

7.1 The *lac* Operon

- The *lac* operon was the first operon discovered
- It contains 3 genes coding for *E. coli* proteins that permit the bacteria to use the sugar lactose
 - Galactoside permease (*lacY*) which transports lactose into the cells
 - β -galactosidase (*lacZ*) cuts the lactose into galactose and glucose
 - Galactoside transacetylase (*lacA*) whose function is unclear

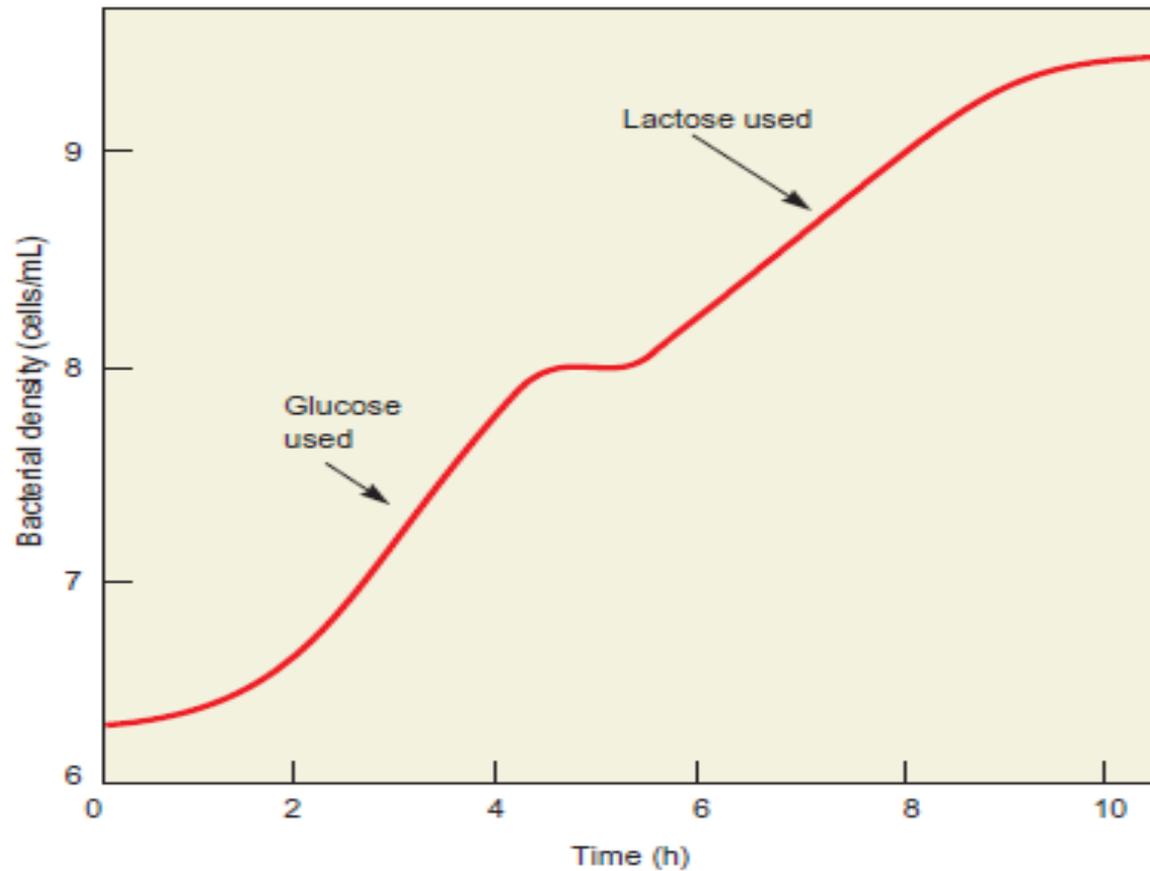


Figure 7.1 Diauxic growth. *E. coli* cells are grown on a medium containing both glucose and lactose, and the bacterial density (number of cells/mL) is plotted versus time in hours. The cells grow rapidly on glucose until that sugar is exhausted, then growth levels off while the cells induce the enzymes needed to metabolize lactose. As those enzymes appear, growth resumes.

Genes of the *lac* Operon

- The genes are grouped together:
 - *lacZ* = β -galactosidase
 - *lacY* = galactoside permease
 - *lacA* = galactoside transacetylase
- All 3 genes are transcribed together producing 1 mRNA, a polycistronic message that starts from a single promoter
 - Each cistron, or gene, has its own ribosome binding site
 - Each cistron can be translated by separate ribosomes that bind independently of each other

Control of the *lac* Operon

- The *lac* operon is tightly controlled, using 2 types of control
 - **Negative control**, like the brake of a car, must remove the repressor from the operator - the “brake” is a protein called the lac repressor
 - **Positive control**, like the accelerator pedal of a car, an activator, additional positive factor responds to low glucose by stimulating transcription of the *lac* operon

Negative Control of the *lac* Operon

- Negative control indicates that the operon is turned on unless something intervenes and stops it
- The off-regulation is done by the *lac* repressor
 - Product of the *lacI* gene
 - Tetramer of 4 identical polypeptides
 - Binds the operator just right of promoter

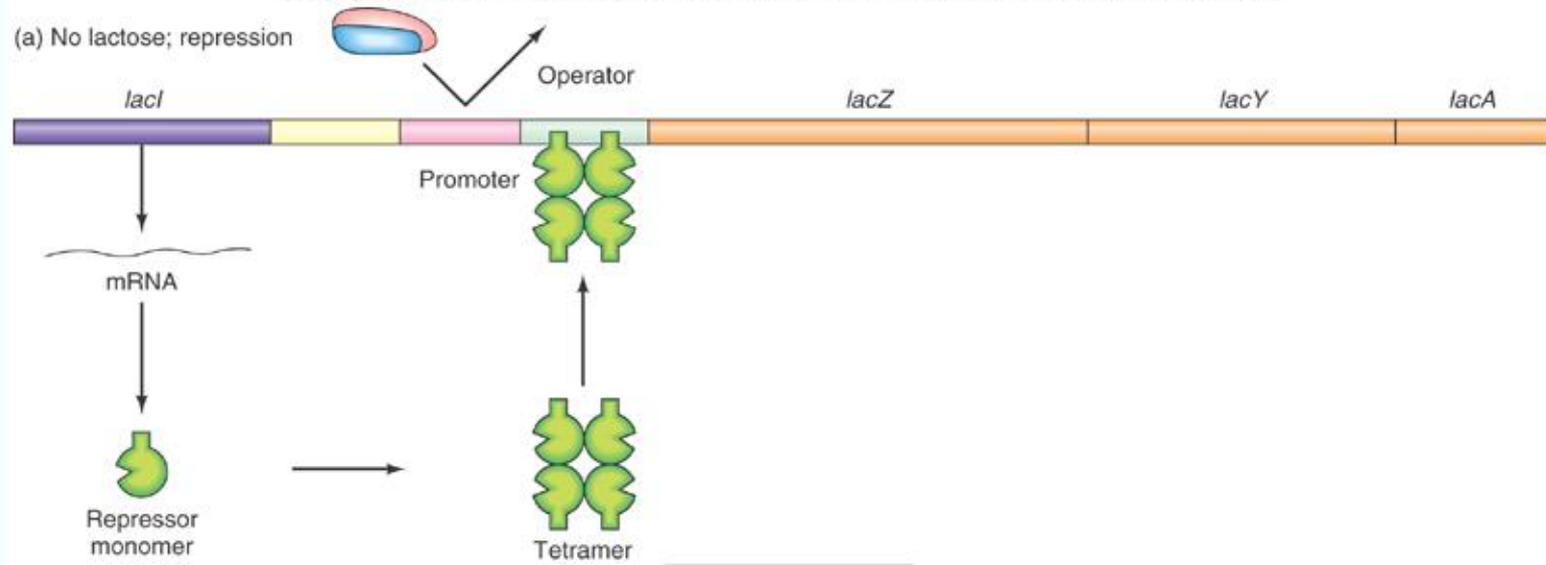
lac Repressor

- When the repressor binds to the operator, the operon is repressed
 - Operator and promoter sequence are contiguous
 - Repressor bound to operator prevents RNA polymerase from binding to the promoter and transcribing the operon
- As long as no lactose is available, the *lac* operon is repressed

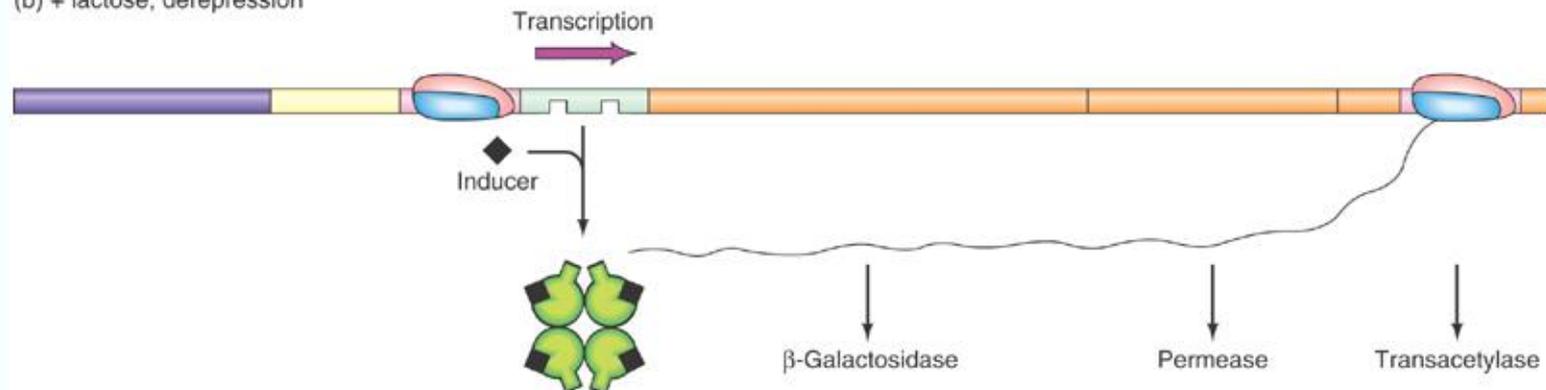
Negative Control of the *lac* Operon

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(a) No lactose; repression



(b) + lactose; derepression

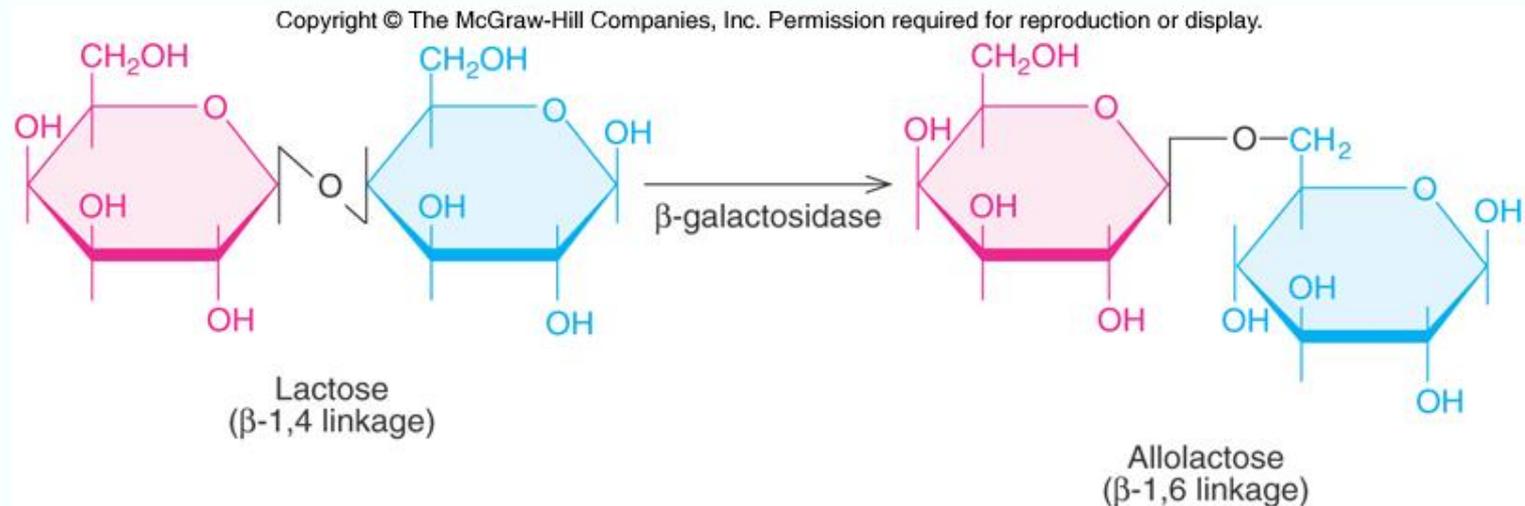


Inducer of the *lac* Operon

- The repressor is an allosteric protein
 - Binding of one molecule to the protein changes shape of a remote site on that protein
 - Altering its interaction with a second molecule
- The inducer binds the repressor
 - Causing the repressor to change conformation that favors release from the operator
- The inducer is allolactose, an alternative form of lactose

Inducer of the *lac* Operon

- The inducer of the *lac* operon binds the repressor
- The inducer is allolactose, an alternative form of lactose



Discovery of the Operon

During the 1940s and 1950s, Jacob and Monod studied the metabolism of lactose by *E. coli*

- Three enzyme activities / three genes were induced together by galactosides
- Constitutive mutants need no induction, genes are active all the time
- Created merodiploids or partial diploid bacteria carrying both wild-type (inducible) and constitutive alleles

Discovery of the Operon

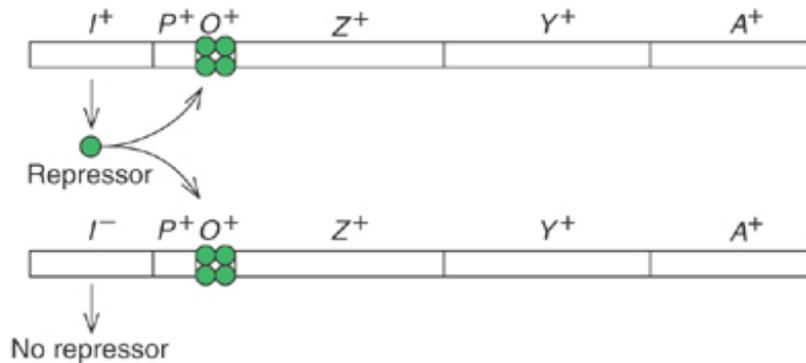
- Using merodiploids or partial diploid bacteria carrying both wild-type and constitutive alleles distinctions could be made by determining whether the mutation was dominant or recessive
- Because the repressor gene produces a repressor protein that can diffuse throughout the cell, it can bind to both operators in a meriploid and is called a ***trans-acting*** gene because it can act on loci on both DNA molecules
- Because an operator controls only the operon on the same DNA molecule it is called a ***cis-acting*** gene

