BI 8 LECTURE 10

BACTERIAL REGULATION, II

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Not all bacterial promoters use the same σ factors, and this provides added regulation capability

Table 7–2 Sigma Factors of *E. coli*

SIGMA FACTOR	PROMOTERS RECOGNIZED
σ^{70}	most genes
σ^{32}	genes induced by heat shock
σ^{28}	genes for stationary phase and stress response
σ^{28}	genes involved in motility and chemotaxis
σ^{54}	genes for nitrogen metabolism
σ^{24}	genes dealing with misfolded proteins in the periplasm

The sigma factor designations refer to their approximate molecular weights, in kilodaltons.

Most sigma factors are related… except σ^{54} which has distinctive functions

It's σ^{54} that is used for positive regulation at Ara and Gln operons

Table 7-2 *Molecular Biology of the Cell* (© Garland Science 2008)

Positive regulation depends on a different starting assumption…

- You need positive regulation only for genes that are not *automatically* going to be transcribed *by default*
  - As we will see, most genes in eukaryotic organisms need positive regulators

- *Lac* operon, *LacI*, *Trp* operon are all in danger of being transcribed all the time

- Using a different sigma factor for E. coli RNA polymerase at certain promoters enables polymerase activity to respond to transcription factors even after it is bound

σ^{54}, like σ^{70} , recruits RNA polymerase to promoter, but does not automatically license polymerase to move: needs TF binding at enhancer too

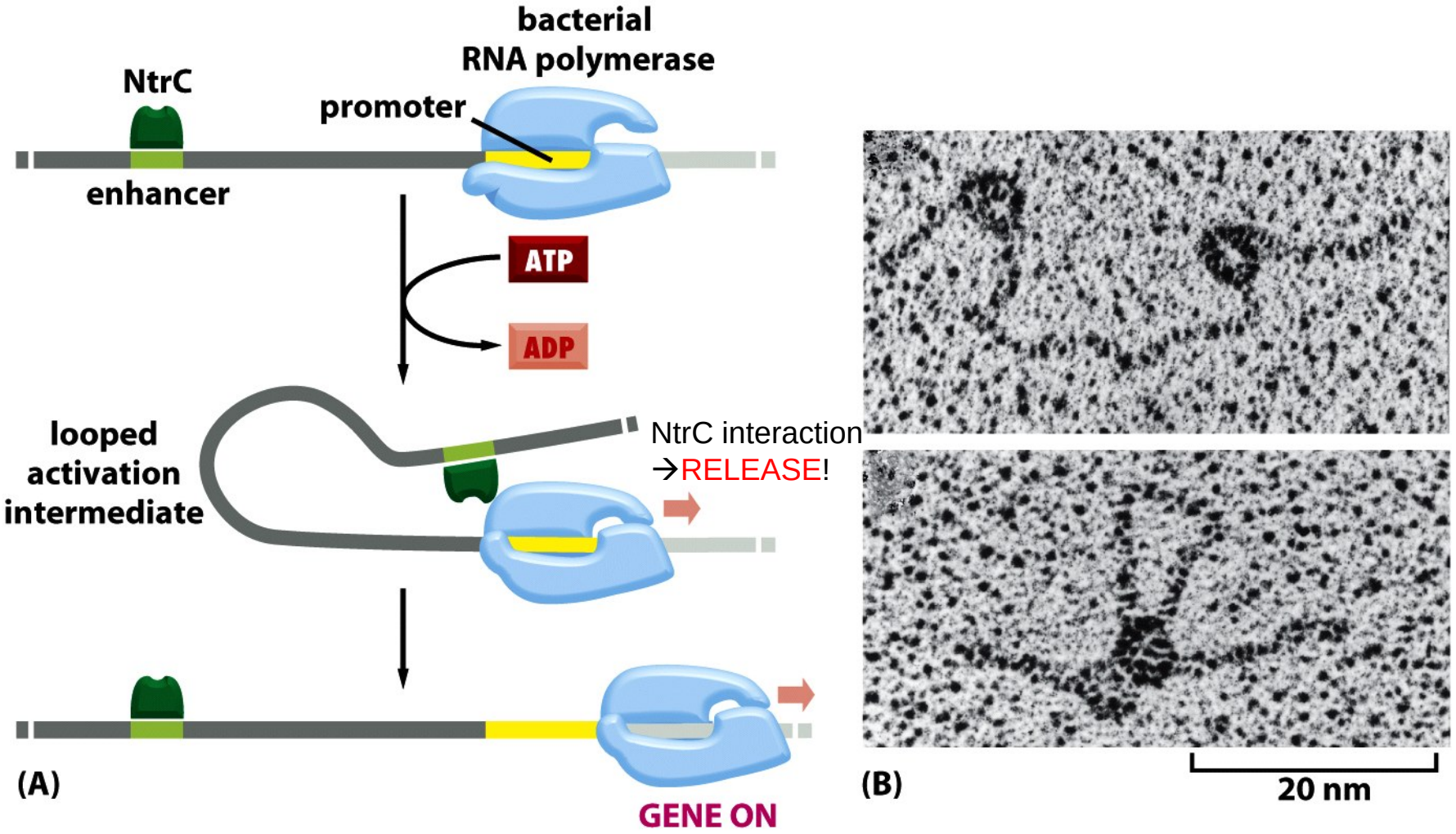
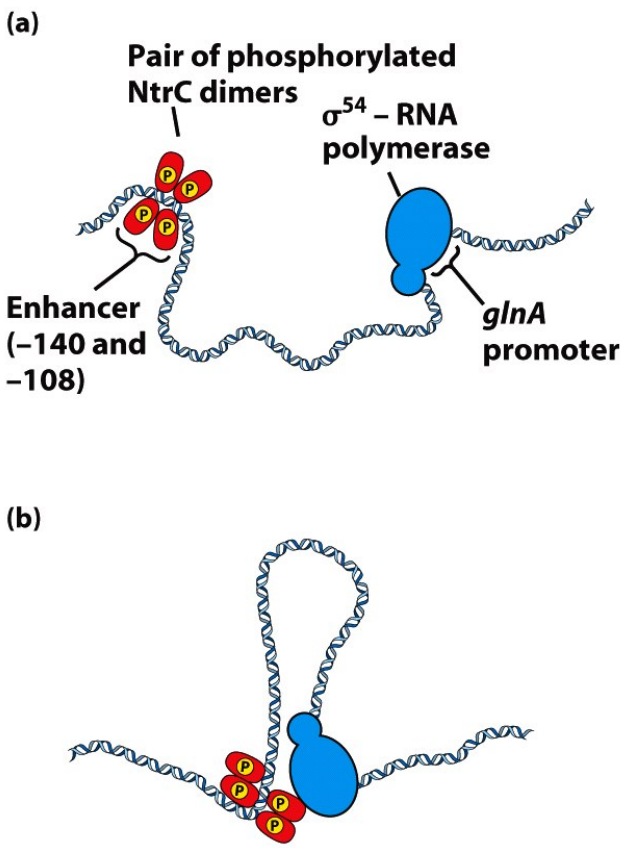


Figure 7-42 *Molecular Biology of the Cell* (© Garland Science 2008)

σ^{54} subunit at the glutamine operon instead of σ^{70}: freezes polymerase at promoter until released by signal-activated transcription factor binding upstream

When glutamine synthesis is needed, transcription factor NtrC is phosphorylated & can now turn on the genes…



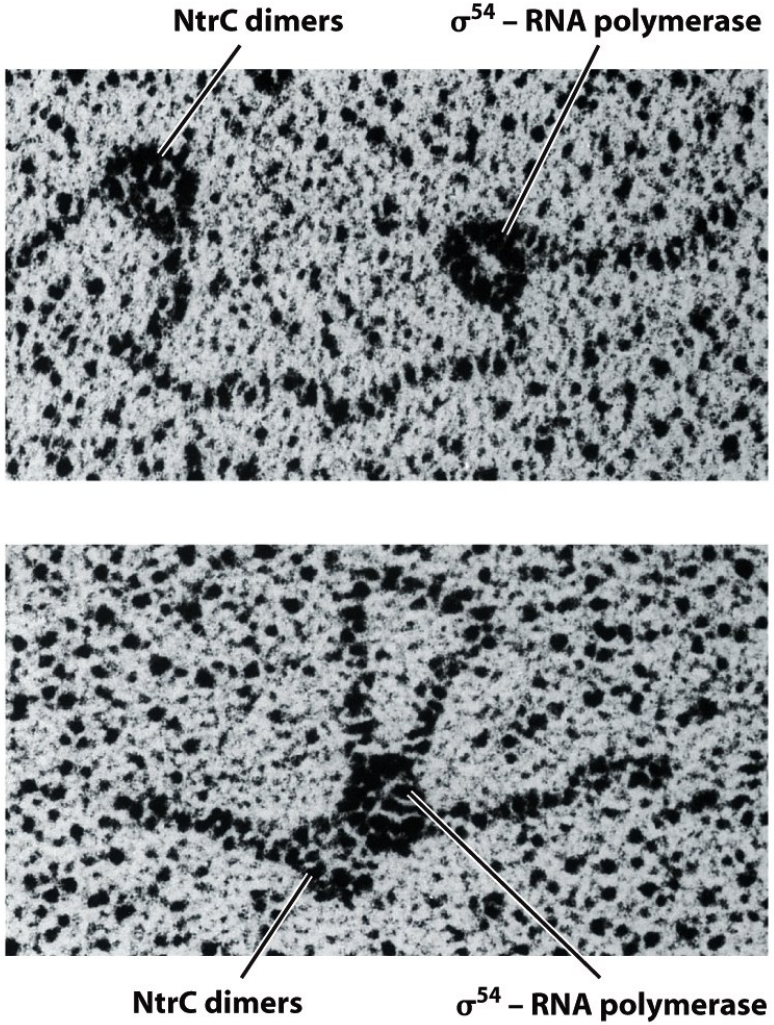


Figure 7-4
Molecular Cell Biology, Sixth Edition

Bacteria can use "extracellular" signal-dependent pathways for transcriptional control, too

