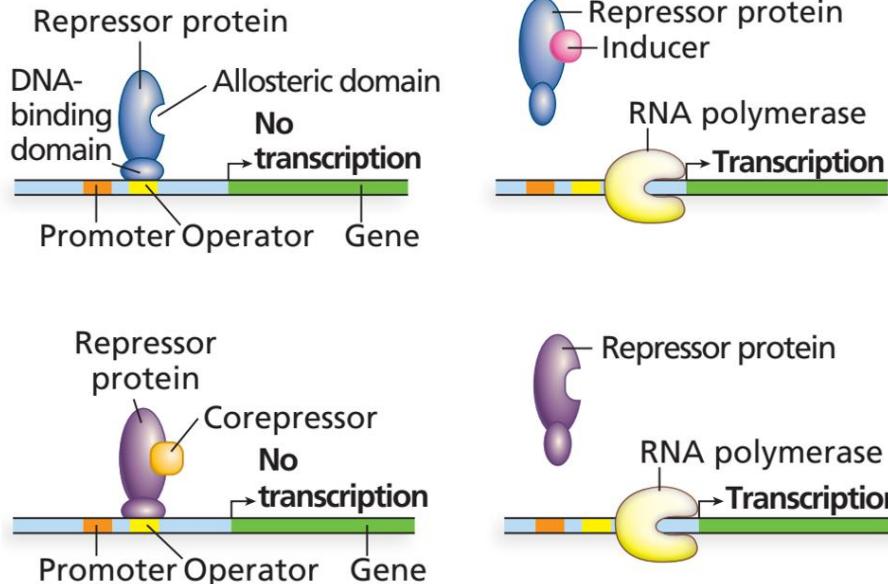
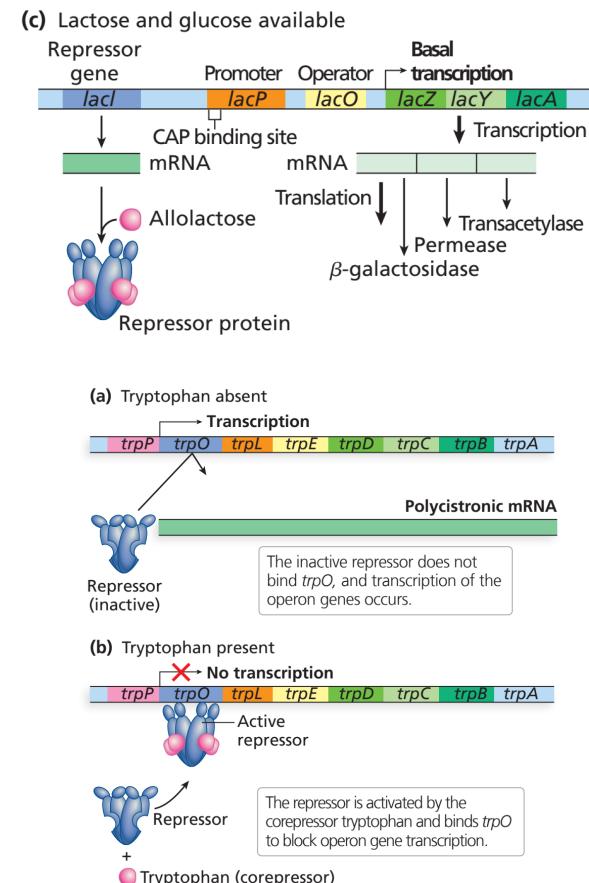