Effects of Regulatory Mutations: Wild-type and Mutated Repressor

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Merodiploid with one wild-type gene and one:

(a) Mutant repressor gene (I^-)



Results

→ No *lac* products in absence of lactose

→ No *lac* products in absence of lactose

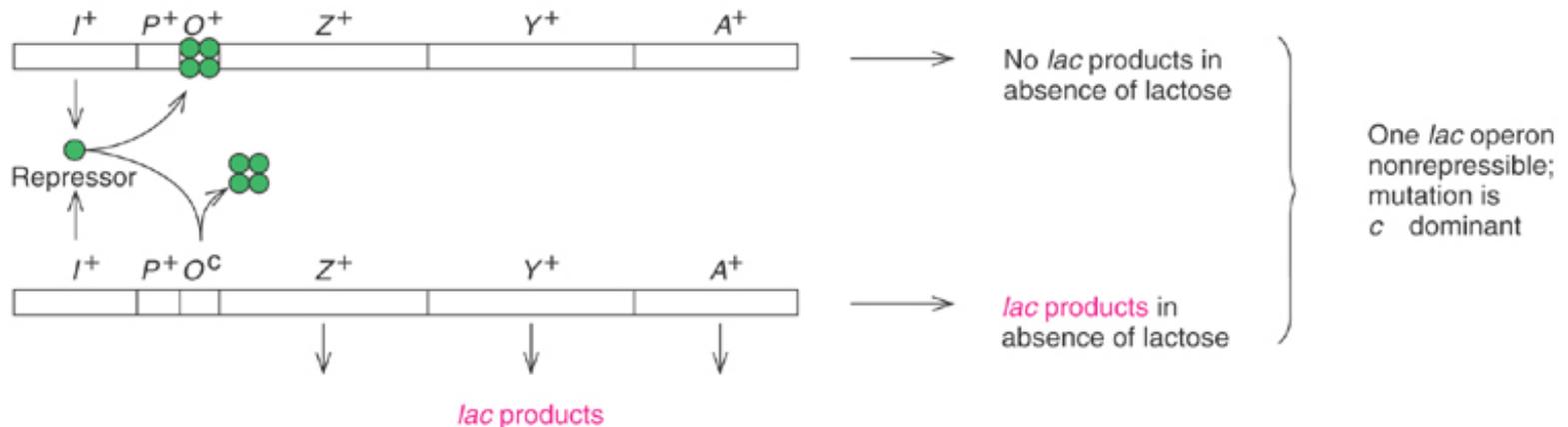
Conclusion

} Both *lac* operons repressible; mutation is recessive

Effects of Regulatory Mutations: Wild-type and Mutated Operator with Defective Binding

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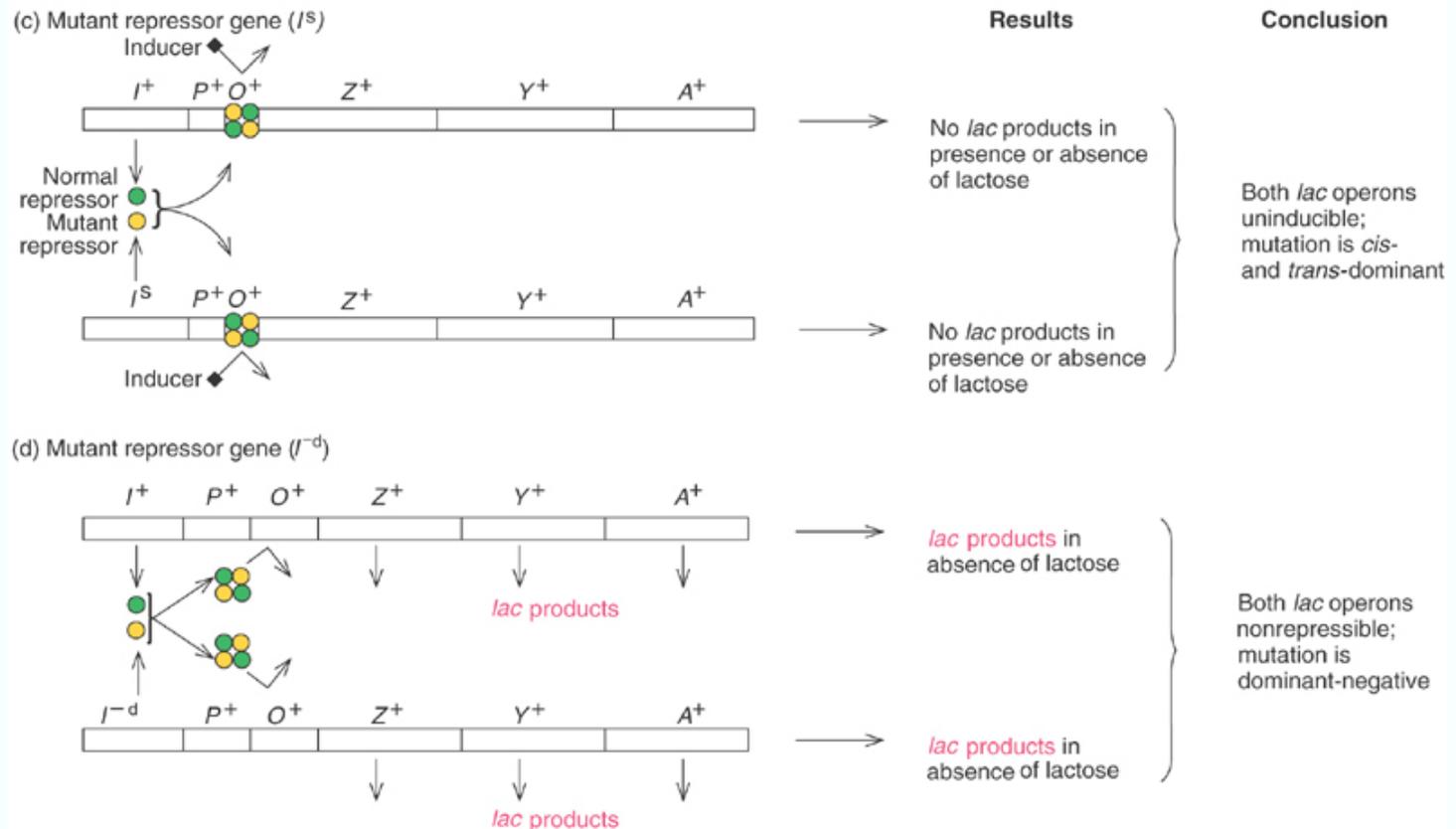
(b) Mutant operator (O^c)



Effects of Regulatory Mutations: Wild-type and Mutated Operon binding Irreversibly

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Merodiploid with one wild-type gene and one:



Jacob and Monod, by skillful genetic analysis, were able to develop the operon concept. They predicted the existence of two key control elements: **the repressor gene** and **the operator**. Deletion mutations revealed a third element (**the promoter**) that was necessary for expression of all three *lac genes*. *Furthermore, they could conclude that all three lac genes (lacZ, Y, and A) were clustered into a single control unit: the lac operon.* Subsequent biochemical studies have amply confirmed Jacob and Monod's beautiful hypothesis.

Repressor-Operator Interactions

Walter Gilbert and Benno Müller-Hill succeeded in partially purifying the *lac repressor*.

The most sensitive assay available to them was binding a labeled synthetic inducer (isopropylthiogalactoside, or IPTG) to the repressor.

But, with a crude extract of wild-type cells, the repressor was in such low concentration that this assay could not detect it.

To get around this problem, Gilbert and Müller-Hill used a mutant *E. coli* strain with a repressor mutation (*lacI^t*) that causes the repressor to bind IPTG more tightly than normal. This tight binding allowed the mutant repressor to bind enough inducer that the protein could be detected even in very impure extracts. Because they could detect the protein, Gilbert and Müller-Hill could purify it.

Melvin Cohn and his colleagues used repressor purified by this technique in operator-binding studies.

- Using a filter-binding assay, the *lac* repressor binds to the *lac* operator

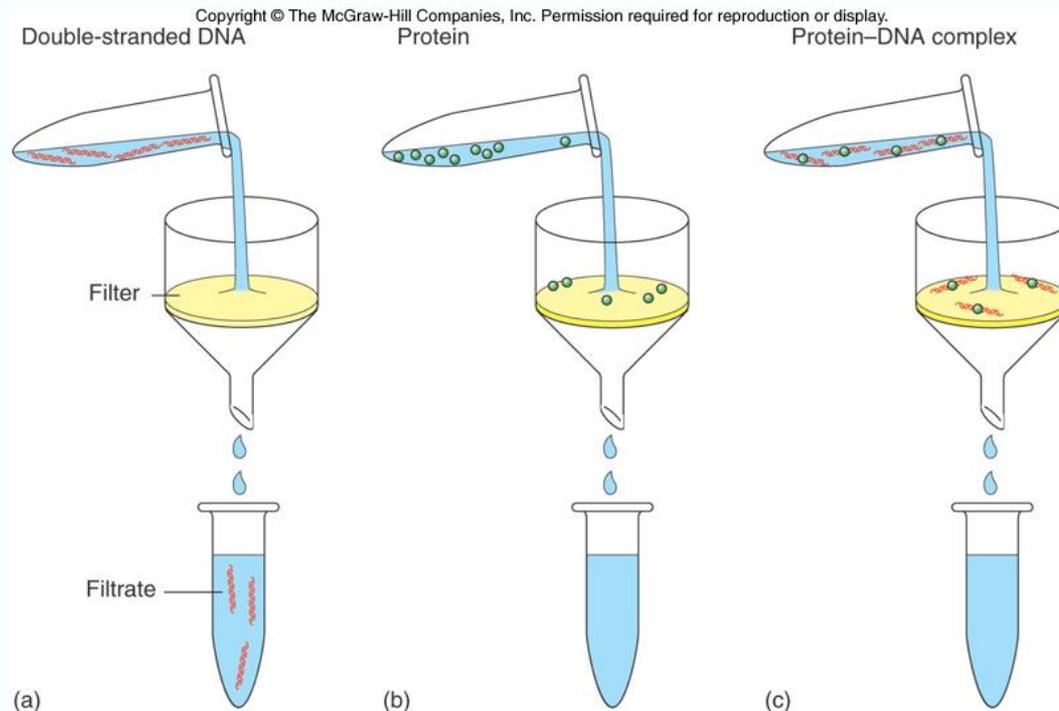
Filter binding is used to measure DNA-protein interaction and based on the fact that double-stranded DNA will not bind by itself to a filter, but a protein-DNA complex will

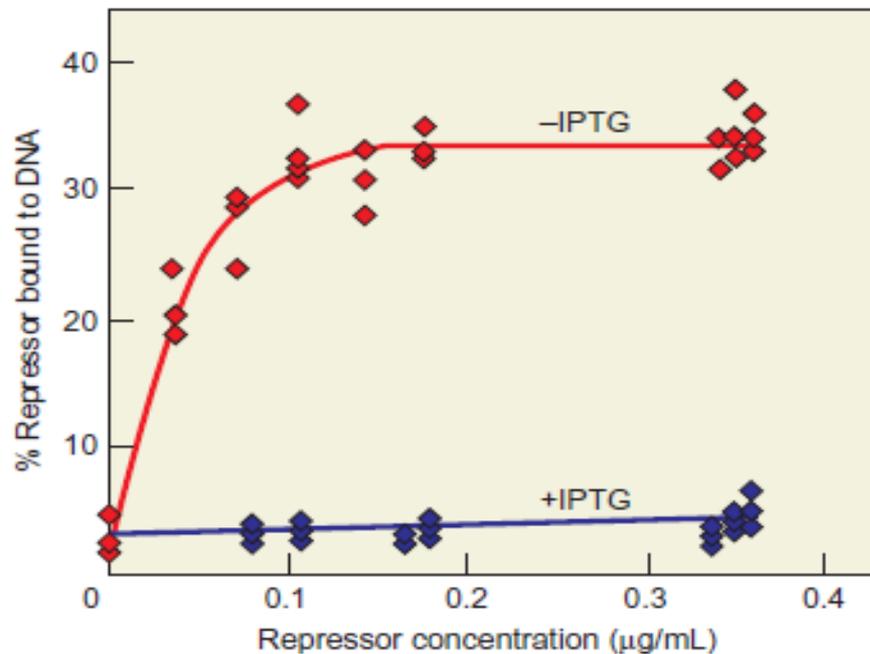
Double-stranded DNA can be labeled and mixed with protein

Assay protein-DNA binding by measuring the amount of label retained on the filter

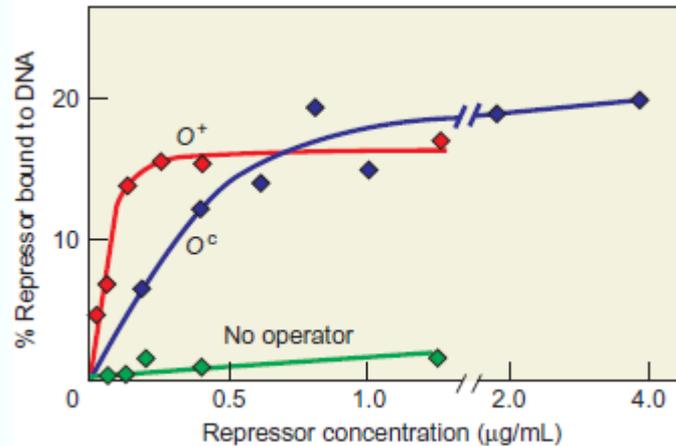
Nitrocellulose Filter-Binding Assay

- dsDNA is labeled and mixed with protein
- Pour dsDNA through a nitrocellulose filter
- Measure amount of radioactivity that passed through filter and retained on filter





Assaying the binding between *lac operator* and *lac* repressor. Cohn and colleagues labeled *lacO*-containing DNA with ^{32}P and added increasing amounts of *lac* repressor. They assayed binding between repressor and operator by measuring the radioactivity attached to nitrocellulose. Only labeled DNA bound to repressor would attach to nitrocellulose. Red: repressor bound in the absence of the inducer IPTG. Blue: repressor bound in the presence of 1 mM IPTG, which prevents repressor–operator binding.