Ability of a transcription factor PhoB to turn on target genes can depend on enzymatic phosphorylation by a membrane-bound ligand sensor

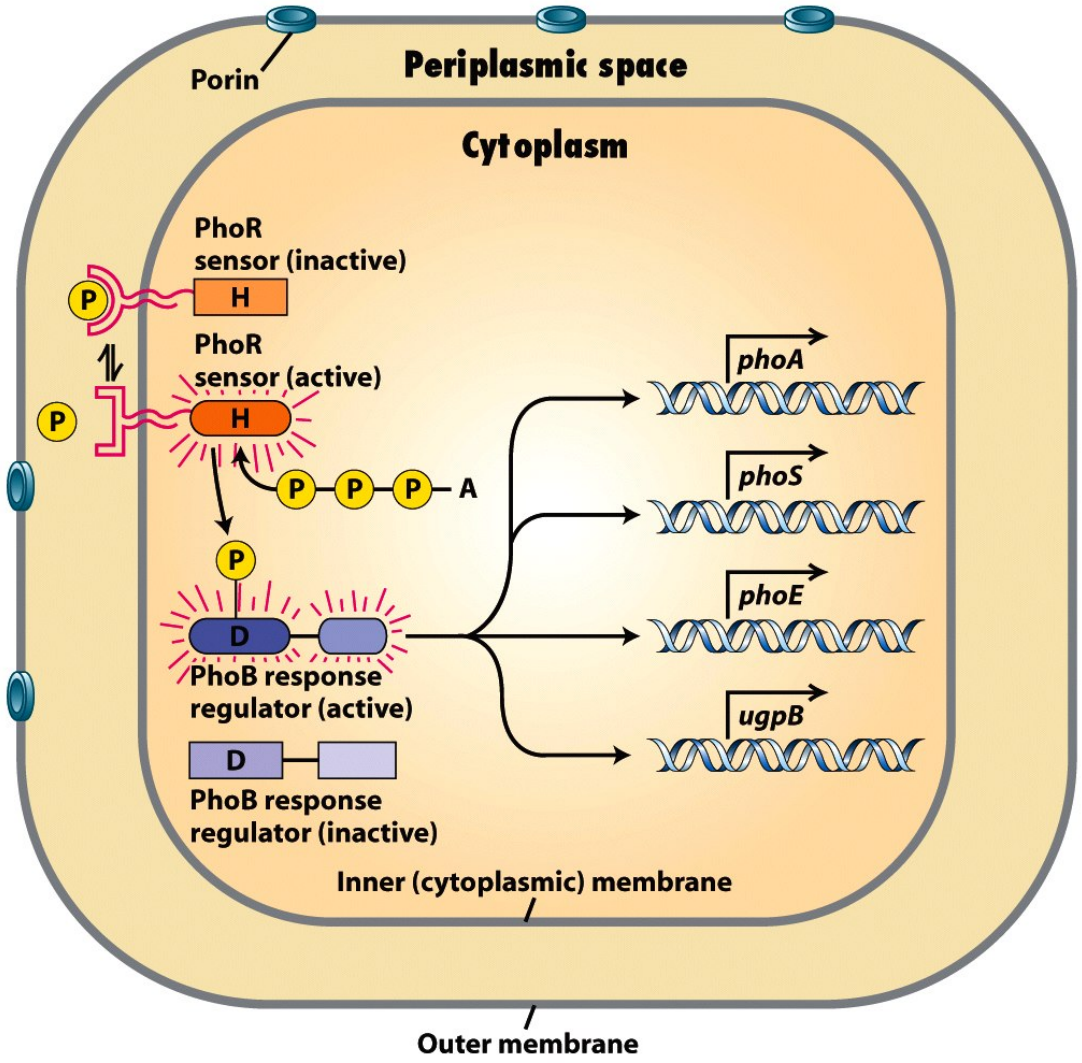


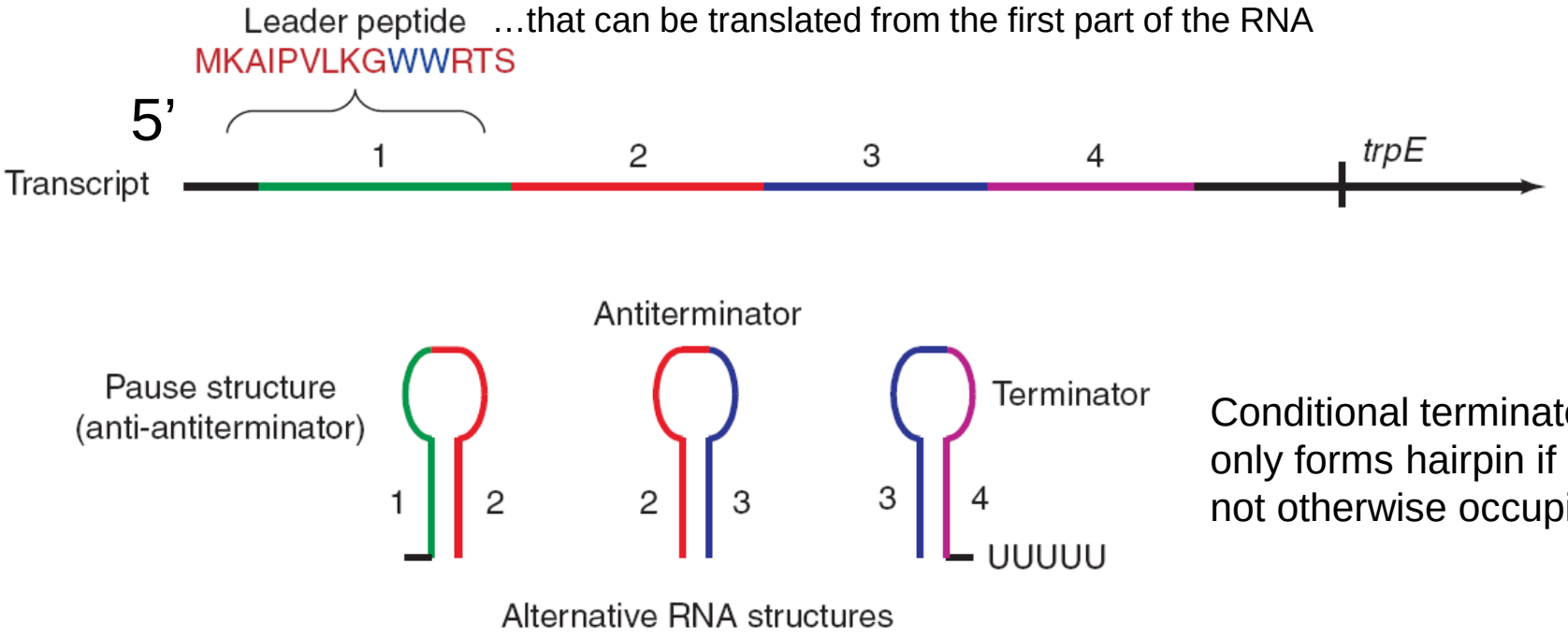
Figure 7-5
Molecular Cell Biology, Sixth Edition

A major paradigm that is exploited by eukaryotic cells

Transcription/translation spatial coupling gives bacteria unique regulatory opportunities

- Ribosome itself can be used as a transcriptional elongation regulatory protein
- Powerful in combination with RNA secondary structure which signals transcriptional termination
- Double control possible – use translational progress vs. pausing to control transcriptional termination!

It could only happen here: a secondary control system for Trp biogenesis in E. coli



Conditional terminator: only forms hairpin if “3” is not otherwise occupied

Charles Yanofsky: Trends Genet. 20, 367-374 (2004)

Secondary control via RNA secondary structure: THREE possible stem/loop hairpins can be formed from this sequence …. If the RNA polymerase gets far enough to synthesize the key sequences