Lecture 15: Bacterial gene regulation

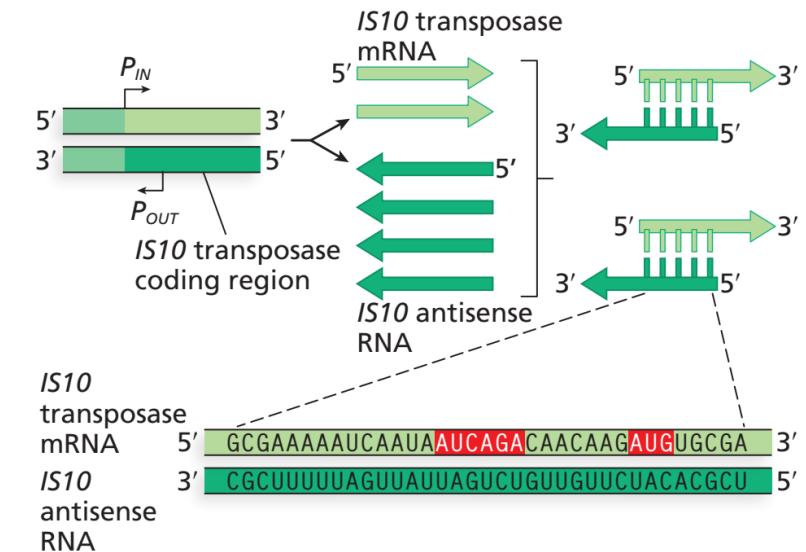
Positive and negative transcriptional regulation



lac and trp operons



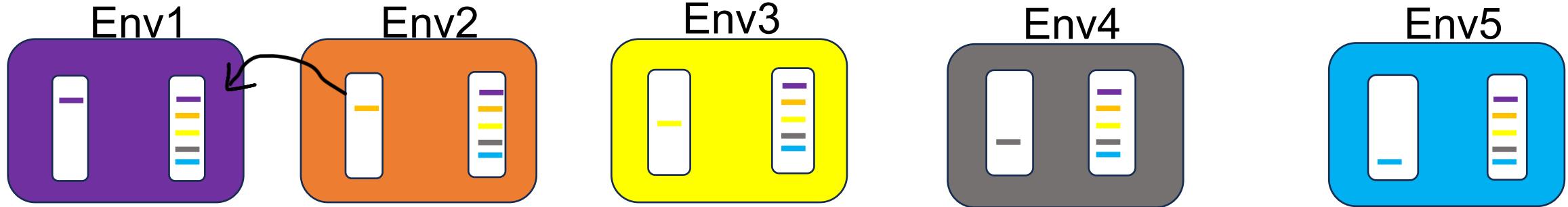
Other mechanisms of regulation



Learning objectives

- 1. Understand why gene regulation is needed and how it is accomplished
- 2. Understand how the lac operon is regulated
- 3. Understand how the trp operon is regulated
- 4. Understand other mechanisms of gene regulation in bacteria

Why regulate gene expression?

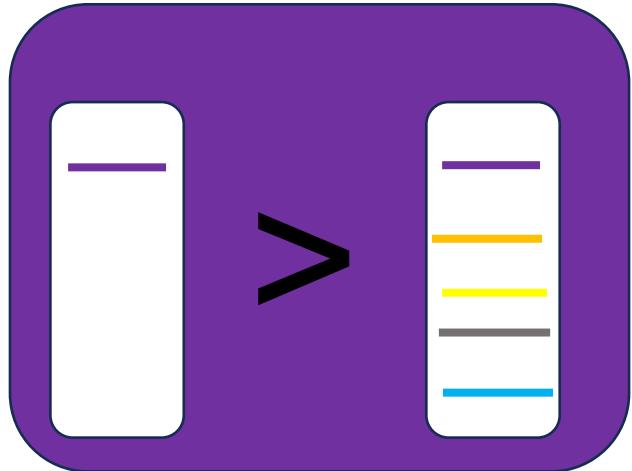


- Regulated gene expression
- Pros:
 - Less metabolically costly, only express genes when you need them
 - Can still respond appropriately to environmental changes given efficient regulation
- Cons:
 - Necessitates accurate sensing of the environmental conditions
 - Response is not instantaneous

- Unregulated gene expression
- Pros:
 - All proteins present at all times
 - No need to respond to environment
- Cons:
 - Metabolically costly
 - Antagonistic action of proteins involved in different metabolic systems