The O^c *lac* operator binds repressor with lower affinity than does the wild-type operator. Cohn and colleagues performed a *lac* operator–repressor binding assay as described in last figure, using three different DNAs as follows: **red, DNA containing a wild type operator (O^+); **blue**, DNA containing an operator-constitutive mutation (O^c) that binds repressor with a lower affinity; **green**, control, If80 DNA, which does not have a *lac* operator.**

Cohn and colleagues demonstrated with a filter-binding assay that *lac repressor binds to lac operator*. Furthermore, this experiment showed that a genetically defined constitutive *lac* operator has lower than normal affinity for the *lac* repressor, demonstrating that the sites defined genetically and biochemically as the operator are one and the same.

The Mechanism of Repression

- The repressor does not block access by RNA polymerase to the *lac* promoter
- Polymerase and repressor can bind together to the *lac* promoter
- Polymerase-promoter complex is in equilibrium with free polymerase and promoter

Ira Pastan and his colleagues had shown as early as 1971 that RNA polymerase could bind tightly to the *lac* promoter, even in the presence of repressor

Susan Straney and Donald Crothers reinforced this view in 1987 by showing that polymerase and repressor can bind together to the *lac promoter*.

If we accept that RNA polymerase can bind tightly to the promoter, even with repressor occupying the operator, how do we explain repression?

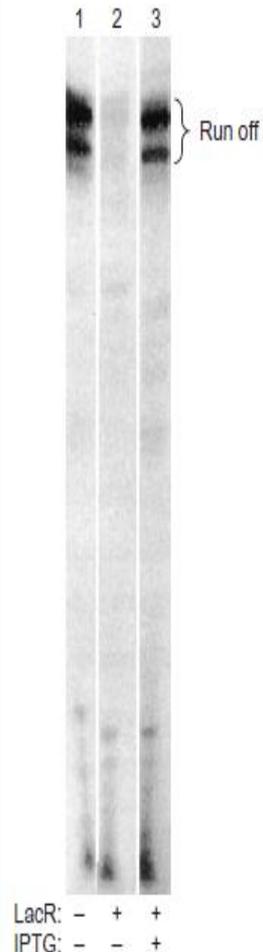
Barbara Krummel and Michael Chamberlin Proposed :
Repressor blocks the transition from the initial transcribing complex state to the elongation state.

In other words, repressor traps the polymerase in a nonproductive state in which it spins its wheels making abortive transcripts without ever achieving promoter clearance.

Jookyung Lee and Alex Goldfarb provided some evidence for this idea. First, they used a run-off transcription Assay to show that RNA polymerase is already engaged on the DNA template, even in the presence of repressor.

The experimental plan was as follows: First, they incubated repressor with a 123-bp DNA fragment containing the *lac* control region plus the beginning of the *lacZ* gene. After allowing 10 min for the repressor to bind to the operator, they added polymerase. Then they added heparin—a polyanion that binds to any RNA polymerase that is free or loosely bound to DNA and keeps it from binding to DNA. They also added all the remaining components of the RNA polymerase reaction except CTP.

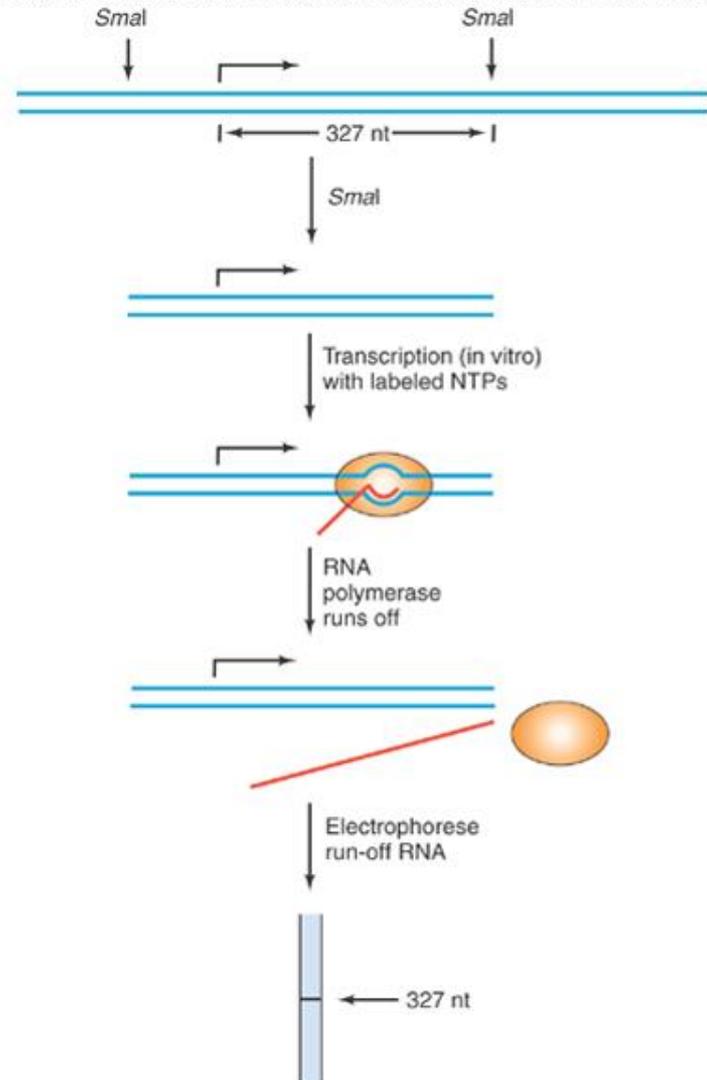
Finally, they added labeled CTP with or without the inducer IPTG. The question is this: Will a run-off transcript be made? If so, the RNA polymerase has formed a heparin resistant (open) complex with the promoter even in the presence of the repressor. In fact, the run-off transcript did appear, just as if repressor had not been present. Thus, under these conditions in vitro, repressor does not seem to inhibit tight binding between polymerase and the *lac promoter*.



Run-Off Transcription

- A good assay to measure the rate of in vitro transcription
- DNA fragment containing gene to transcribe is cut with restriction enzyme in middle of transcription region
- Transcribe the truncated fragment in vitro using labeled nucleotides, as polymerase reaches truncation it “runs off” the end
- Measure length of run-off transcript compared to location of restriction site at 3'-end of truncated gene

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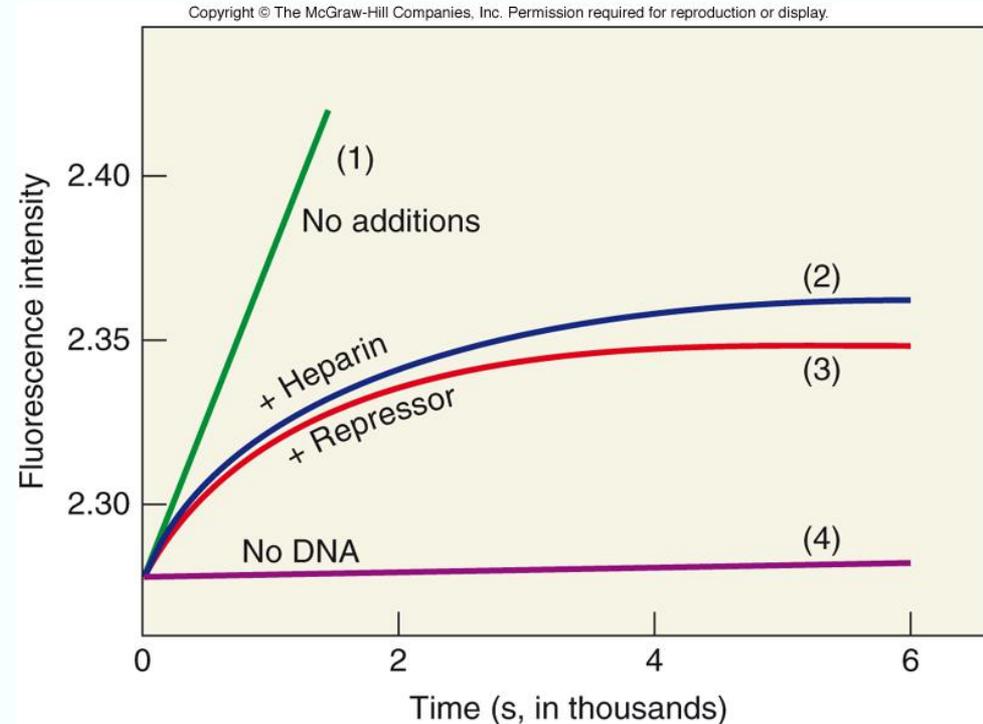
Record and colleagues

Made complexes between RNA polymerase and DNA containing the *lac* promoter–operator region. Then they allowed the complexes to synthesize abortive transcripts in the presence of a UTP analog

fluorescently labeled in the γ -phosphate. As the polymerase incorporates UMP from this analog into transcripts, the labeled pyrophosphate released increases in fluorescence intensity

lac Repressor and Dissociation of RNA Polymerase from *lac* Promoter

- Without competitor, dissociated polymerase returns to promoter
- Heparin and repressor prevent reassociation of polymerase and promoter
- Repressor prevents reassociation by binding to the operator adjacent to the promoter
- This blocks access to the promoter by RNA polymerase

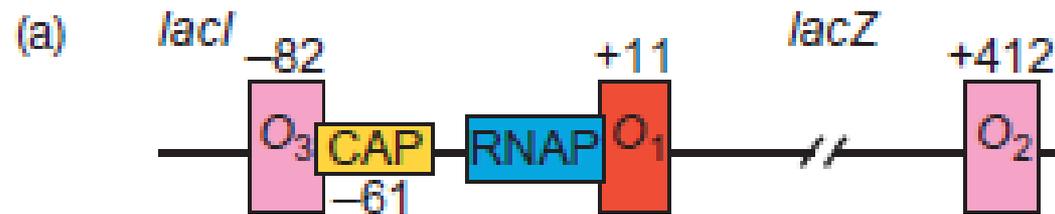


Mechanism Summary

- Two competing hypotheses of mechanism for repression of the *lac* operon
 - RNA polymerase can bind to lac promoter in presence of repressor
 - Repressor will inhibit transition from abortive transcription to processive transcription
 - The repressor, by binding to the operator, blocks access by the polymerase to adjacent promoter

lac Operators

- There are three *lac* operators
 - The major *lac* operator lies adjacent to promoter
 - Two auxiliary *lac* operators - one upstream and the other downstream
- All three operators are required for optimum repression
- The major operator produces only a modest amount of repression



(b)

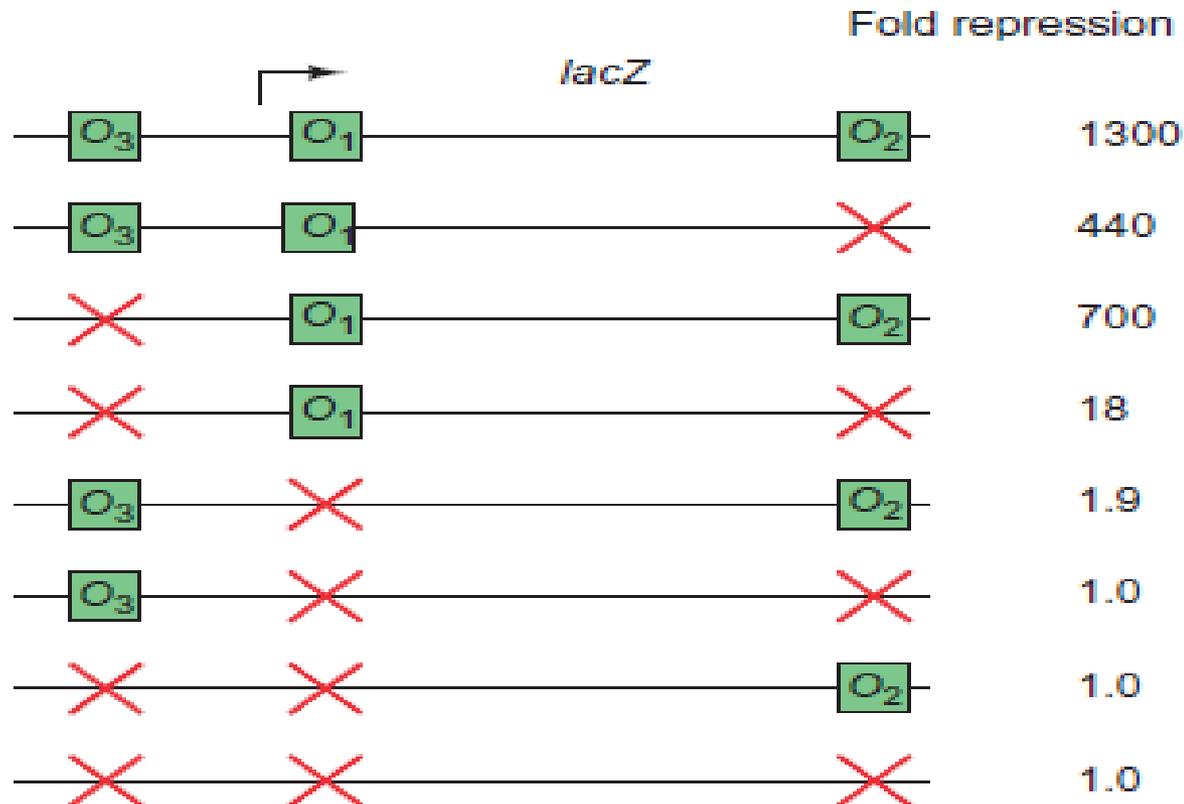
O_1	5'	AATTGTGAGCGGATAACAATT	3'
O_2	5'	AAaTG T GAGCGagTAACAacc	3'
O_3	5'	ggcaGT G GAGCGcAacgCAATT	3'

Figure 7.10 The three *lac* operators. (a) Map of the *lac* control region. The major operator (O_1) is shown in red; the two auxiliary operators are shown in pink. The CAP and RNA polymerase binding sites are in yellow and blue, respectively. CAP is a positive regulator of the *lac* operon discussed in the next section of this chapter.