A few numbers…
the *E. coli* advantage in translational speed

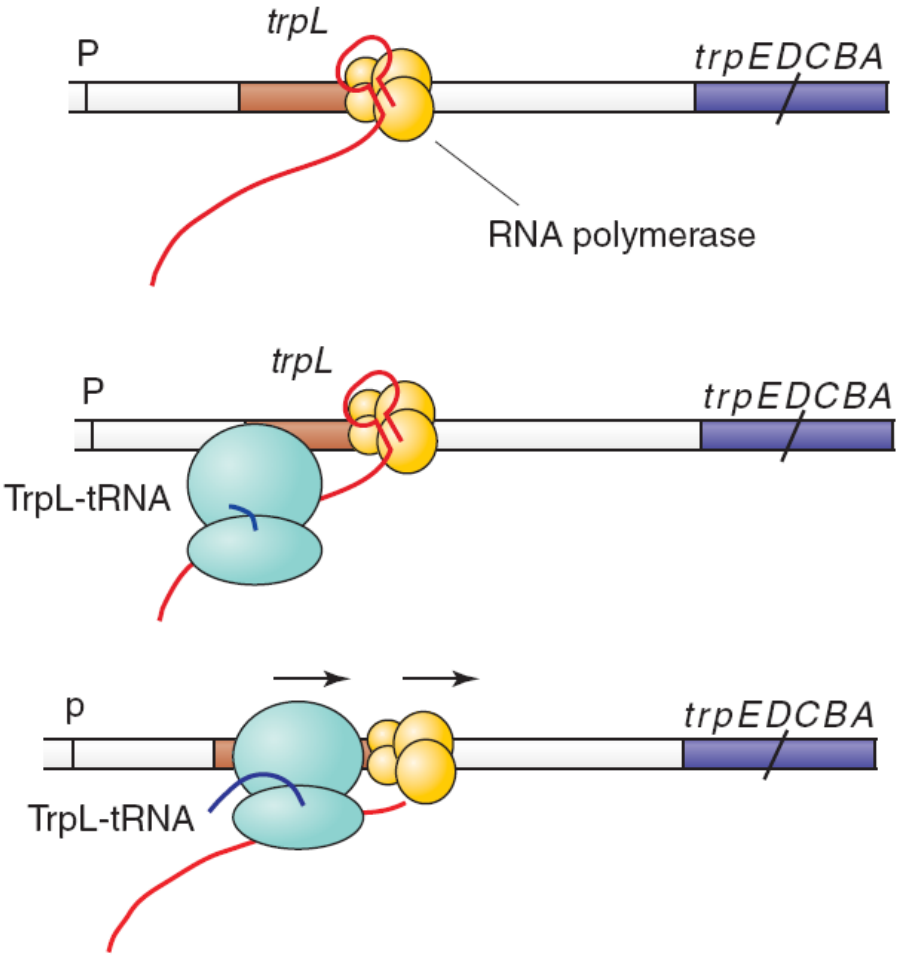
- Average rate of transcriptional elongation in eukaryotes at 37˚C: 30-40 nt/sec

- Average rate of translational elongation in eukaryotes at 37˚C: 2-4 amino acids/sec

- Average rate of transcriptional elongation in prokaryotes at 37˚C: 50-100 nt/sec (~17-33 codons/sec)

- Average rate of translational elongation in prokaryotes at 37˚C: 20 aa/sec

Polymerase running into secondary structure, waits for ribosome to catch up…



Step 1
Transcription begins
and polymerase pauses

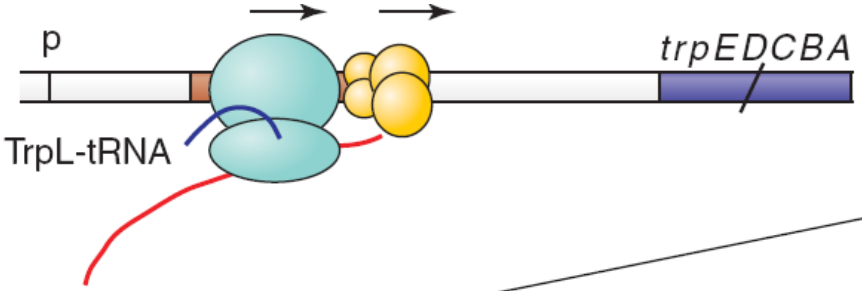
Step 2
Translation begins

Step 3
Moving ribosome releases
the paused polymerase

Ribosome translating across “1” breaks up “1”-”2” hairpin

Ribosomal translation as an RNA synthesis “elongation factor”!
…But only for a little bit longer…

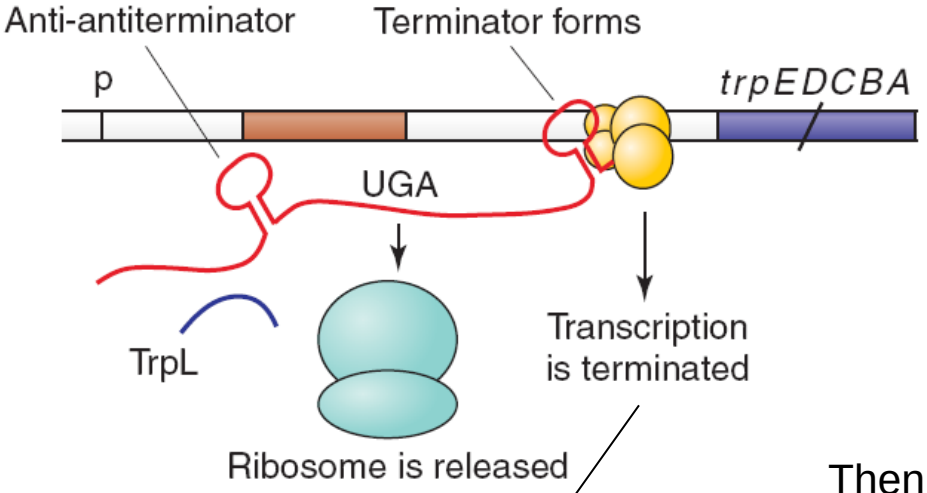
How to get transcription to abort… *if* Trp-tRNA is sufficient



Step 3
Moving ribosome releases the paused polymerase

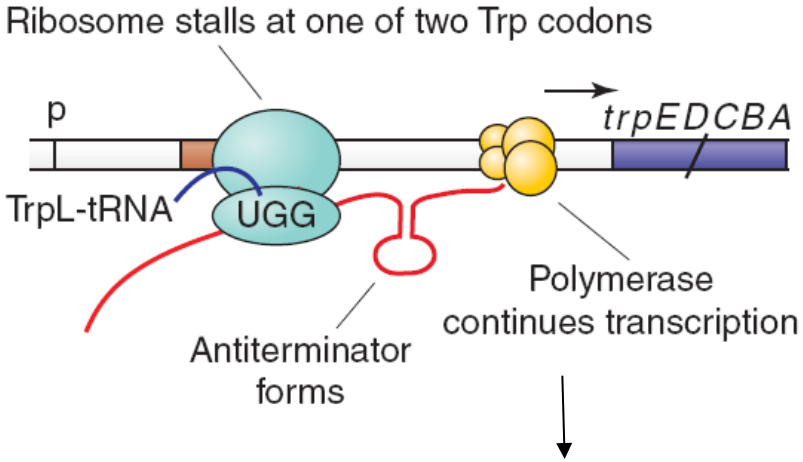
Now ***ribosomal stalling*** enables polymerase to continue!

Step 4a
Adequate charged $tRNA^{Trp}$.
Transcription termination



"Attenuation"

Step 4b
Insufficient charged $tRNA^{Trp}$.
Transcription antitermination

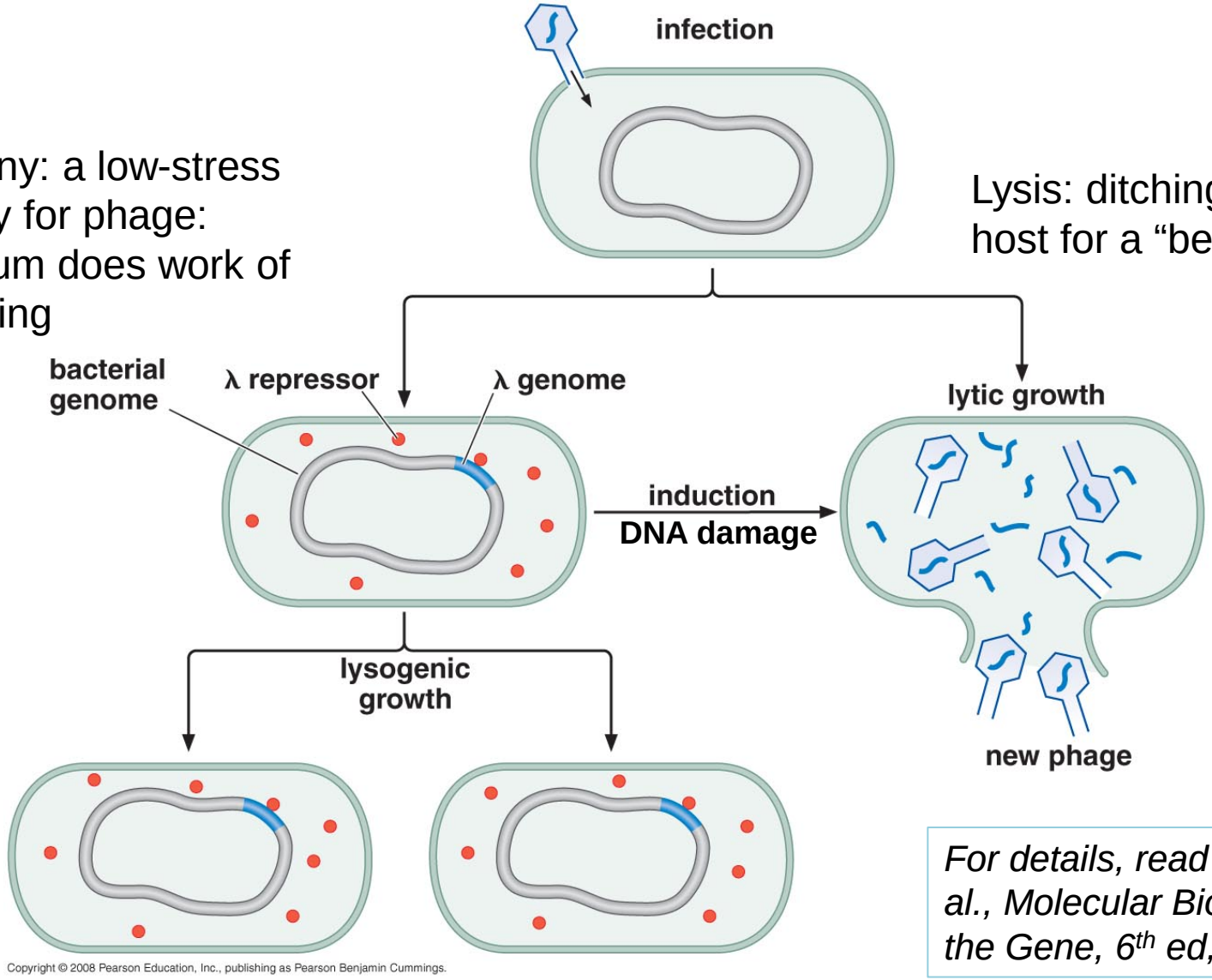


Then trp operon gene products, eventually translated, enable more Trp to be generated… until there is enough to trigger repressor or "attenuation" again