Gene regulation circa 1950s-1960s

- It was hypothesized that the reduced metabolic costs of regulated gene expression would be evolutionarily favored because it is more energetically efficient
- To confirm this, examples of regulated gene expression needed to be identified and studied
- Around this time, the lac operon and the trp operon were characterized, demonstrating that gene expression is regulated
- Jacques Monod, Andre Lwoff, and Francois Jacob performed key experiments and were awarded the Nobel prize for their work on the lac operon in 1965



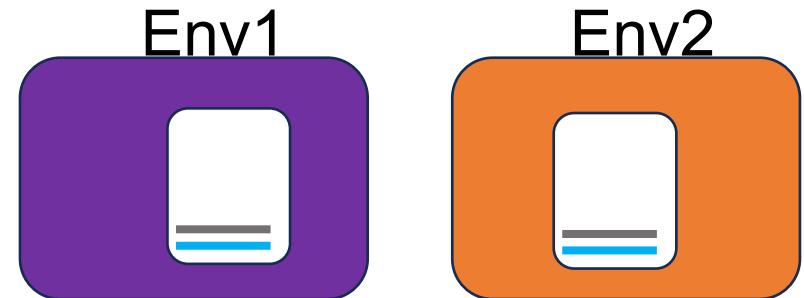
Monod Lwoff Jacob



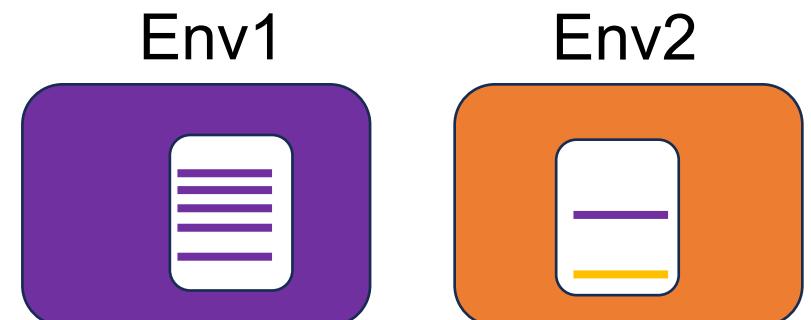
Gene regulation terminology

- Some genes in bacteria are always transcribed = constitutive expression
 - These are usually housekeeping genes that are always needed to maintain proper cellular function
- Other genes are not always needed
- These are only expressed under certain environmental conditions = regulated transcription
- Regulation of transcription occurs at two levels:
 - 1. Regulation of transcription initiation (on/off)
 - 2. Regulation of the amount of transcription (magnitude if on)
- Gene regulation also occurs at other levels:
 - mRNA stability
 - Translation
 - post translational modifications (ex phosphorylation)