(b) Sequences of the three operators. The sequences are aligned, with the central G of each in boldface. Sites at which the auxiliary operator sequences differ from the major operator are lower case in the O_2 and O_3 sequences.

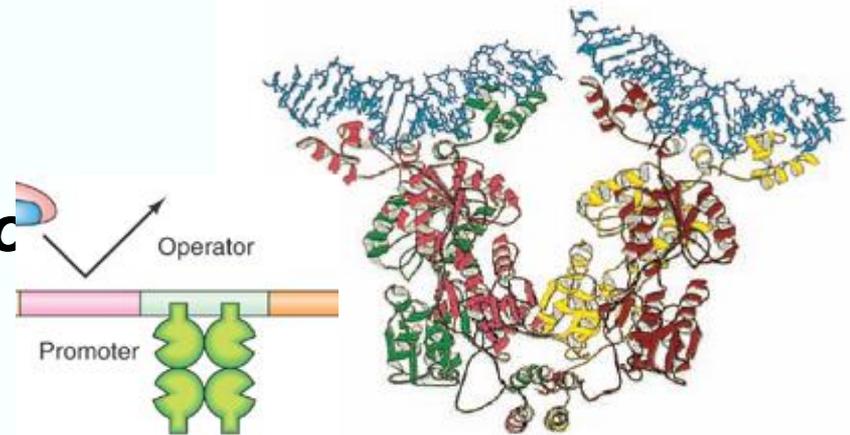
Effects of mutations in the three *lac* operators. Müller-

Hill and colleagues placed wild-type and mutated *lac operon fragments* on λ phage DNA and allowed these DNAs to lysogenize *E. coli* cells (Chapter 8). This introduced these *lac* fragments, containing the three operators, the *lac* promoter, and the *lacZ* gene, into the cellular genome. The cell contained no other *lacZ* gene, but it had a wild-type *lacI* gene. Then Müller-Hill and coworkers assayed for β -galactosidase produced in the presence and absence of the inducer IPTG.



- In 1996, Mitchell Lewis and coworkers provided a structural basis for this cooperativity among operators.
- They determined the crystal structure of the *lac* repressor and its complexes with 21-bp DNA fragments containing operator sequences.

Structure of the *lac* repressor tetramer bound to two operator fragments.



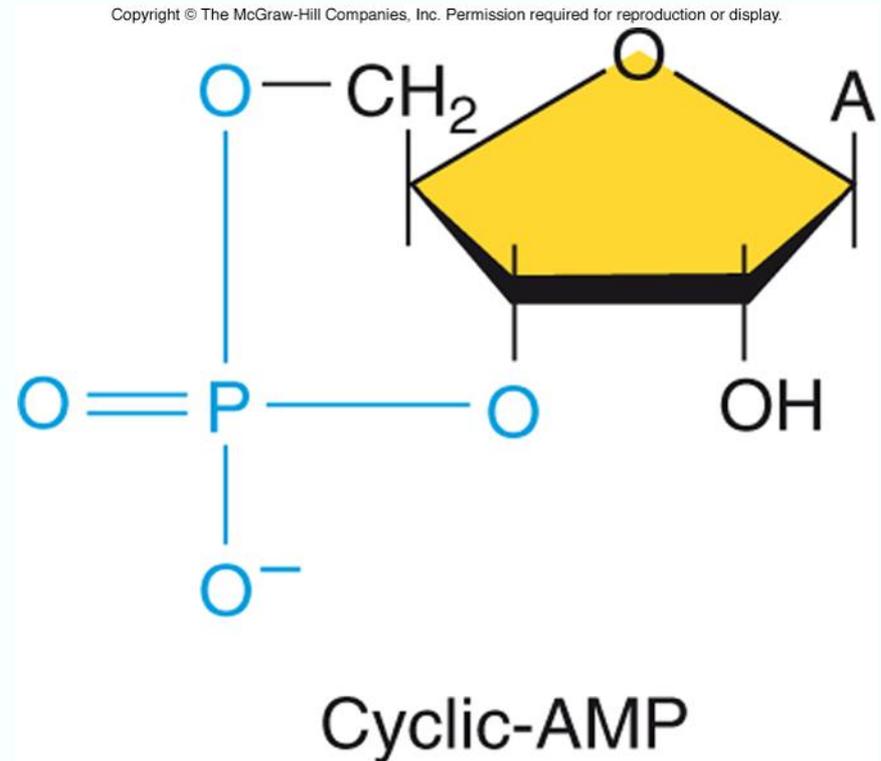
The structure presents the four repressor monomers in pink, green, yellow, and red, and the DNA fragments in blue. Two repressor dimers interact with each other at bottom to form tetramers. Each of the dimers contains two DNA-binding domains that can be seen interacting with the DNA major grooves at top. The structure shows clearly that the two dimers can bind independently to separate *lac* operators.

Catabolite Repression of the *lac* Operon

- When glucose is present, the *lac* operon is in a relatively inactive state
- Selection in favor of glucose attributed to role of a breakdown product, catabolite
- Process known as catabolite repression uses a breakdown product to repress the operon

Positive Control of *lac* Operon

- Positive control of the *lac* operon by a substance sensing lack of glucose that responds by activating the *lac* promoter
 - The concentration of a nucleotide, cyclic-AMP (cAMP), rises as the concentration of glucose drops



Catabolite Activator Protein

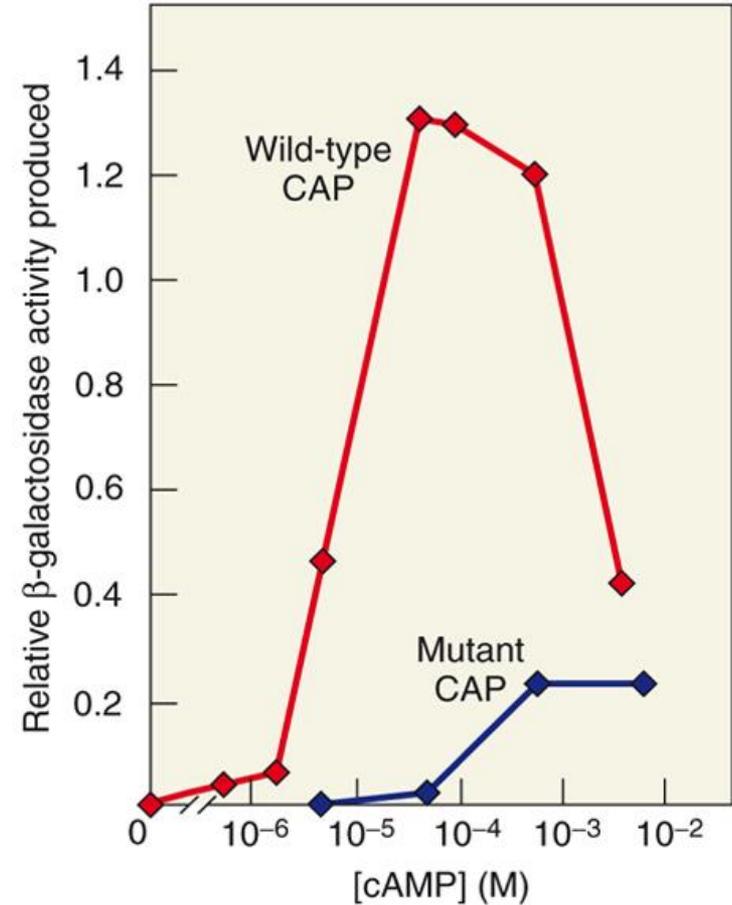
- cAMP added to *E. coli* can overcome catabolite repression of the *lac* operon
- The addition of cAMP leads to the activation of the *lac* gene even in the presence of glucose
- Positive controller of *lac* operon has 2 parts:
 - cAMP
 - A protein factor is known as:
 - Catabolite activator protein or **CAP** by Geoffrey Zubay and coworkers
 - Cyclic-AMP receptor protein or **CRP** by Pastan and colleagues
 - Gene encoding this protein is *crp*

Stimulation of *lac* Operon

CAP-cAMP complex positively controls the activity of β -galactosidase

- CAP binds cAMP tightly
- Mutant CAP not bind cAMP tightly prepared
- Compare activity and production of β -galactosidase using both complexes
- Low activity with mutant CAP-cAMP

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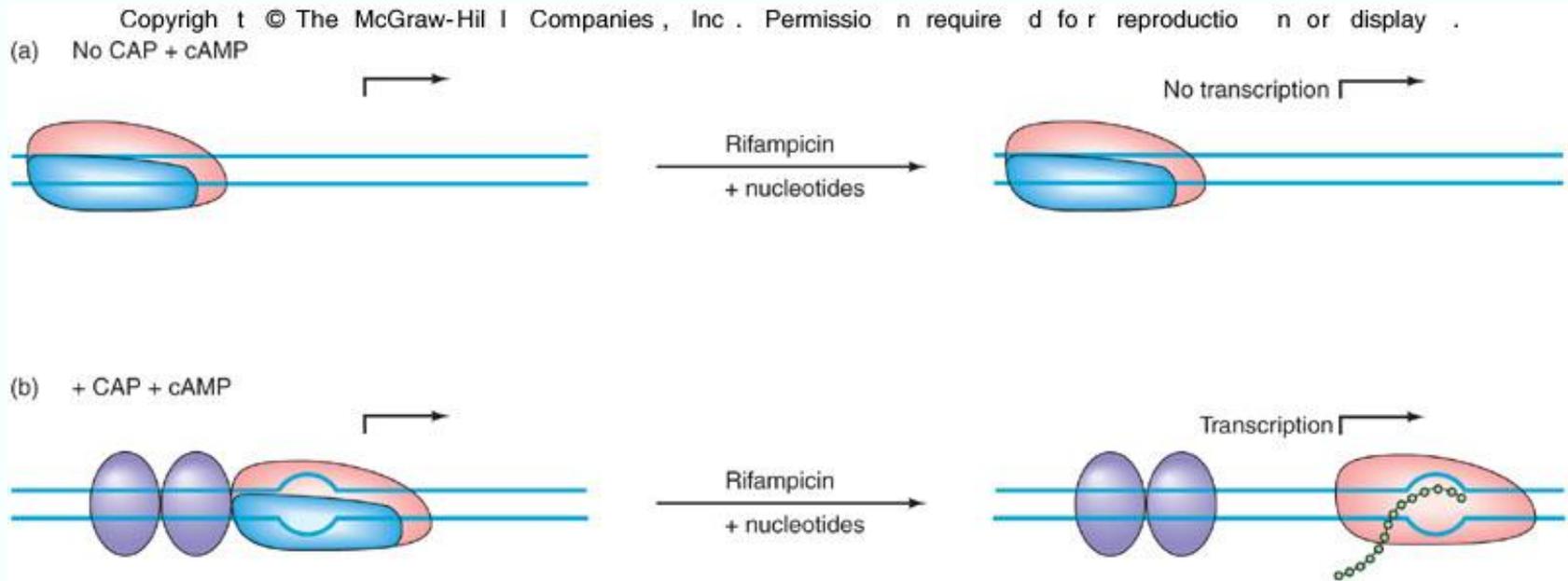


The Mechanism of CAP Action

- The CAP-cAMP complex binds to the *lac* promoter
 - Mutants whose *lac* gene is not stimulated by complex had the mutation in the *lac* promoter
 - Mapping the DNA has shown that the activator-binding site lies just upstream of the promoter
- Binding of CAP and cAMP to the activator site helps RNA polymerase form an open promoter complex

CAP plus cAMP allow formation of an open promoter complex

- The open promoter complex does not form, even if RNA polymerase has bound the DNA, unless the CAP-cAMP complex is also bound



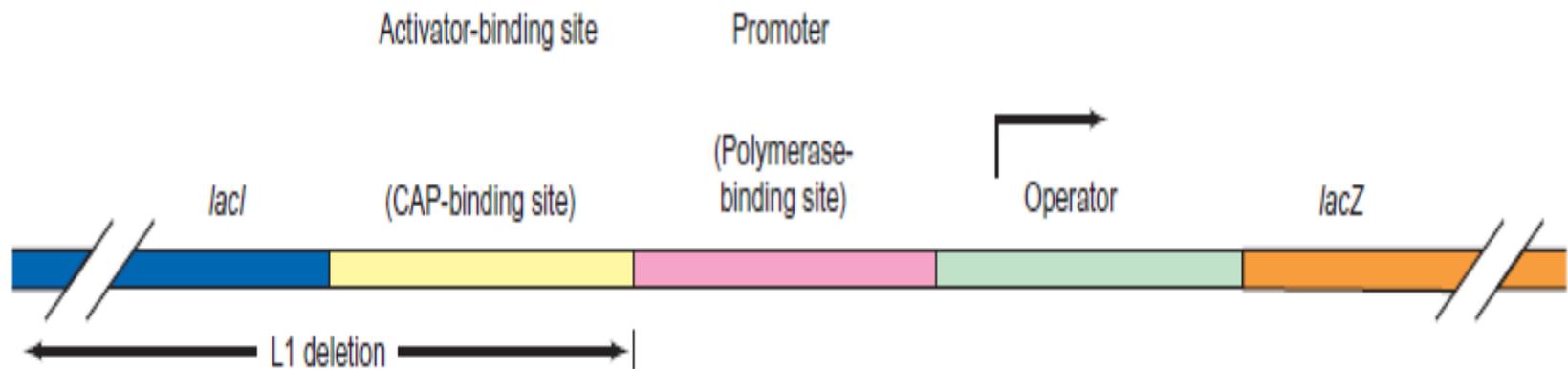


Figure 7.16 The *lac* control region. The activator-promoter region, just upstream of the operator, contains the activator-binding site, or CAP-binding site, on the left (yellow) and the promoter, or polymerase-binding site, on the right (pink). These sites have been defined by footprinting experiments and by genetic analysis. An

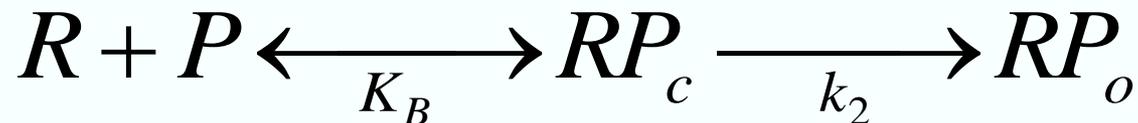
example of the latter approach is the L1 deletion, whose right-hand end is shown. The L1 mutant shows basal transcription of the *lac* operon, but no stimulation by CAP and cAMP. Thus, it still has the promoter, but lacks the activator-binding site.