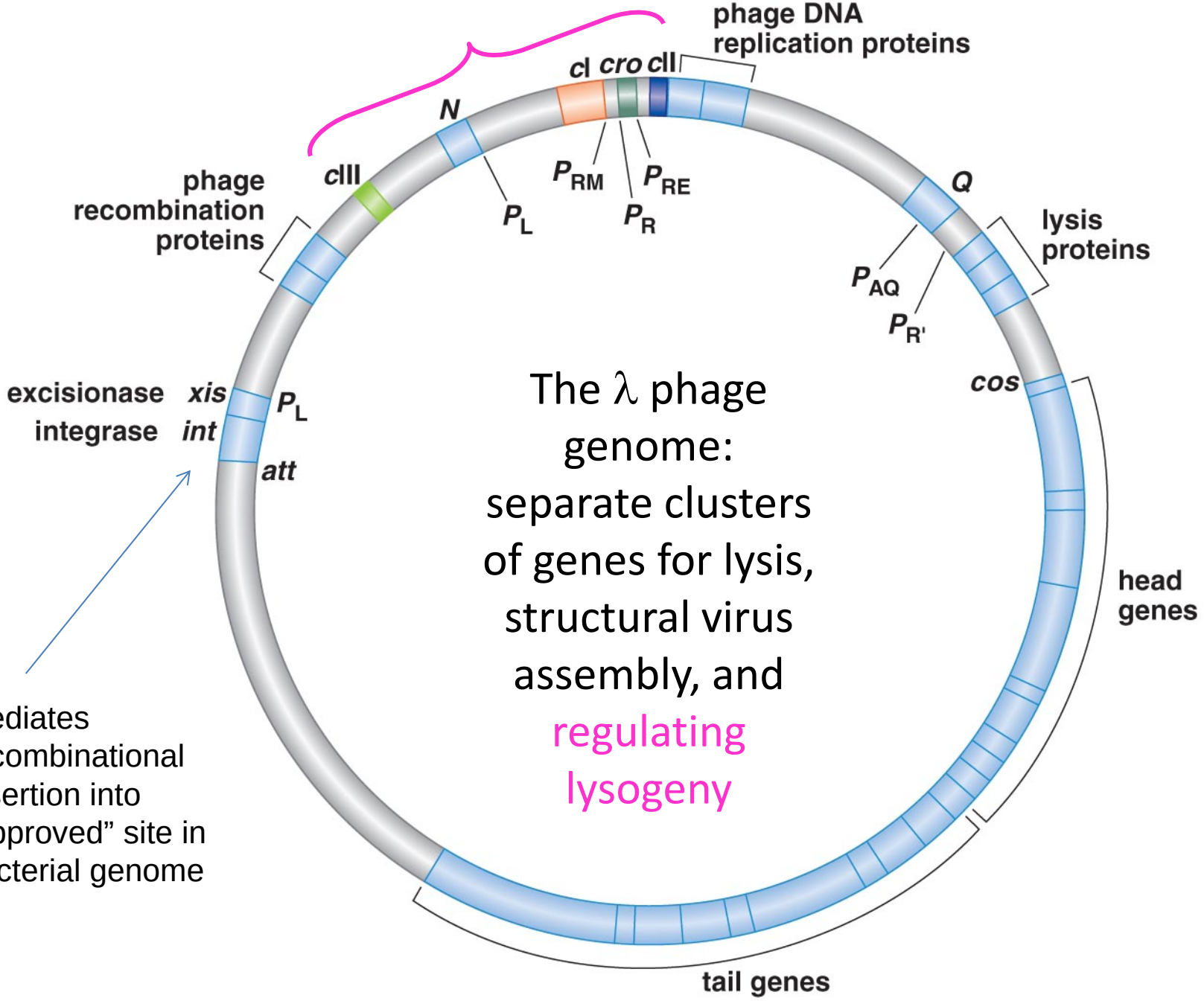
A life vs. death role for repression: Lifestyles of the temperate bacteriophage λ

Lysogeny: a low-stress strategy for phage: bacterium does work of replicating

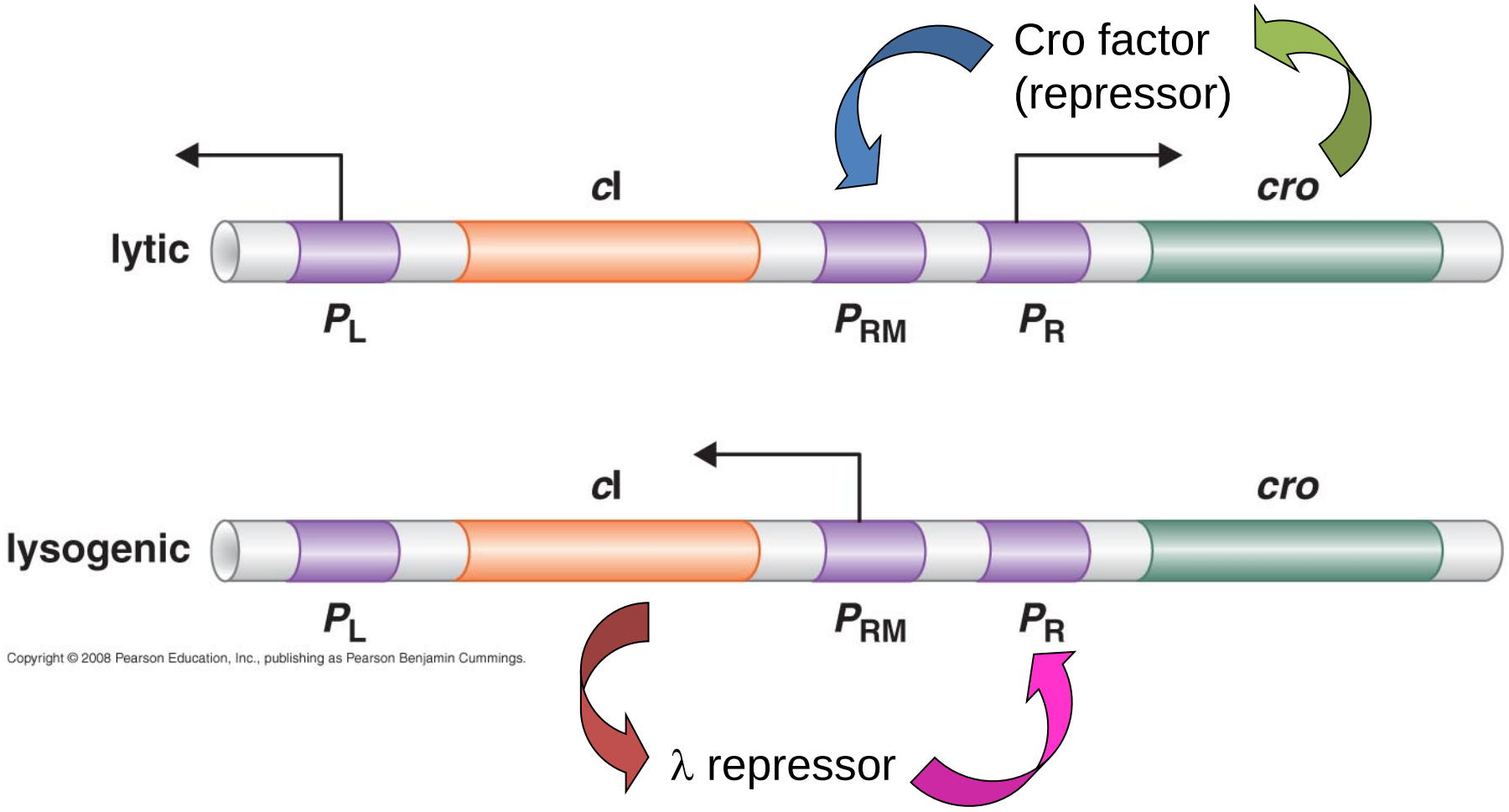
Lysis: ditching "this old" host for a "better" one



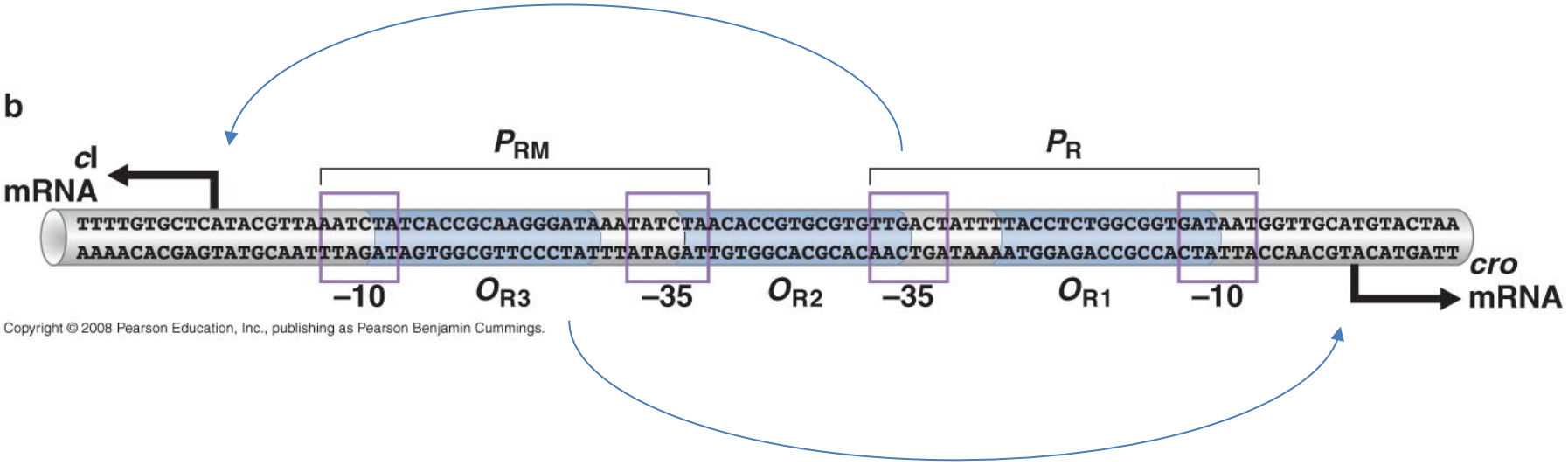
For details, read Watson et al., Molecular Biology of the Gene, 6th ed, Ch. 16