Constitutive expression

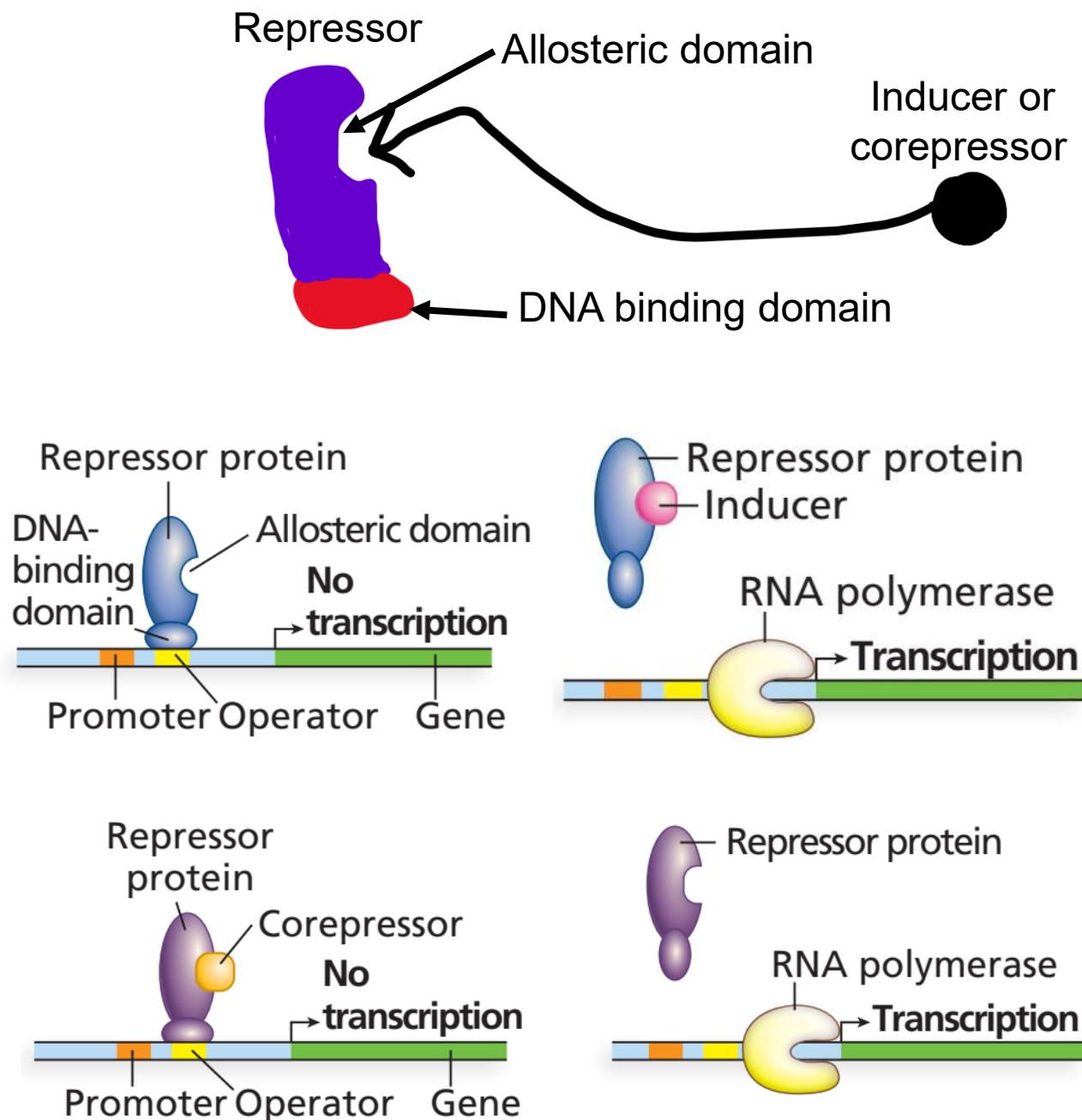


Regulated expression



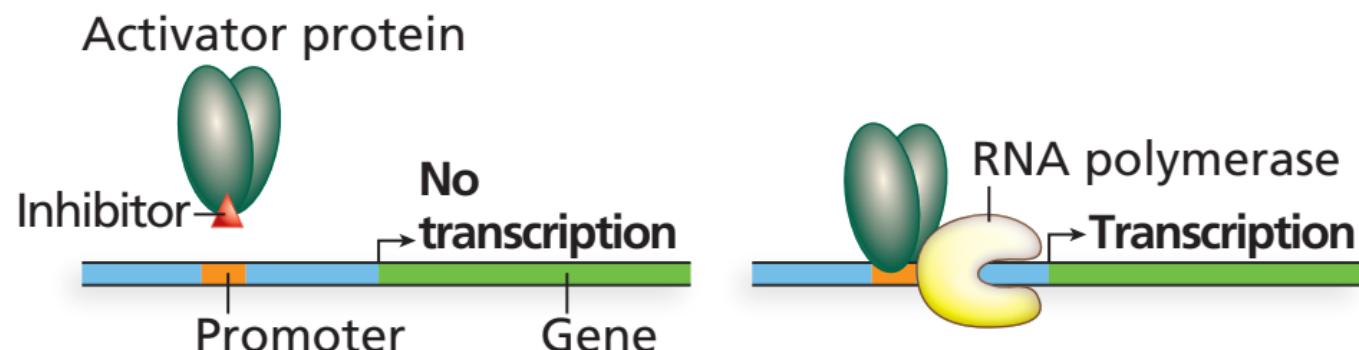
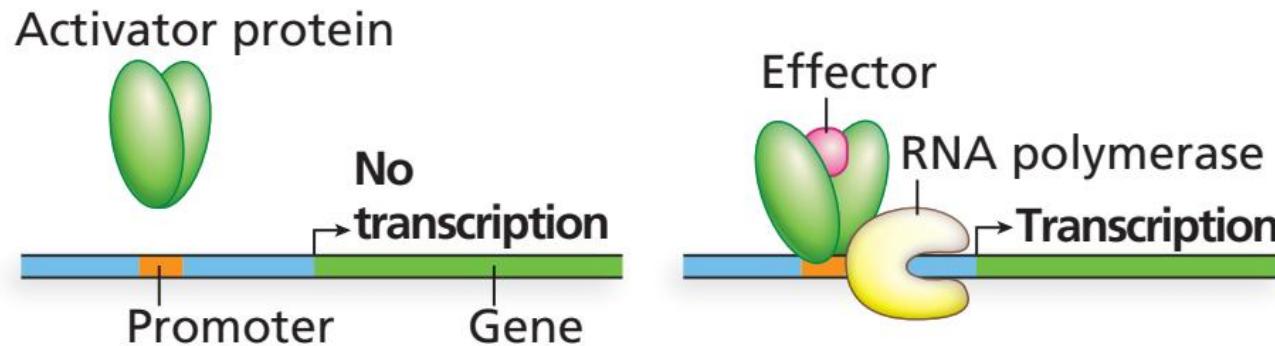
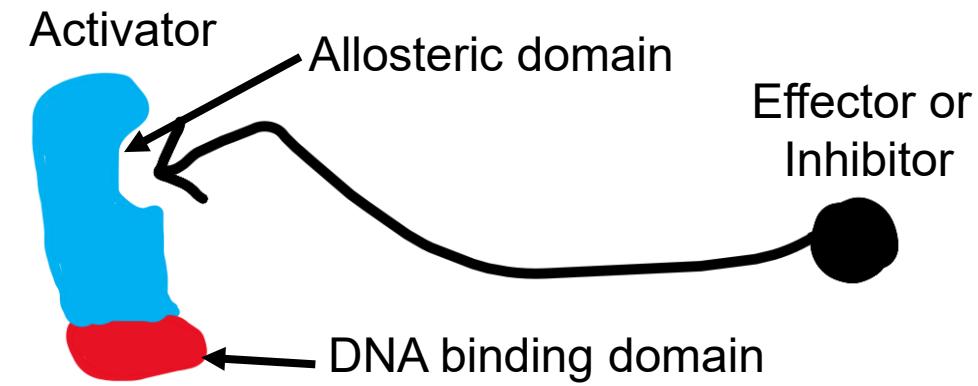
Negative control of transcription

- Negative control = binding a repressor protein to DNA with the consequence of preventing transcription
- Repressors:
 - DNA binding proteins (DNA binding domain)
 - Blocks transcription initiation
 - Occupies the space where RNA polymerase would normally bind or prevents formation of the open promoter complex
 - Can be activated or inactivated via interactions with other compounds (allosteric domain)
- Allostery = binding in one site of a protein alters the conformation and function of another site
- Allosteric domains of repressors operate in two ways:
 - Inducers: bind repressor, cause release of DNA
 - Corepressors: bind repressor and induce DNA binding