CAP

- Binding sites for CAP in *lac*, *gal* and *ara* operons all contain the sequence TGTGA
 - Sequence conservation suggests an important role in CAP binding
 - Binding of CAP-cAMP complex to DNA is tight
- CAP-cAMP activated operons have very weak promoters
 - Their -35 boxes are quite unlike the consensus sequence
 - If these promoters were strong they could be activated even when glucose is present

Recruitment

- CAP-cAMP recruits polymerase to the promoter in two steps:
 - Formation of the closed promoter complex
 - Conversion of the closed promoter complex into the open promoter complex



- where R is RNA polymerase, P is the promoter, RP_c is the closed promoter complex, and RP_o is the open promoter complex.

McClure and coworkers devised kinetic methods of distinguishing between the two steps and determined that CAP-cAMP acts directly to stimulate the first step by increasing K_B . CAP-cAMP has little if any effect on k_2

- CAP-cAMP bends its target DNA by about 100° when it binds

How does binding CAP–cAMP to the activator-binding site facilitate binding of polymerase to the promoter?

One long-standing hypothesis is that CAP and RNA polymerase actually touch as they bind to their respective DNA targetsites and therefore they bind cooperatively.

This hypothesis has much experimental support.

First, CAP and RNA polymerase cosediment on ultracentrifugation in the presence of cAMP, suggesting that they have an affinity for each other.

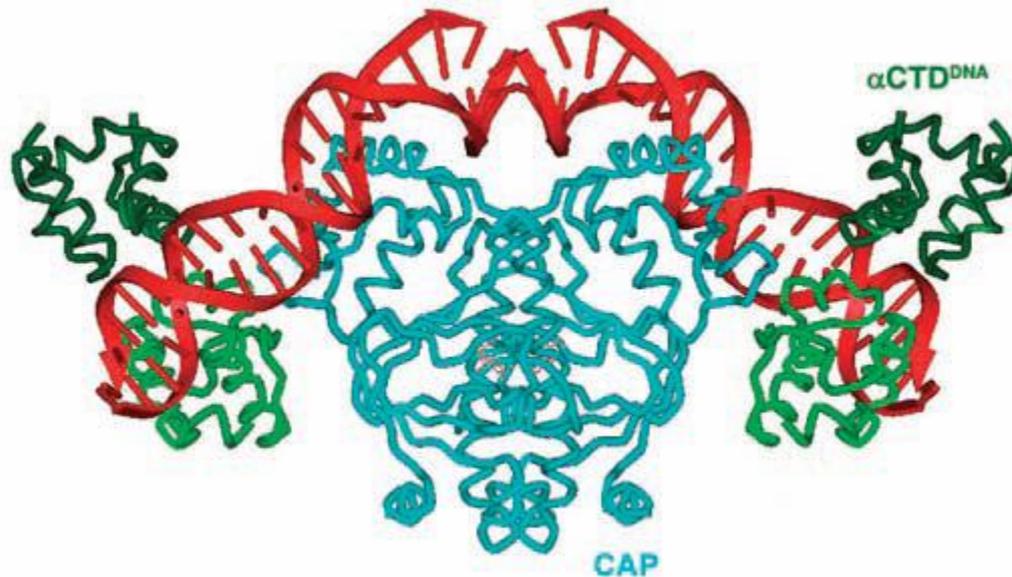
Second, CAP and RNA polymerase, when both are bound to their DNA sites, can be chemically cross-linked to each other, suggesting that they are in close proximity.

Third, DNase footprinting experiments show that the CAP–cAMP footprint lies adjacent to the polymerase footprint. Thus, the DNA binding sites for these two proteins are close enough that the proteins could interact with each other as they bind to their DNA sites.

Fourth, several CAP mutations decrease activation without affecting DNA binding (or bending), and some of these mutations alter amino acids in the region of CAP (**activation region I [ARI]**) that is thought to interact with polymerase.

Fifth, the polymerase site that is presumed to interact with ARI on CAP is the carboxyl terminal domain of the α -subunit (the α CTD), and deletion of the α CTD prevents activation by CAP–cAMP.

Sixth, Richard Ebright and colleagues performed x-ray crystallography in 2002 on a complex of DNA, CAP– cAMP, and the α CTD of RNA polymerase. They showed that the ARI site on CAP and the α CTD do indeed touch in the crystal structure, although the interface between the two proteins is not large.



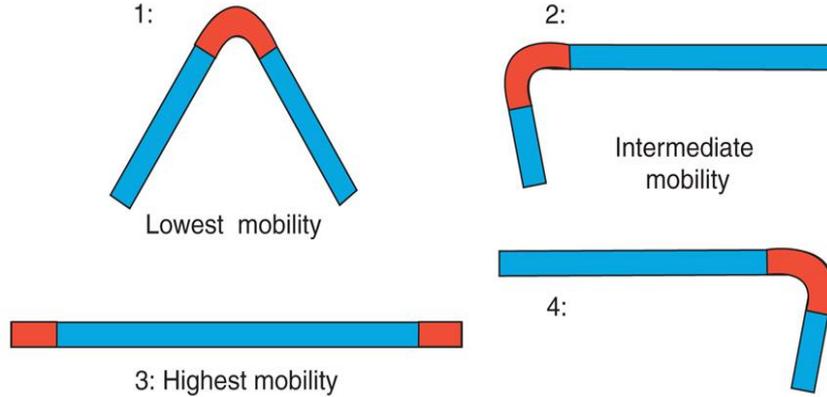
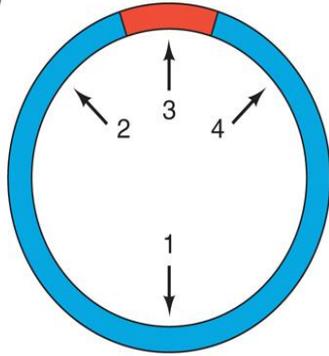
Crystal structures of the CAP–cAMP– α CTD–DNA complex

DNA is in red, CAP is in cyan, with cAMP represented by thin red lines, α CTD^{DNA} is in dark green, and α CTD^{CAP,DNA} is in light green.

CAP-cAMP Promoter Complexes

Fig. 7.18
(a)

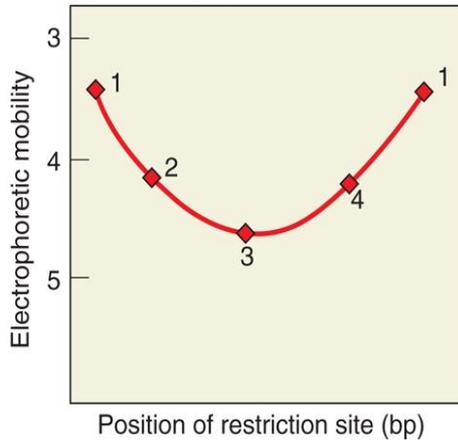
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(b)



(b) Results of cutting DNA in panel (a) with each restriction enzyme, then adding a DNA binding protein, which bends DNA.

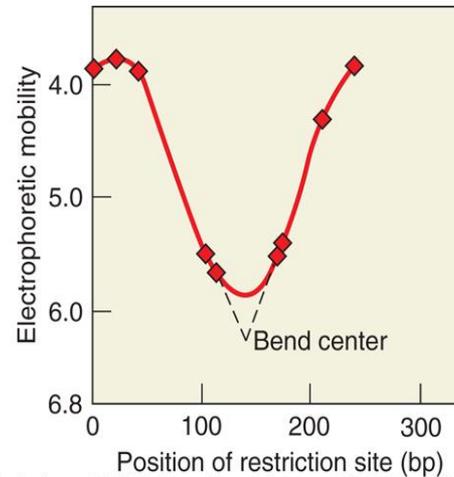
(c)

Theoretical:



(d)

Electrophoresis results:



(d) Actual electrophoresis results

© Wu, H.M., and D.M. Crothers, The locus of sequence-directed and protein-induced DNA bending. Nature 308:511, 1984.

(c) Theoretical curve showing the relationship between electrophoretic mobility and bent DNA, with the bend at various sites along the DNA.

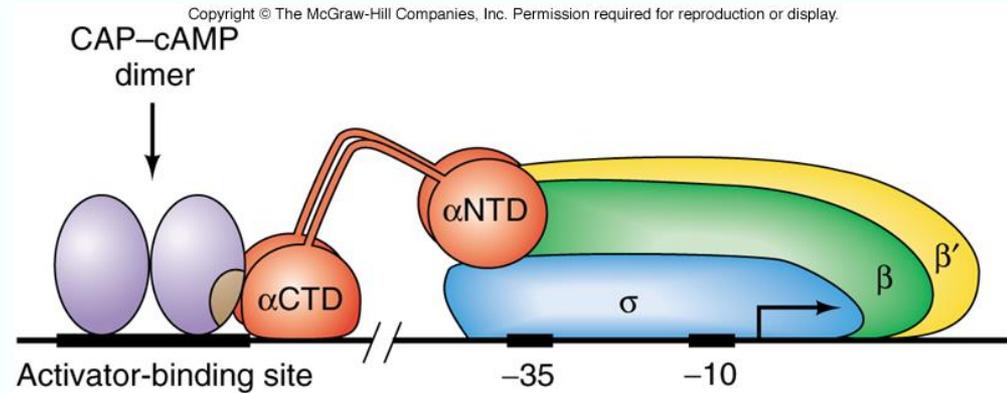
Table 7.2 Activation of *lac* P1 Transcription by CAP–cAMP

Enzyme	Transcripts (cpm)				P1/UV5 (%)		Activation (fold)
	–cAMP–CAP		+cAMP–CAP		–cAMP–CAP	+cAMP–CAP	
	P1	UV5	P1	UV5			
α-WT	46	797	625	748	5.8	83.6	14.4
α-256	53	766	62	723	6.9	8.6	1.2
α-235	51	760	45	643	6.7	7.0	1.0

results of run-off transcription from a CAP–cAMP-dependent *lac promoter* (*P1*) and a CAP–cAMP-independent *lac promoter* (*lacUV5*) with reconstituted polymerases containing the wild-type or truncated α-subunits in the presence and absence of CAP–cAMP.

Proposed CAP-cAMP Activation of *lac* Transcription

- The CAP-cAMP dimer binds to its target site on the DNA
- The α CTD (α -carboxy terminal domain) of polymerase interacts with a specific site on CAP
- Binding is strengthened between promoter and polymerase



Summary

- CAP-cAMP binding to the *lac* activator-binding site recruits RNA polymerase to the adjacent *lac* promoter to form a closed complex
- CAP-cAMP causes recruitment through protein-protein interaction with the α CTD of RNA polymerase

7.2 The *ara* Operon

- The *ara* operon of *E. coli* codes for enzymes required to metabolize the sugar arabinose
- It is another catabolite-repressible operon

The *araCBAD* Operon

The *ara* operon is also called the *araCBAD* operon for its 4 genes

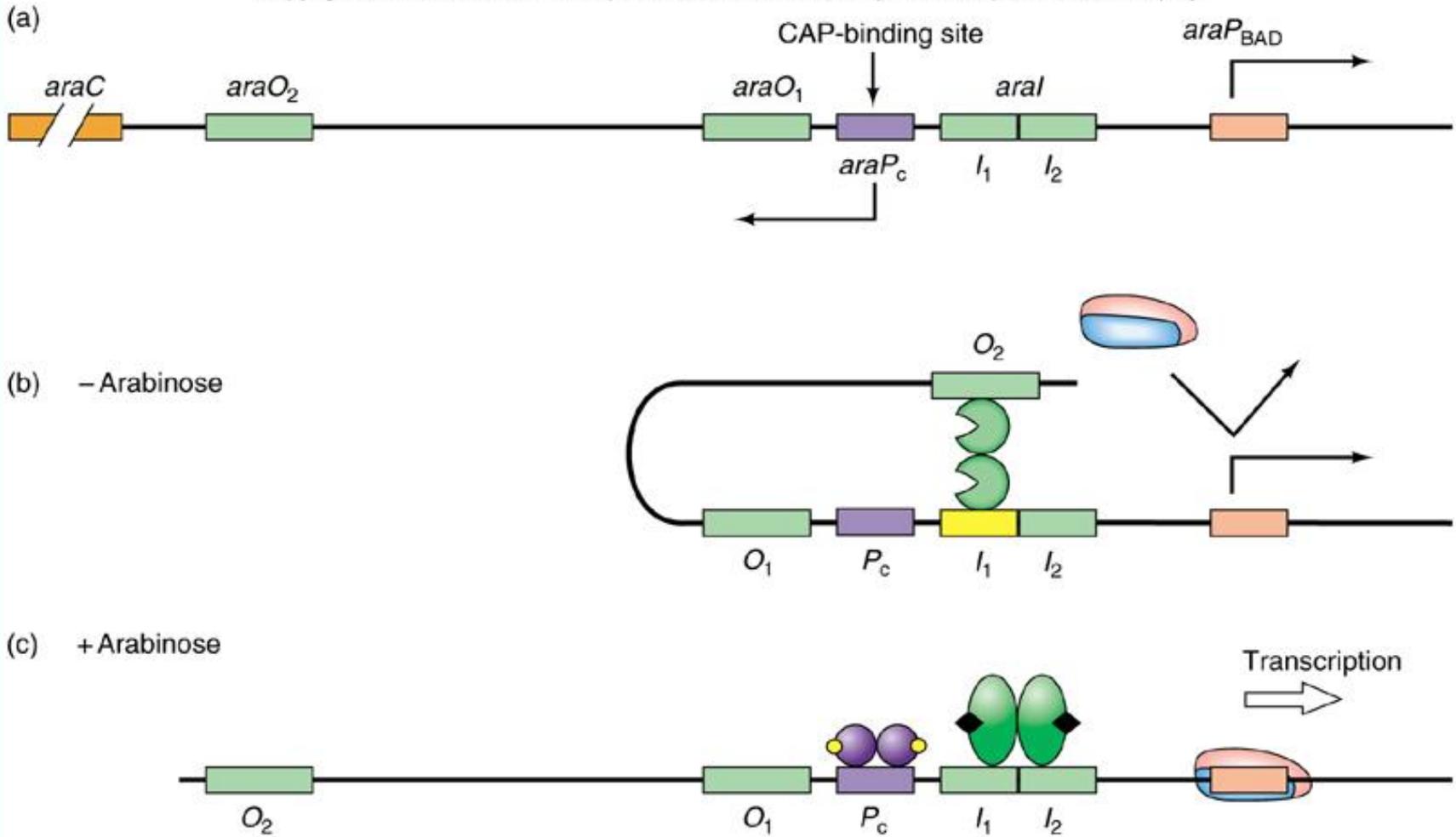
- Three genes, *araB*, *A*, and *D*, encode the arabinose metabolizing enzymes
- These are transcribed rightward from the promoter *araP*_{BAD}
- Other gene, *araC*
 - Encodes the control protein AraC
 - Transcribed leftward from the *araP*_c promoter