Regulatory complex of divergent promoters that control lysis vs. lysogeny decision



The heart of the lysis/lysogeny switch is a two promoter regulatory element with adjacent, opposite-pointing promoters and crossed positive regulatory sites for TF binding

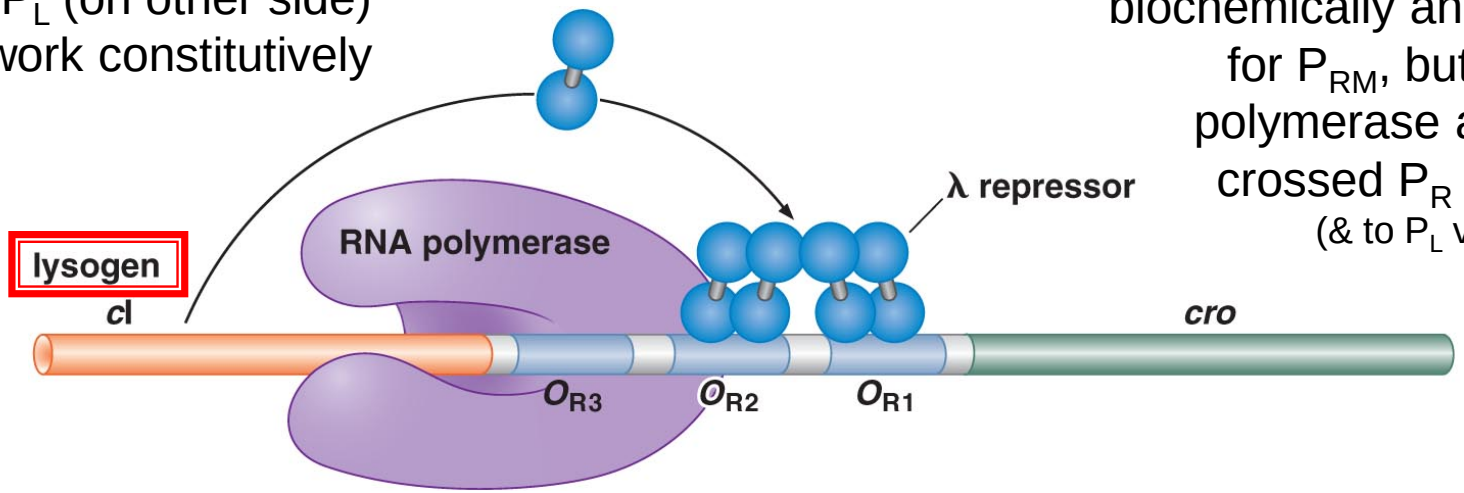


P = promoter, polymerase binding
O = binding sites for regulatory proteins

λ repressor: a "repressor" of λ phage replication, but biochemically an activator

Without λ repressor, both P_R and P_L (on other side) would work constitutively

The "λ repressor" is biochemically an *activator* for P_{RM}, but it blocks polymerase access to crossed P_R promoter (& to P_L via O_{L1}, O_{L2})



Crossed promoter structure in the DNA makes the repression: The λ repressor is just an obstruction for lysis-related genes…

…its binding to activate one promoter passively "gets in the way" of any polymerase trying to load in the opposite direction

λ repressor: a bifunctional protein opposed by repressor Cro

Without λ repressor, both P_R and P_L (on other side) would work constitutively

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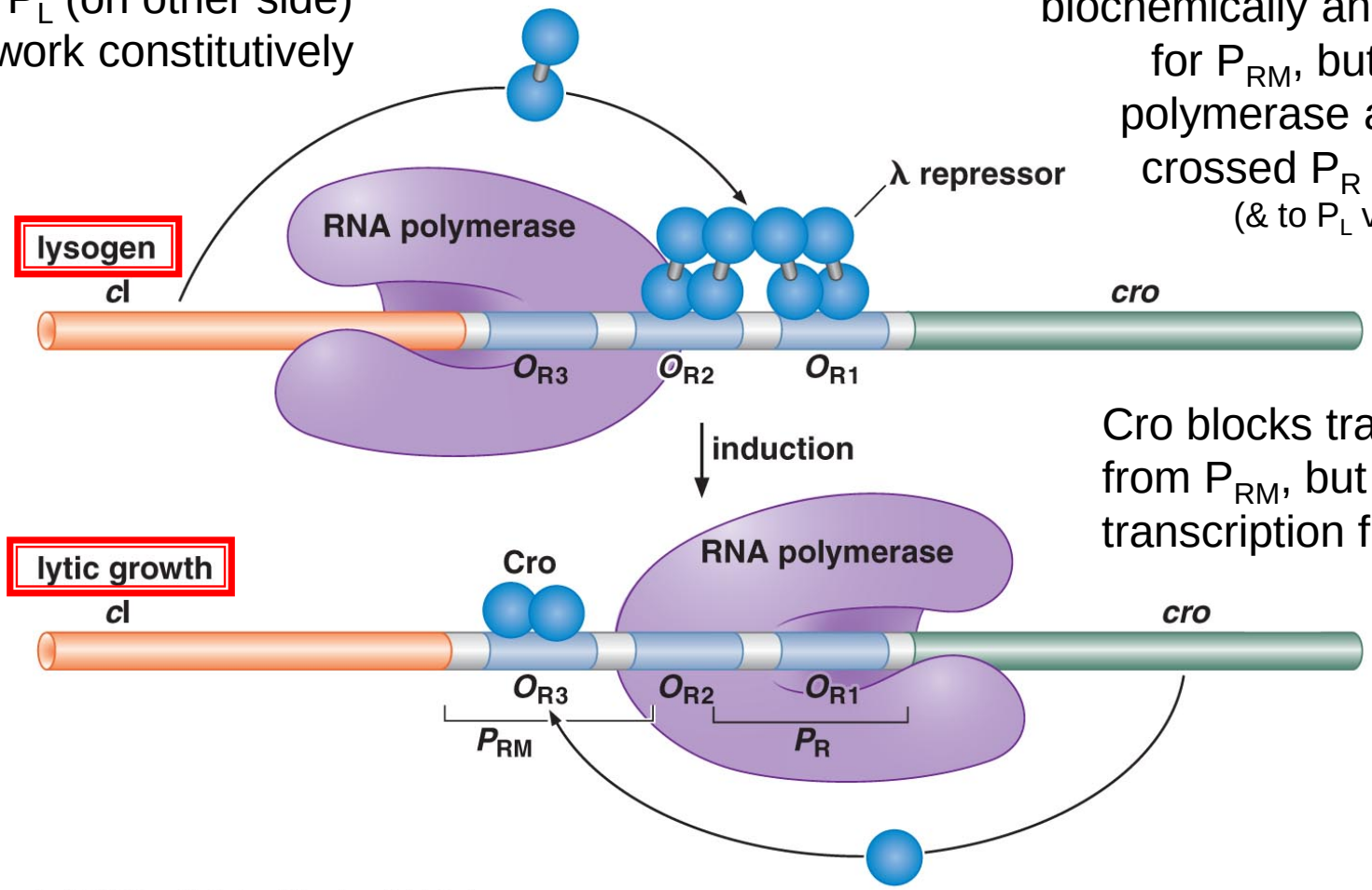


Cro blocks transcription from P_{RM}, but allows transcription from P_R

Some crucial tools to establish transcription factor protein binding to particular DNA site

- Abundant and pure transcription factor proteins
  - CLONING and EXPRESSION of transcription factor cDNAs

- Binding assays
  - Oligonucleotide probes with radiolabel incorporated
  - Binding to purified protein or nuclear extract
  - Gel electrophoresis to separate bound from free: “gel shift” complex

- Quantitation of binding
  - Titration of binding relative to added protein
  - Distinguish specific from nonspecific binding: nonspecific competitor
  - Distinguish *affinities of binding* by competing with specific competitors

