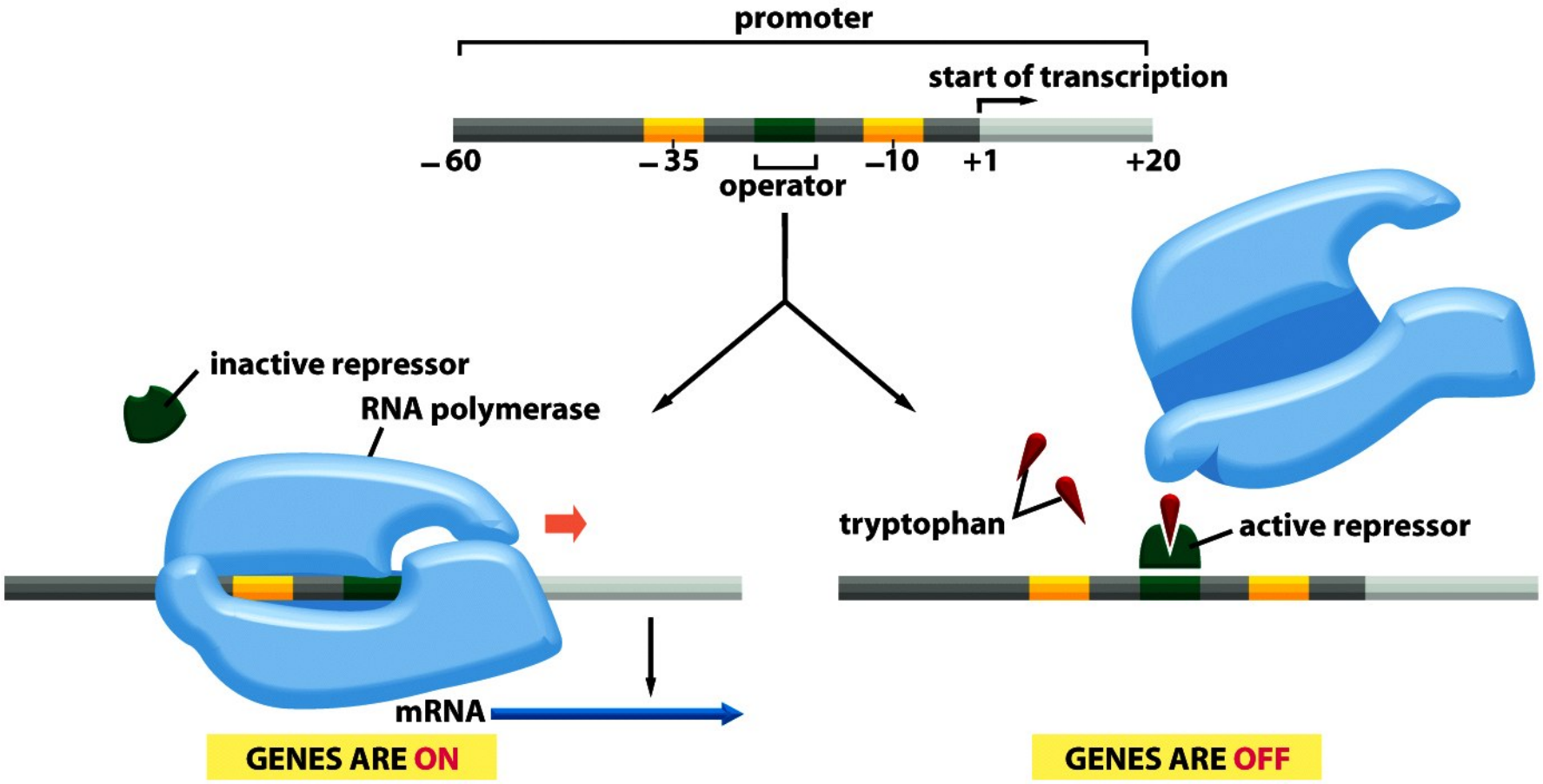


Figure 7-3
Molecular Cell Biology, Sixth Edition

These help keep local concentration of LacI protein high near Lac operon, so that only ***ten molecules*** per E. coli cell are enough to keep genes repressed

The other repression paradigm: ligand-dependent repression at the Trp operon



For feedback *negative* control…
homeostasis

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

A subtle ligand-dependent conformational change in the Trp repressor alters recognition helix orientation… promotes DNA binding