AraC Control of the *ara* Operon

- In absence of arabinose, no araBAD products are needed, AraC exerts negative control
 - Binds to *araO*₂ and *araI*₁
 - Loops out the DNA in between
 - Represses the operon
- Presence of arabinose, AraC changes conformation
 - It can no longer bind to *araO*₂
 - Occupies *araI*₁ and *araI*₂ instead
 - Repression loop broken
 - Operon is derepressed

Control of the *ara* Operon

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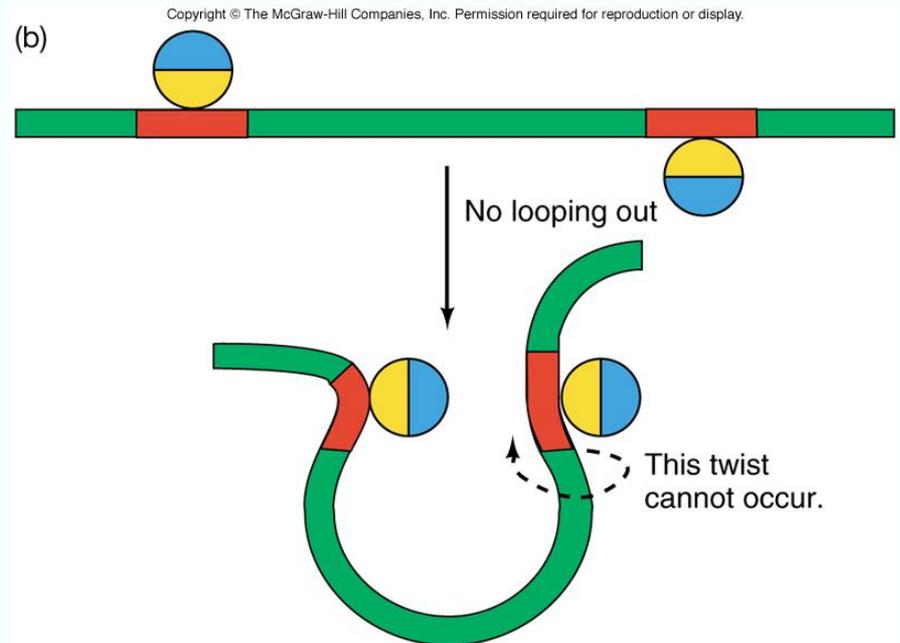
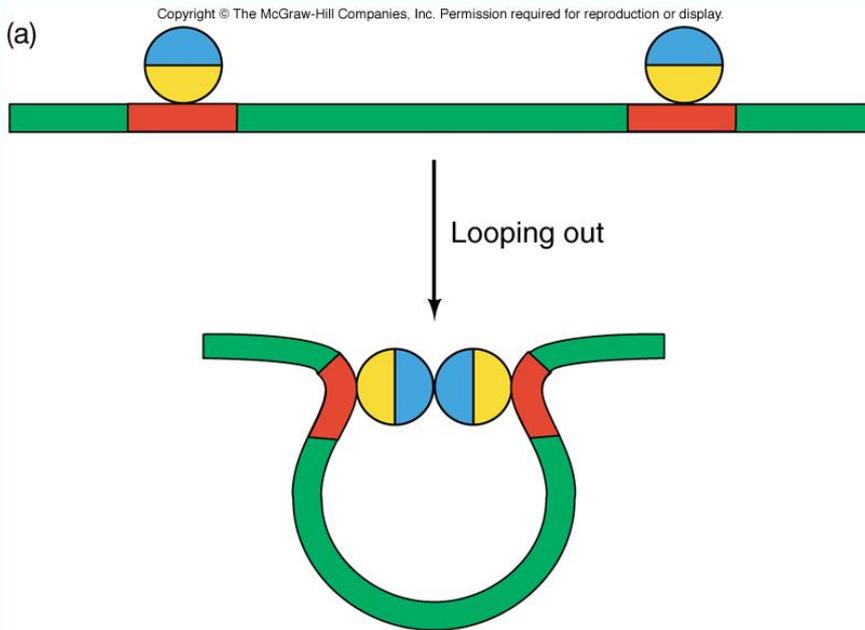


Features of the *ara* Operon

- Two *ara* operators exist:
 - *araO*₁ regulates transcription of a control gene called *araC*
 - *araO*₂ is located far upstream of the promoter it controls (P_{BAD})
- The CAP-binding site is 200 bp upstream of the *ara* promoter, yet CAP stimulates transcription
- This operon has another system of negative regulation mediated by the AraC protein

The *ara* Operon Repression Loop

- The araO_2 operator controls transcription from a promoter 250 downstream
- Does the DNA between the operator and the promoter loop out?

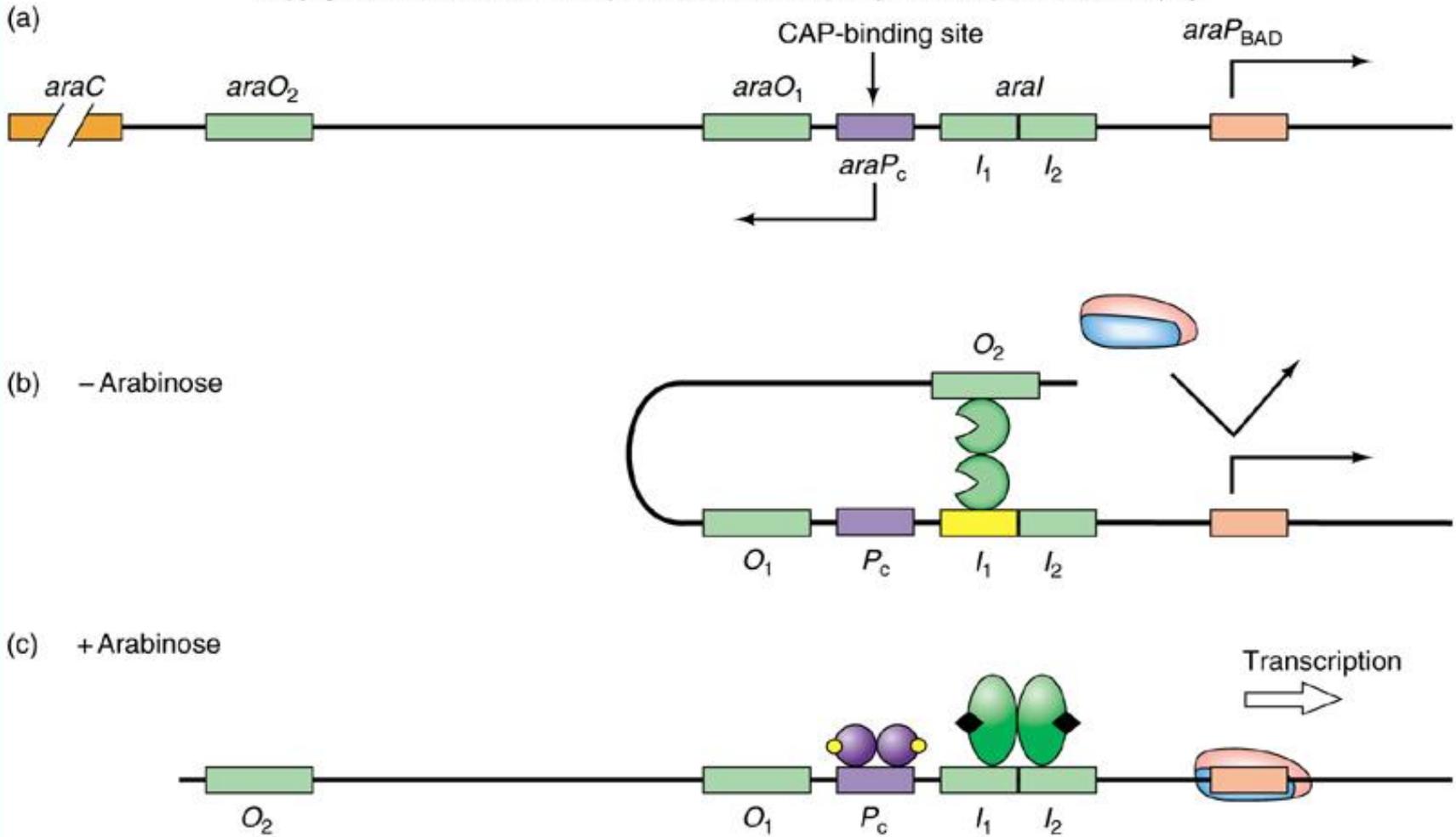


The *ara* Control Protein

- The AraC, *ara* control protein, acts as both a positive and negative regulator
- There are 3 binding sites
 - Far upstream site, *araO*₂
 - *araO*₁ located between -106 and -144
 - *araI* is really 2 half-sites
 - *araI*₁ between -56 and -78
 - *araI*₂ -35 to -51
 - Each half-site can bind one monomer of AraC

Control of the *ara* Operon

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Positive Control of the *ara* Operon

- Positive control is also mediated by CAP and cAMP
- The CAP-cAMP complex attaches to its binding site upstream of the *araBAD* promoter
- DNA looping would allow CAP to contact the polymerase and thereby stimulate its binding to the promoter

Autoregulation of *araC*

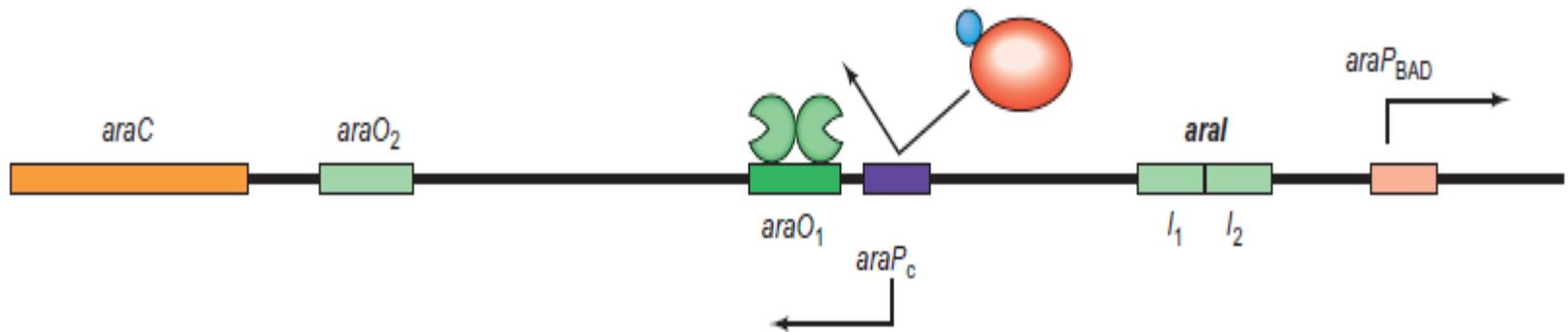


Figure 7.24 Autoregulation of *araC*. AraC (green) binds to *araO₁* and prevents transcription leftward from *P_c* through the *araC* gene. This can presumably happen whether or not arabinose is bound to AraC, that is, with the control region either unlooped or looped.

ara Operon Summary

- The *ara* operon is controlled by the AraC protein
 - Represses by looping out the DNA between 2 sites, *araO*₂ and *araI*₁ that are 210 bp apart
- Arabinose can derepress the operon causing AraC to loosen its attachment to *araO*₂ and bind to *araI*₂
 - This breaks the loop and allows transcription of operon
- CAP and cAMP stimulate transcription by binding to a site upstream of *araI*
 - AraC controls its own synthesis by binding to *araO*₁ and prevents leftward transcription of the *araC* gene

7.3 The *trp* Operon

- The *E. coli trp* operon contains the genes for the enzymes the bacterium needs to make the amino acid tryptophan
- The *trp* operon codes for anabolic enzymes, those that build up a substance
- Anabolic enzymes are typically turned off by a high level of the substance produced
- This operon is subject to negative control by a repressor when tryptophan levels are elevated
- *trp* operon also exhibits attenuation

Tryptophan's Role in Negative Control of the *trp* Operon

- Five genes code for the polypeptides in the enzymes of tryptophan synthesis
- The *trp* operator lies wholly within the *trp* promoter
- High tryptophan concentration is the signal to turn off the operon
- The presence of tryptophan helps the *trp* repressor bind to its operator