Some crucial tools, continued

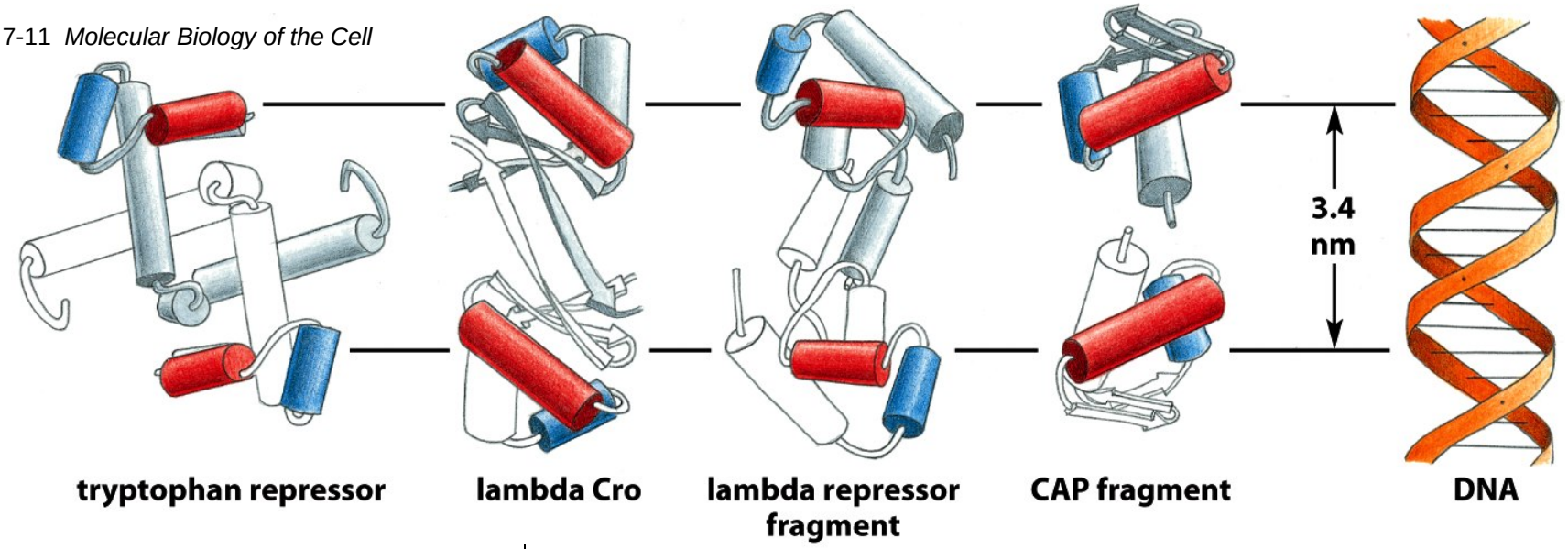
- Identification of binding proteins by antibody interference

- Fine mapping of binding sites by footprinting
  - In vitro footprinting: bind excess protein to *end-labeled* probe
  - Digest lightly with DNase & run on high-resolution gel (+/- 1 bp)
  - Every length of DNA is seen except those under protein "shadow"

- ***Complementary genetics***:
  - Effect of mutation of target DNA site ("cis mutation") should be mimicked by mutation of factor coding gene ("trans factor mutation")
  - ***subject to and/or logic rules for particular gene**]*

λ repressor and λ Cro: two structurally related factors that bind related, overlapping sites

Figure 7-11 *Molecular Biology of the Cell*

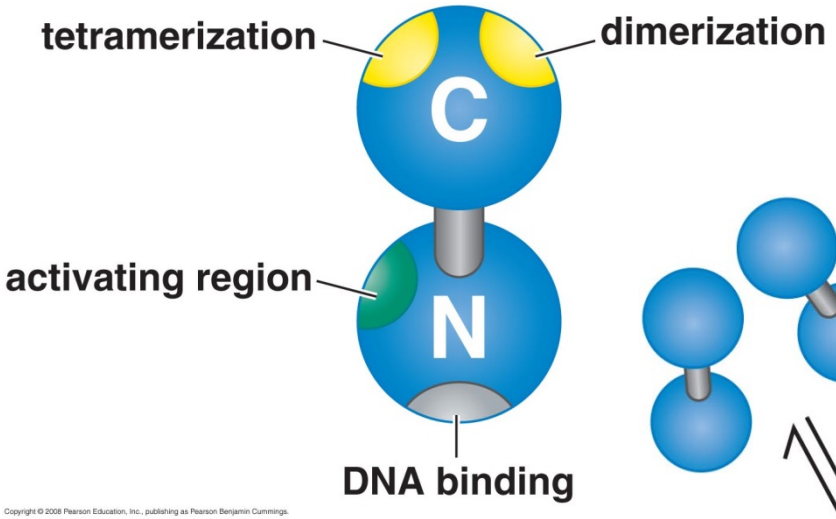


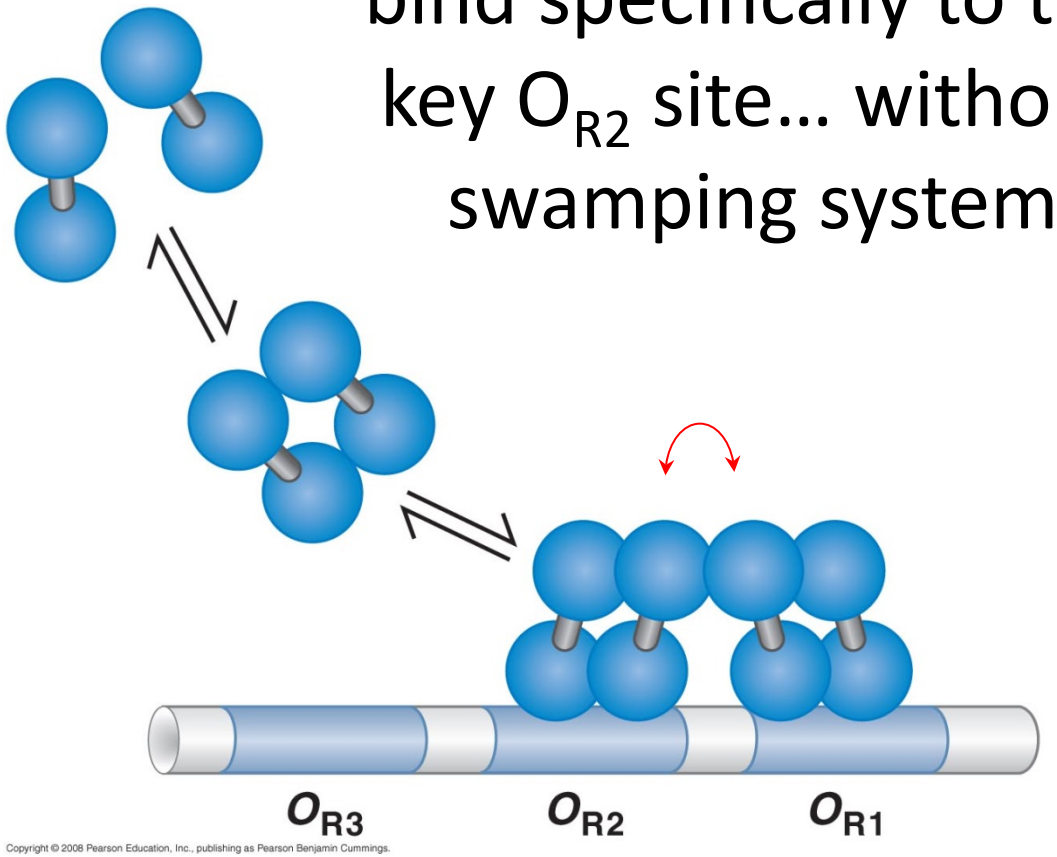
Cro likes O_{R3} 10x better than O_{R1} or O_{R2}

λ Rep likes O_{R1} 10x better than O_{R2} or O_{R3}

Binding of λ repressor to O_{R2} excludes Cro from O_{R3}

Repressor uses weak protein-protein interactions to help it bind specifically to the key O_{R2} site… without swamping system

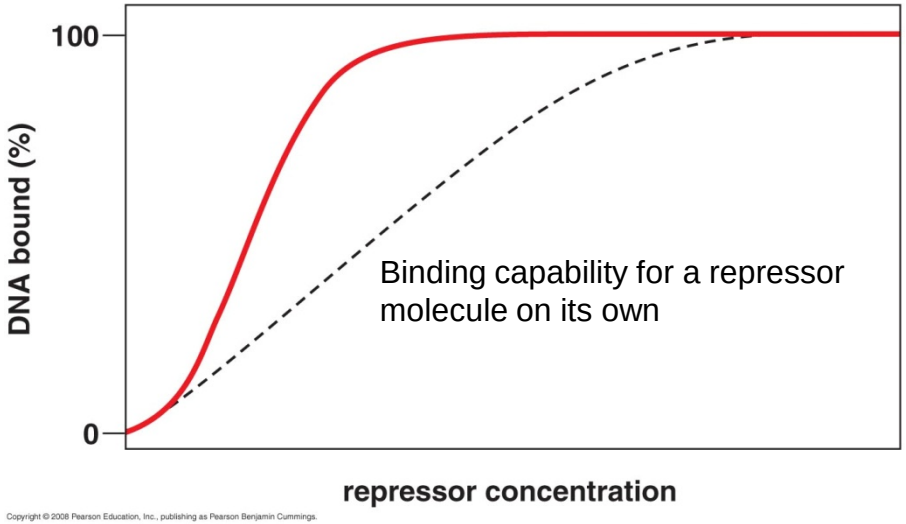




weak strong

Cooperativity in DNA binding by λ repressor at O_{R1} & O_{R2} creates switch-like behavior and helps to maintain lysogeny

Cooperativity: Much steeper binding curve relative to repressor concentration, without increasing background binding to "wrong" sites, if two copies of repressor stabilize each other's binding