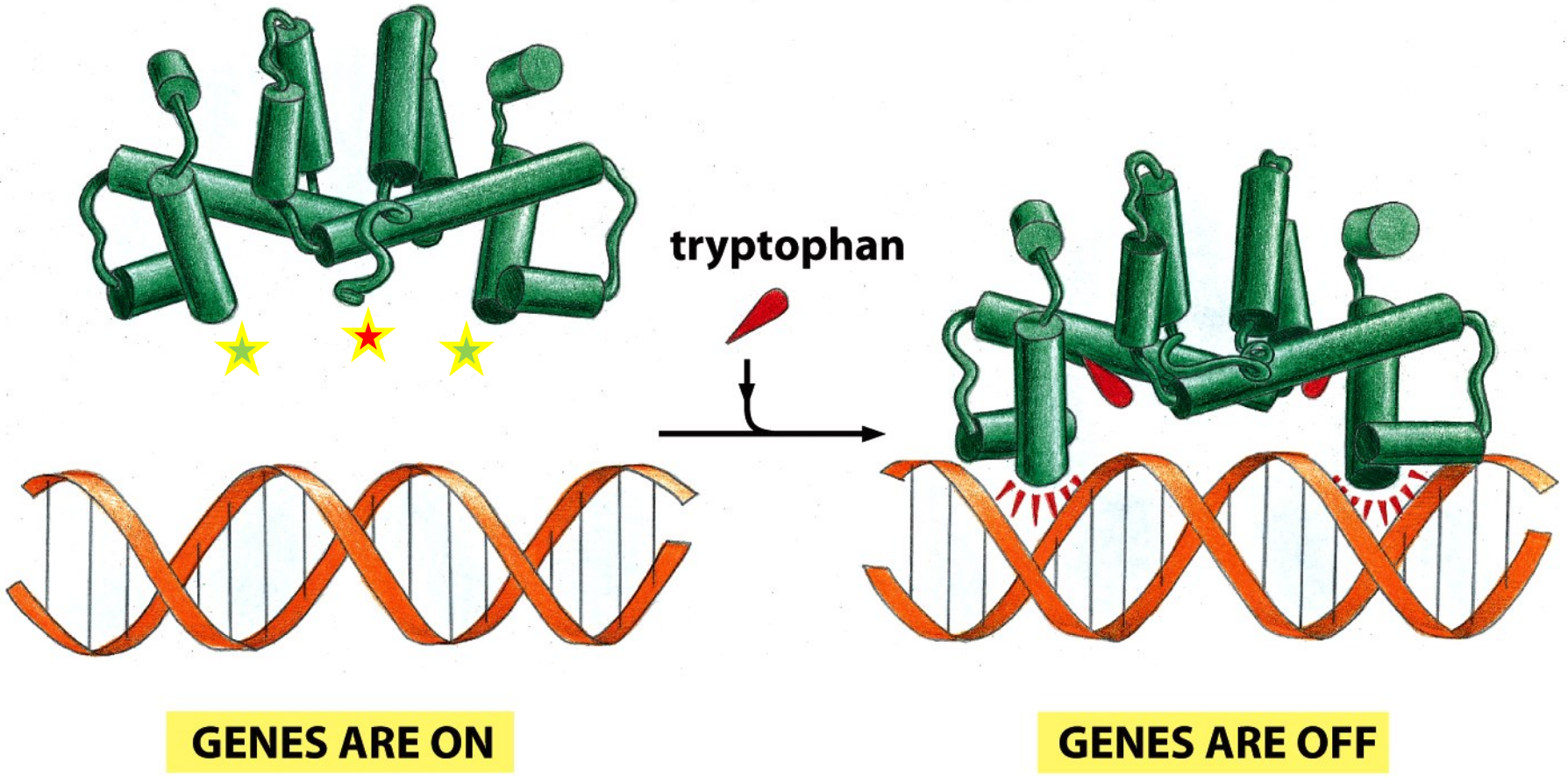


Figure 7-36 *Molecular Biology of the Cell* (© Garland Science 2008)

Opposite sign of effect, similar philosophy

- This is only part of the regulation of the Trp operon… more to come
- But strictly transcriptional regulation component is like mirror image Lac operon
  - Repressor as obstruction for polymerase
  - Direct ligand-dependent control of repressor binding
  - Default is for RNA polymerase to work

Next time: read C. Yanofsky, Trends Genet 20: 367-274 2004