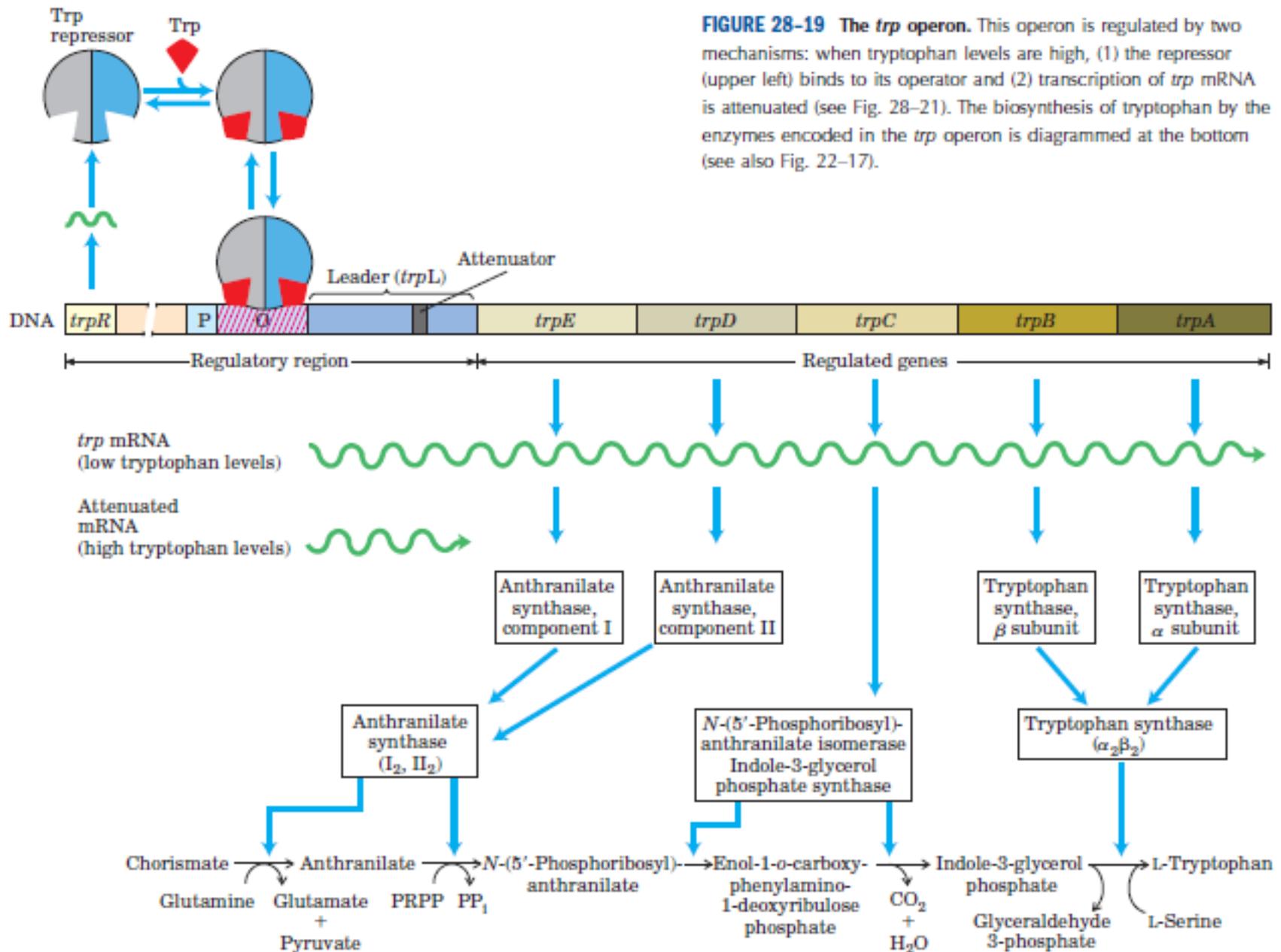
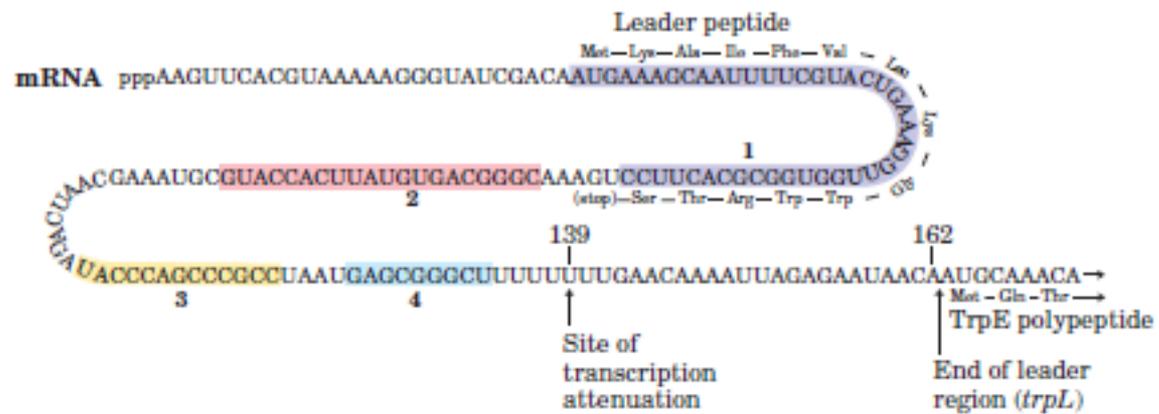
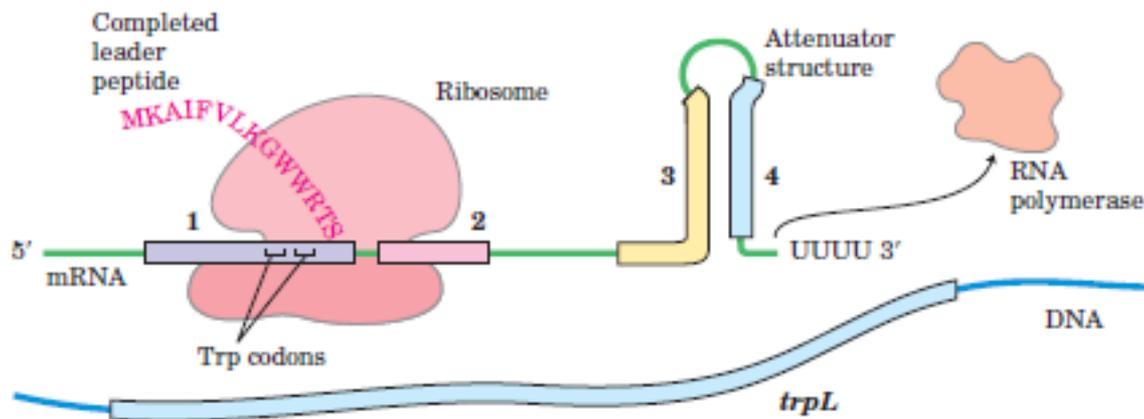


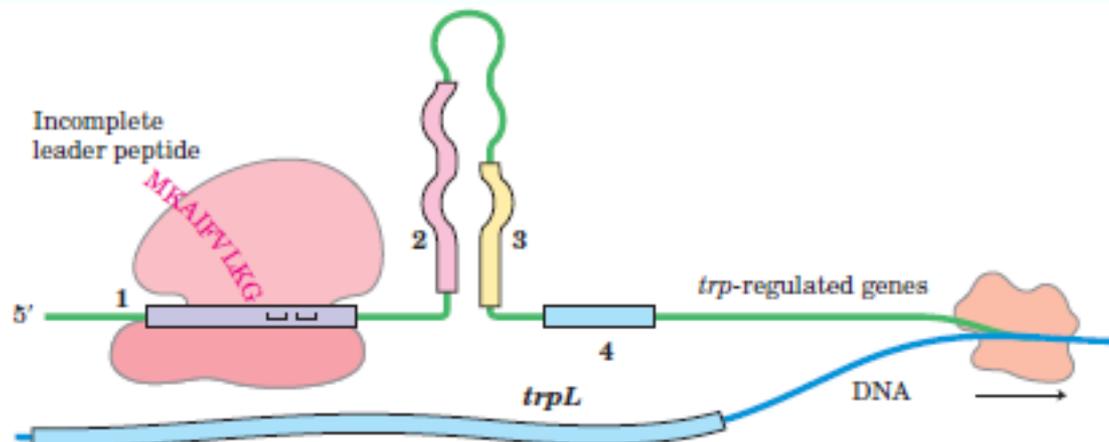
FIGURE 28-19 The *trp* operon. This operon is regulated by two mechanisms: when tryptophan levels are high, (1) the repressor (upper left) binds to its operator and (2) transcription of *trp* mRNA is attenuated (see Fig. 28-21). The biosynthesis of tryptophan by the enzymes encoded in the *trp* operon is diagrammed at the bottom (see also Fig. 22-17).



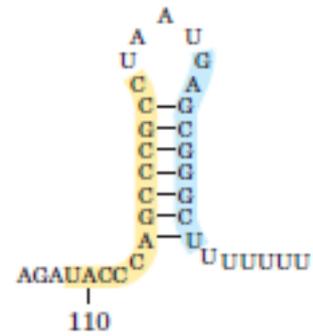
(a)



When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.

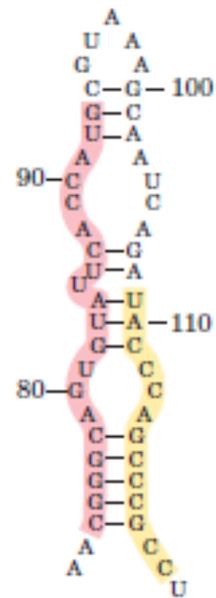


When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.



110

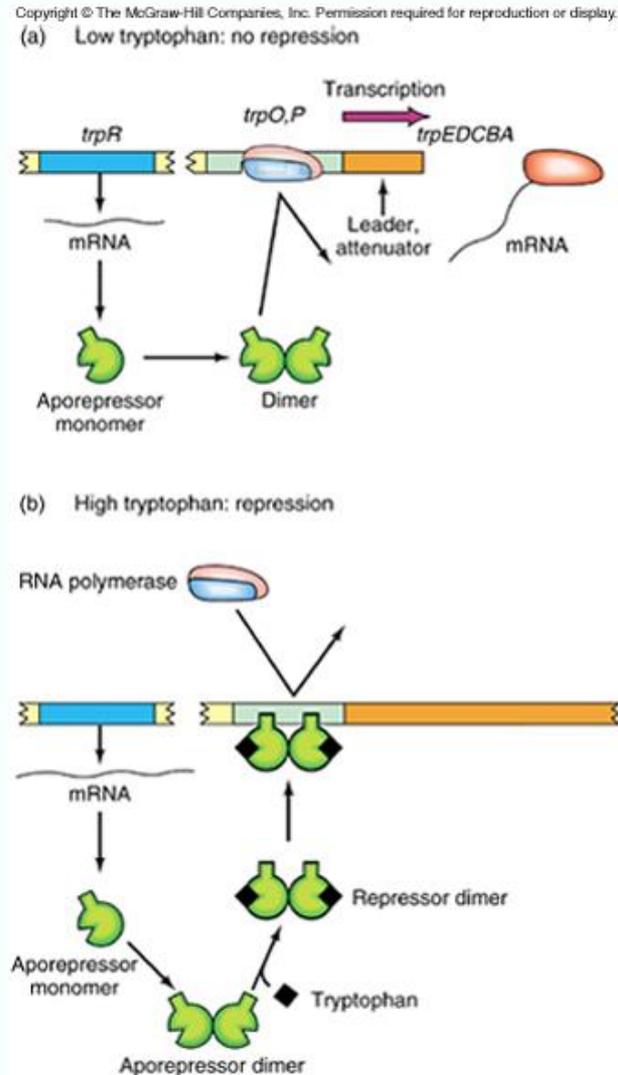
3:4 Pair
(attenuator)



2:3 Pair

Negative Control of the *trp* Operon

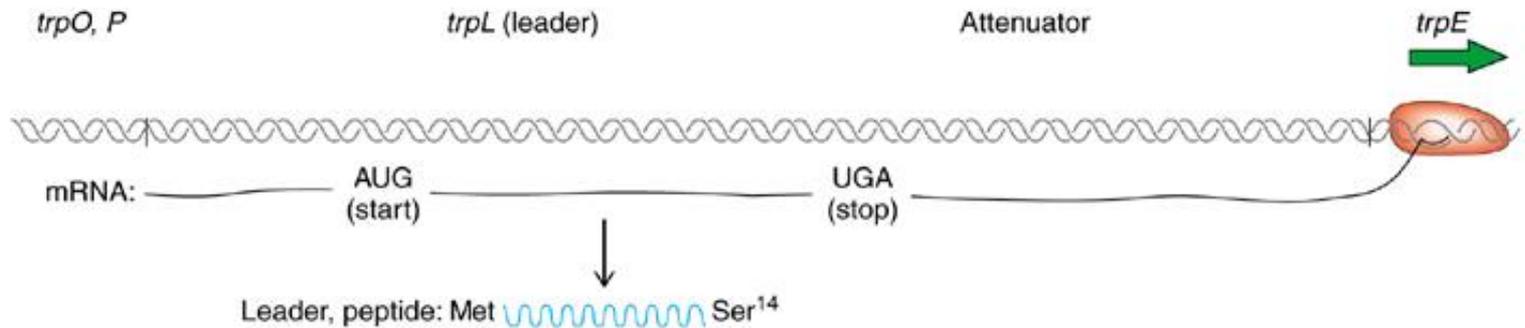
- Without tryptophan no *trp* repressor exists, just the inactive protein, aporepressor
- If aporepressor binds tryptophan, changes conformation with high affinity for *trp* operator
- Combine aporepressor and tryptophan to form the *trp* repressor
- Tryptophan is a corepressor



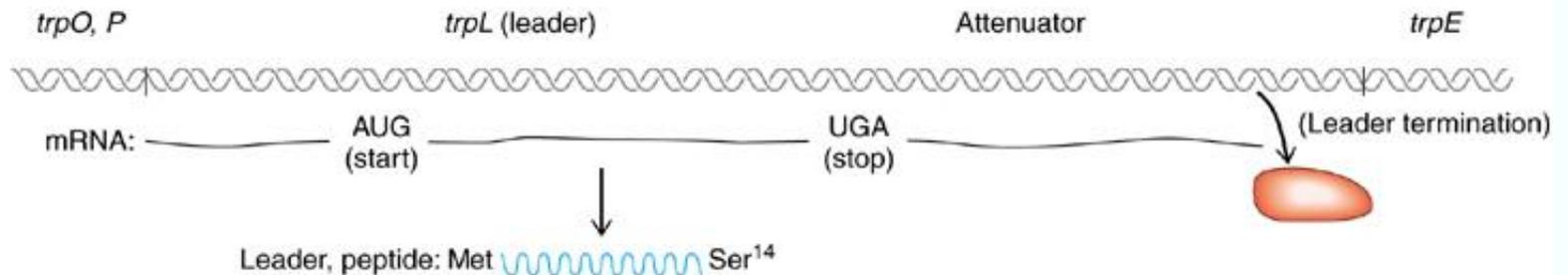
Attenuation in the *trp* Operon

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(a) Low tryptophan: transcription of *trp* structural genes