Repressor functionality range with vs. without cooperativity

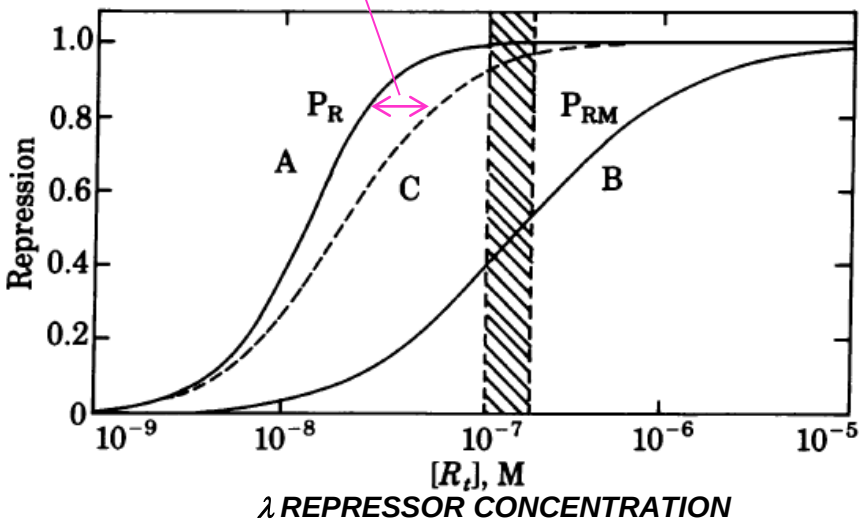
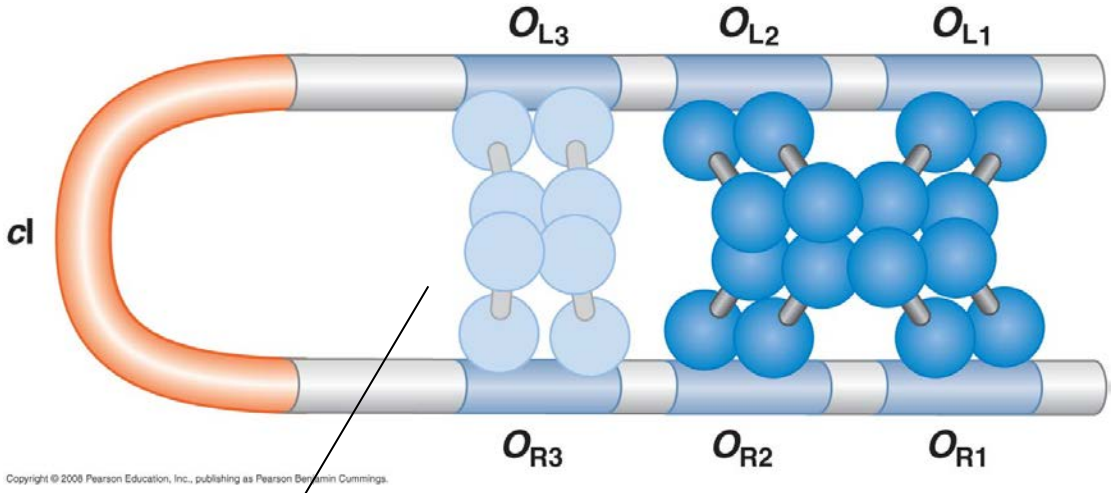


FIG. 2. Predicted repression curves at P_R and P_{RM}. Curve A represents f_{P_R}, the probability that an operator template will have O_R1, O_R2, or both occupied (each of these three configurations shuts off transcription from P_R) as a function of R_t in units of monomer. This curve includes all effects of cooperative interaction between repressor dimers bound to adjacent operator sites and also of repressor dimer dissociation into inactive monomers. Curve B represents $f_{P_{RM}}$, the probability that O_R3 is occupied (required for shutoff of transcription at P_{RM}), including all effects. Curve C, repression curve for P_R devoid of cooperative interactions. ▨, Approximate concentration of repressor in a lysogenic cell.

As λ repressor level rises, it becomes capable of occupying more sites for stable repression of lysis and limitation of its own expression

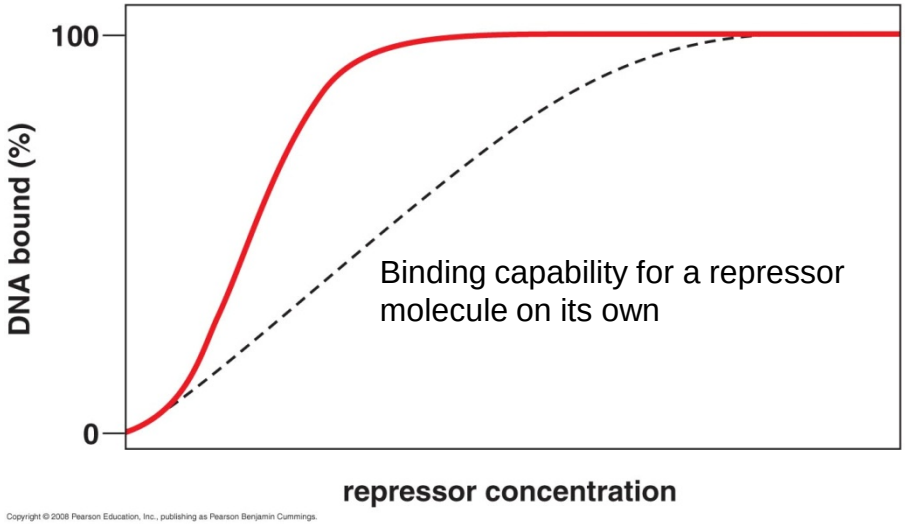


As λ repressor protein levels decrease gradually, OR3 becomes free again and *cI* gene can again be transcribed

Existing λ repressor protein is broken down en masse to switch to lysis mode when cell undergoes DNA damage (destroyed by repair trigger “RecA”)

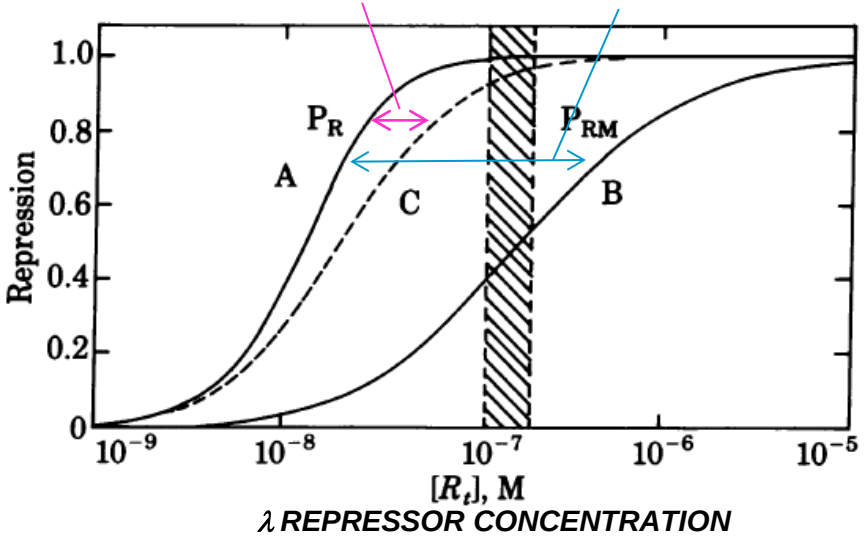
Cooperativity in DNA binding by λ repressor at O_{R1} & O_{R2} creates switch-like behavior and helps to maintain lysogeny

Cooperativity: Much steeper binding curve relative to repressor concentration, without increasing background binding to "wrong" sites, if two copies of repressor stabilize each other's binding



Repressor functionality range with vs. without cooperativity

Working range for repressor before auto-shutoff



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Major take-home messages from λ phage system that apply to many other complex systems (including eukaryotes)

- Transcription factors may be bifunctional… depends on context of sites that they bind to

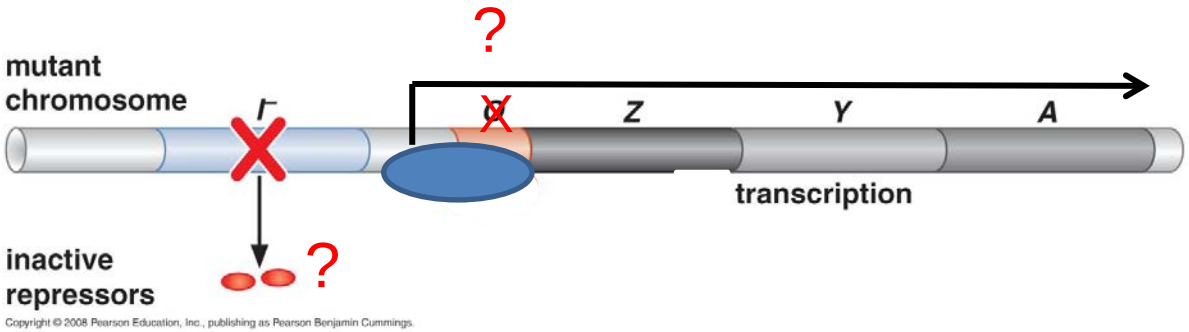
- Binding of multiple copies of a factor to neighboring sites can yield much sharper regulatory responses to transcription factor dose than single binding events… provided that bound factors interact with each other to stabilize (***cooperativity***)

- Biological switches often use mutually antagonistic factors that each enhance their own expression while blocking the others'… causes winner-take-all system behavior