(b) High tryptophan: attenuation, premature termination



Mechanism of Attenuation

- Attenuation imposes an extra level of control on an operon
- Operates by causing premature termination of the operon's transcript when product is abundant
- In the presence of low tryptophan concentration, the RNA polymerase reads through the attenuator so the structural genes are transcribed
- In the presence of high tryptophan concentration, the attenuator causes premature termination of transcription

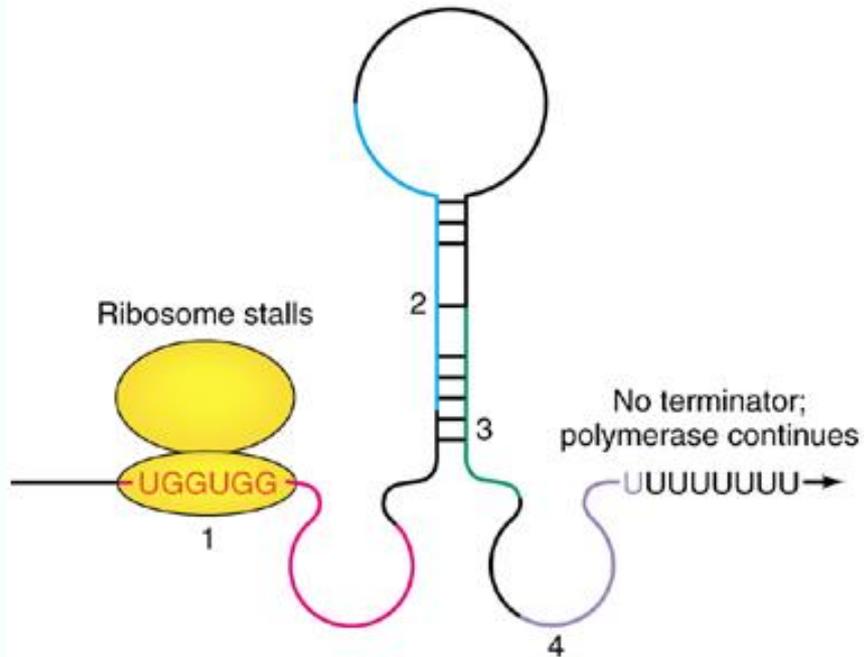
Defeating Attenuation

- Attenuation operates in the *E. coli trp* operon as long as tryptophan is plentiful
- If amino acid supply low, ribosomes stall at the tandem tryptophan codons in the *trp* leader
- *trp* leader being synthesized as stalling occurs, stalled ribosome will influence the way RNA folds
 - Prevents formation of a hairpin
 - This is part of the transcription termination signal which causes attenuation

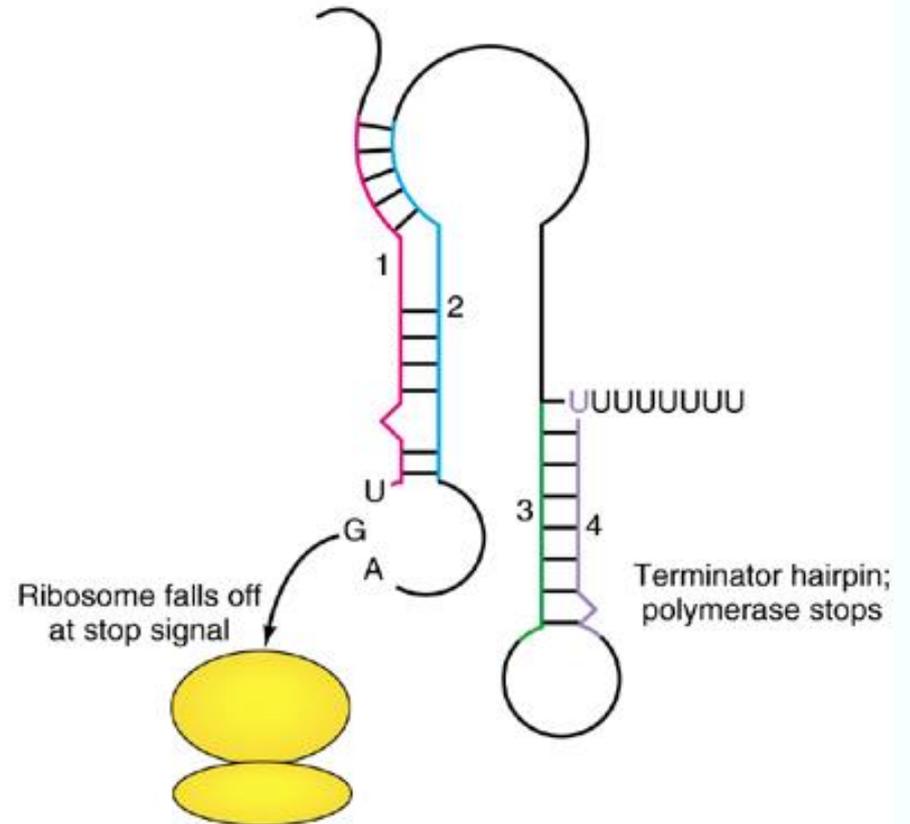
Overriding Attenuation

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(a) Tryptophan starvation



(b) Tryptophan abundance



7.4 Riboswitches

- Small molecules can act directly on the 5'-UTRs of mRNAs to control their expression
- Regions of 5'-UTRs capable of altering their structures to control gene expression in response to ligand binding are called riboswitches

Riboswitch Action

- Region that binds to the ligand is an aptamer
- An expression platform is another module in the riboswitch which can be:
 - Terminator
 - Ribosome-binding site
 - Another RNA element that affects gene expression
- Operates by depressing gene expression
 - Some work at the transcriptional level
 - Others can function at the translational level

ribD operon in B. subtilis.

This operon controls the synthesis and transport of the vitamin riboflavin and one of its products, flavin mononucleotide (FMN).

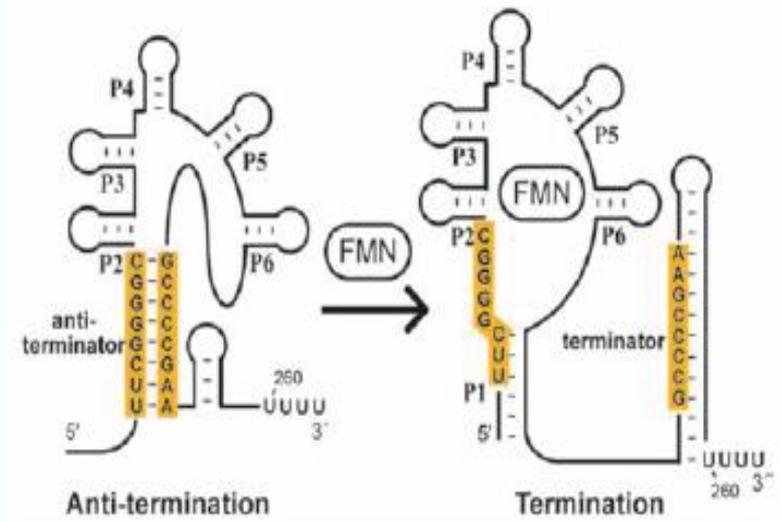
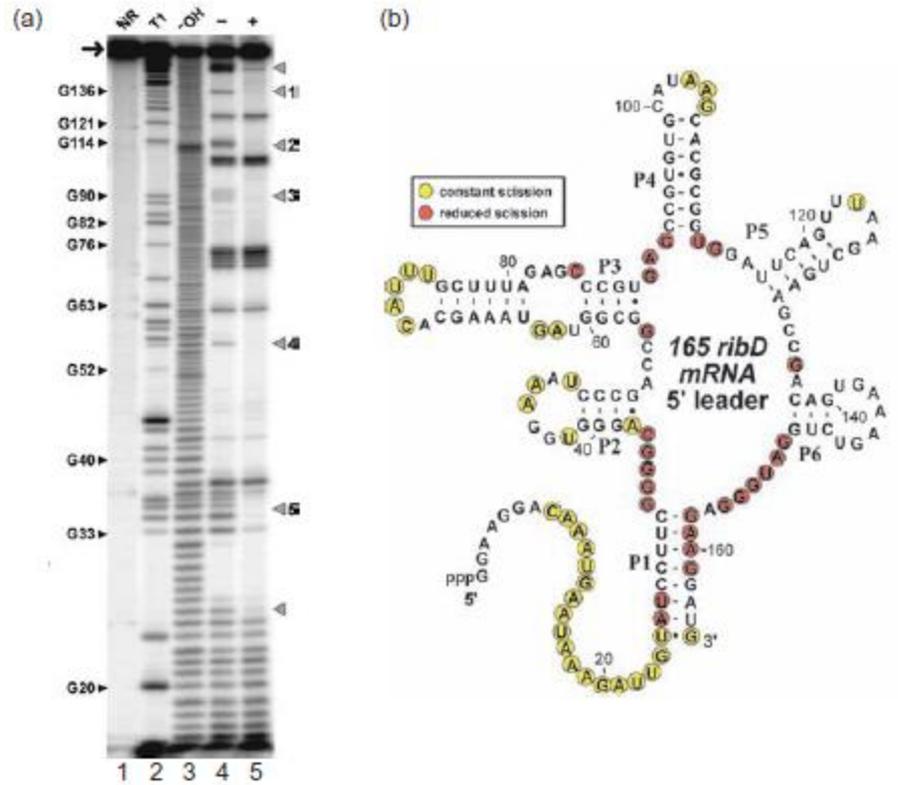
To test the hypothesis that the *RFN element* is an aptamer that binds directly to FMN, Ronald Breaker and colleagues used a technique called **in-line probing**.

This method relies on the fact that efficient hydrolysis (breakage) of a phosphodiester bond in RNA needs a 180-degree (“in-line”) arrangement among the attacking nucleophile (water), the phosphorus atom in the phosphodiester bond, and the leaving hydroxyl group at the end of one of the RNA fragments created by the hydrolysis.

Unstructured RNA can easily assume this in-line conformation, but RNA that is constrained by secondary structure (intramolecular base pairing) or by binding to a ligand cannot.

Thus, spontaneous cleavage of linear, unstructured RNA will occur much more readily than will cleavage of a structured RNA with lots of base pairing or with a ligand bound to it.

Breaker and colleagues incubated a labeled RNA fragment containing the *RFN* element in the presence and absence of FMN

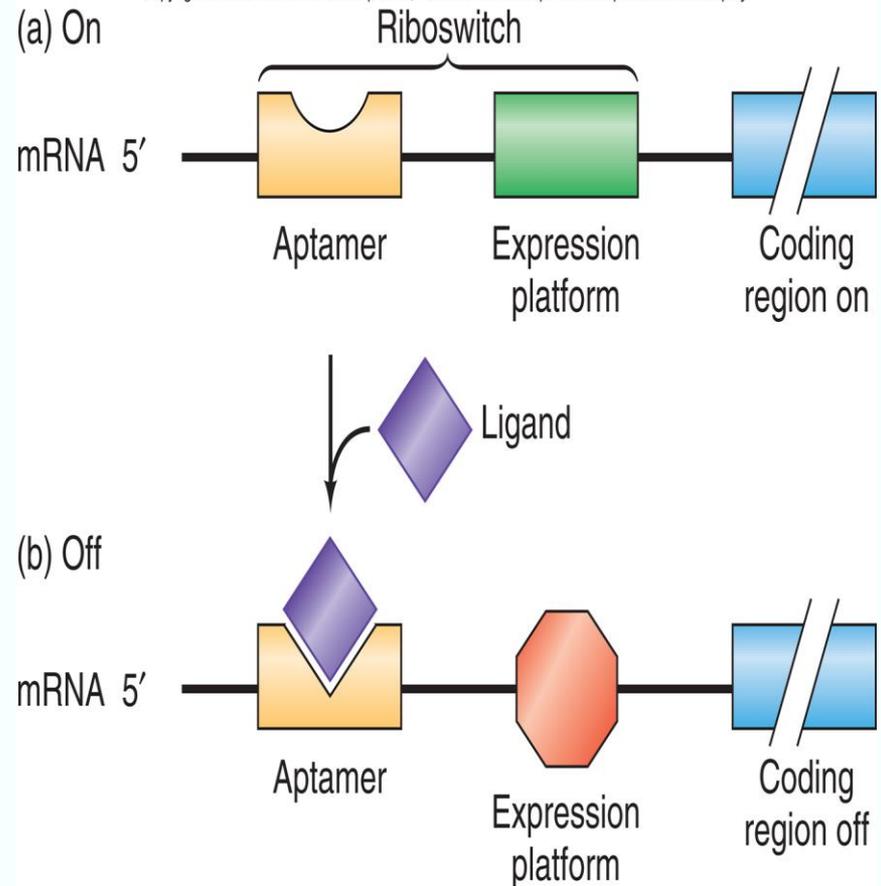


Model of Riboswitch Action

- FMN binds to aptamer in a riboswitch called the RFN element in 5'-UTR of the *ribD* mRNA
- Binding FMN, base pairing in riboswitch changes to create a terminator
- Transcription is attenuated
- Saves cell energy as FMN is a product of the *ribD* operon

Fig. 7.31

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Breaker and colleagues discovered another riboswitch in a conserved region in the 5'-untranslated region (5'-UTR) of the *glmS* gene of *Bacillus subtilis* and at least 17 other Gram-positive bacteria.

This gene encodes an enzyme known as **glutamine-fructose-6-phosphate amidotransferase**, whose product is the sugar **glucosamine-6-phosphate (GlcN6P)**. Breaker and colleagues found that the riboswitch in the 5'-UTR of the *glmS* mRNA is a ribozyme (an RNase) that can cleave the mRNA molecule itself. It does this **at a low rate when concentrations of GlcN6P are low**.

However, **when the concentration of GlcN6P rises**, the sugar binds to the riboswitch in the mRNA and changes its conformation to make it a much better RNase (about 1000-fold better). **This RNase destroys the mRNA, so less of the enzyme is made, so the GlcN6P conc falls.**⁷⁻⁷⁹

Some other Riboswitches also found

hammerhead ribozyme in the 3'-UTRs of rodent C-type lectin type II (*Clec2*) mRNAs.