Key concept: cis-trans test
a classic genetic hypothesis testing approach

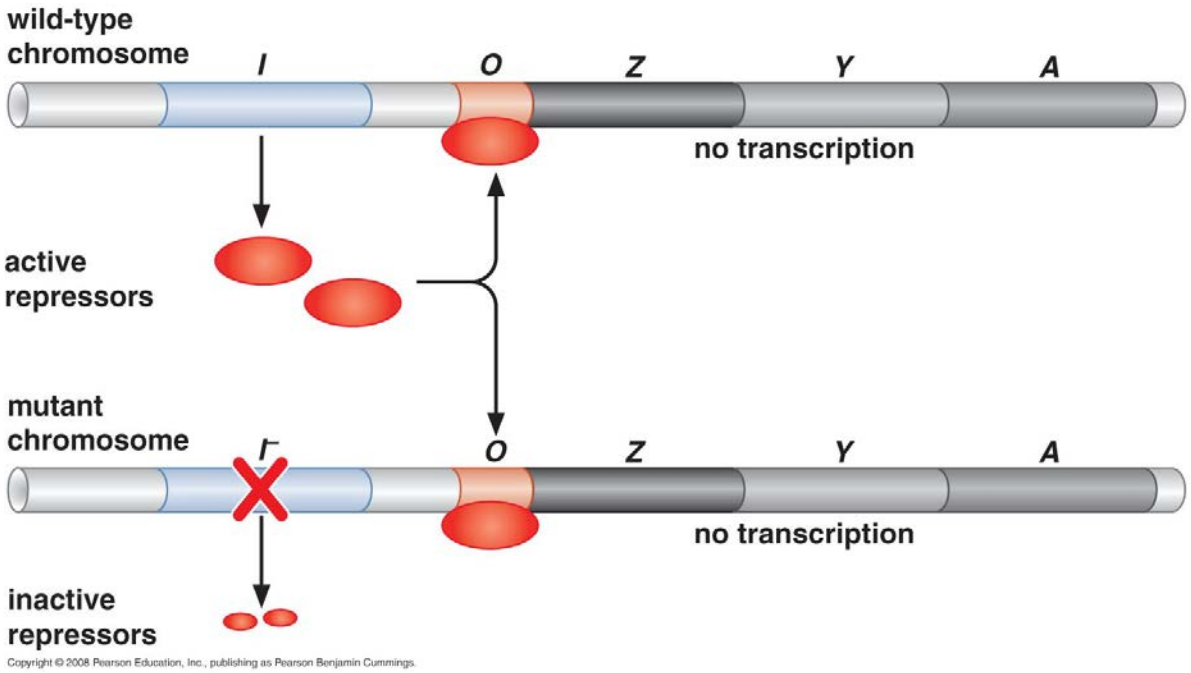
- You have found a mutation that destroys correct regulation of gene X
  - You know that gene X still codes for a protein, but problem is whether RNA for gene X is made correctly
- Is the mutated site tightly linked to the coding sequence of that gene, or in a different part of the genome?
  - Option 1: the problem is in a cis-regulatory element of gene X
  - Option 2: the problem is in a separate gene that codes for a transcription factor that works by binding to the control elements of X
  - Option 3: the problem is in a gene that codes for a signaling component needed to modify (activate/deactivate) some transcription factor for X

Originally this was done with the lac operon…
studying the wildtype function of
REPRESSION



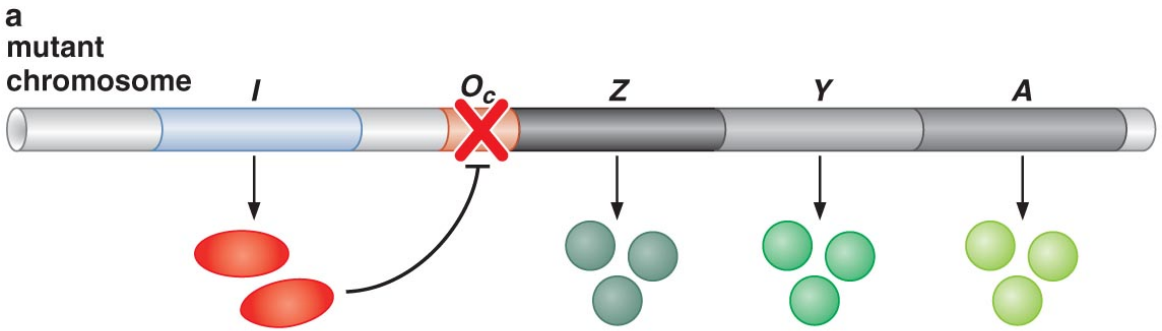
- Elements of strategy
  - You have a collection of mutants that express *lac* genes ALL THE TIME – failure of repression
  - Lac repressor problem or Lac operator problem??
  - Construct plasmid with WILDTYPE version of *lac* operon complex, here including a wildtype copy of the lac repressor gene, *lacI*
  - Make partial diploid by introducing this plasmid into each mutant cell line: is the endogenous gene now correctly controlled?

If wildtype repression wins…



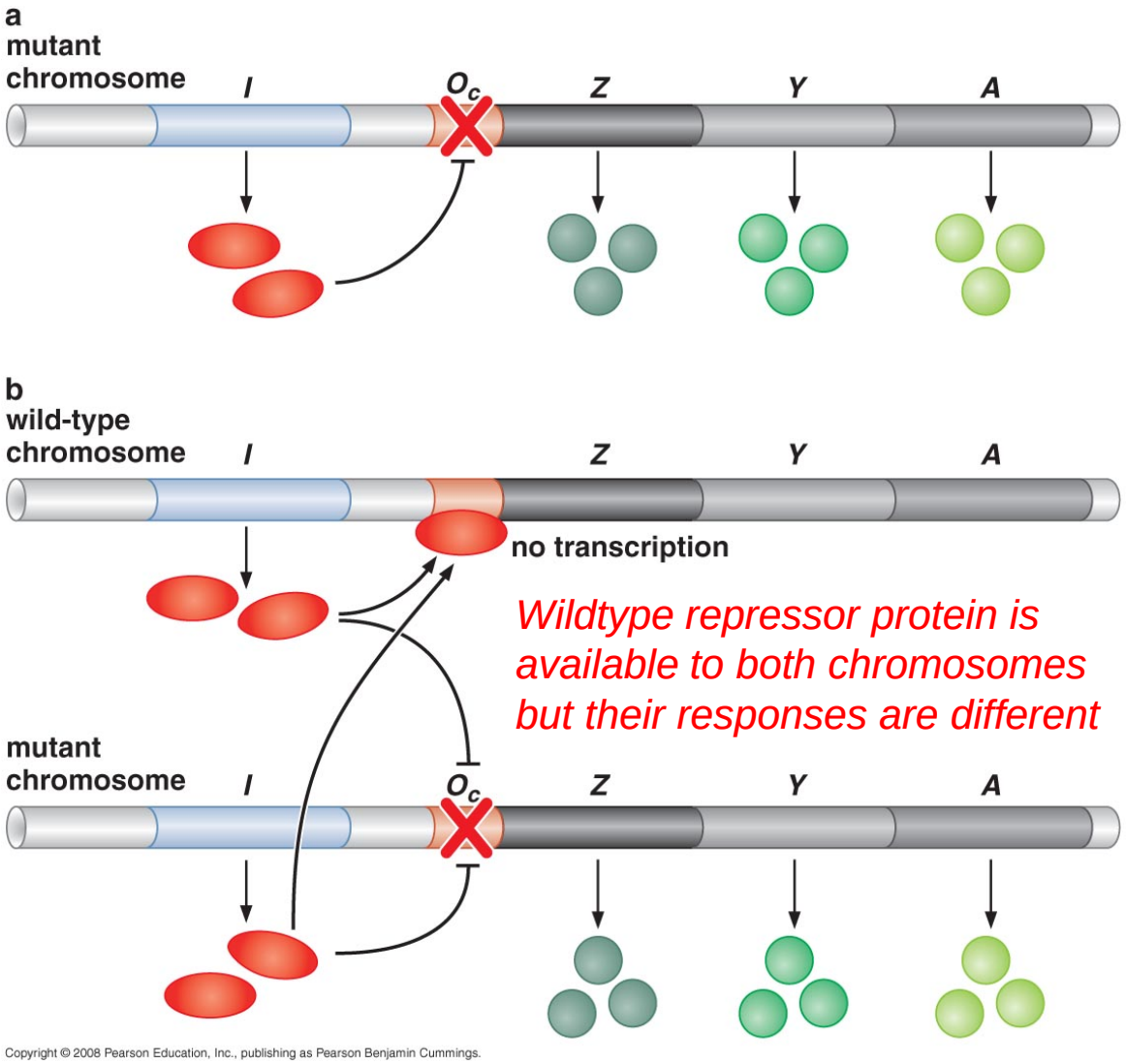
- Neat thing about this strategy
  - Repression by repressor is ***dominant***
  - Therefore addition of wildtype repressor will silence (=“correct”) any *lac* operon, mutant host as well as wildtype transgene
  - Repression occurs as long as the *lacO* cis-regulatory sequence is intact
  - This result proves that the reason for the mutant’s constitutive expression of lac operon was a defective repressor gene

A different result if the defective expression control in mutant is due to defect in cis-reg sequence



A different result if the defective expression control in mutant is due to defect in cis-reg sequence

- Repression is only dominant if repressor has a site to bind to
- Diploid in which mutant locus has cis-regulatory mutation cannot be shut off by wildtype repressor



Wildtype repressor protein is available to both chromosomes but their responses are different

Since you know the wildtype chromosome will be silenced by the wildtype repressor, you can deduce that it's the mutant lac operator that is still defective

Extensions to other systems

- In eukaryotes, most of the interesting cases of regulation are positive regulation
- Ability of a gene to be turned on correctly still depends on BOTH its own cis-regulatory sequences and the presence of the right activator transcription factors (we will discuss more)
- Details of assays are different
  - Usually here the genes encoding the necessary transcription factors are far away in the genome
  - For positive regulation, need to distinguish expression of wildtype allele of “gene X” from expression of mutant’s allele of “gene X”
  - Technical aspects of cis-trans tests are therefore more complex
- Idea – that each gene is controlled by its own linked regulatory sequences, interacting with proteins encoded elsewhere in the genome -- remains the same