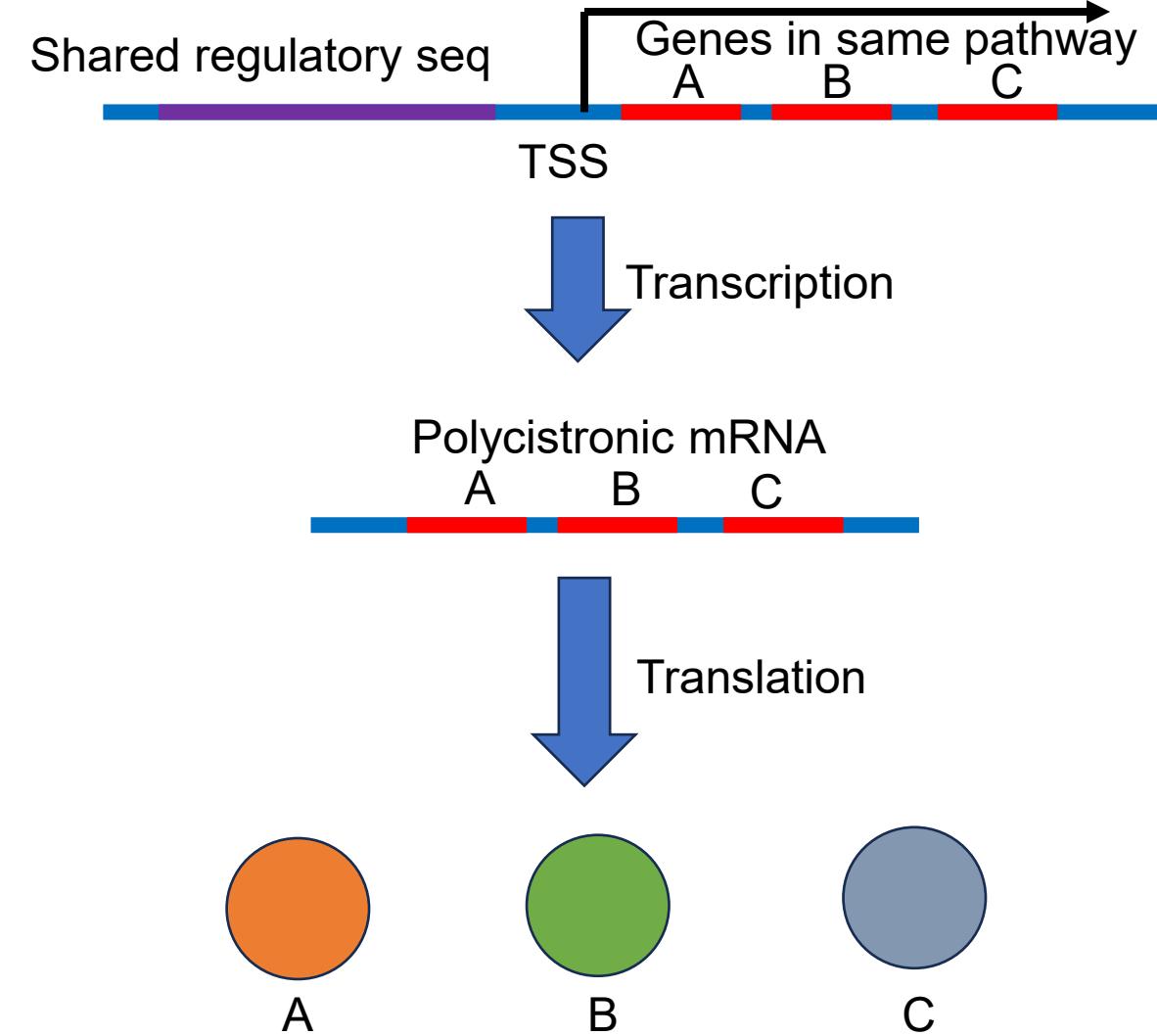
Positive control of transcription

- Positive control = binding an activator with the result of facilitating transcription initiation
- Activators:
 - DNA binding proteins (DNA binding domain)
 - Facilitate transcription initiation
 - Can be activated or inactivated via interactions with other compounds (allosteric domain)
- Allosteric domains of activators operate in two ways:
 - Allosteric effector compounds = allows binding
 - Inhibitors = prevent binding



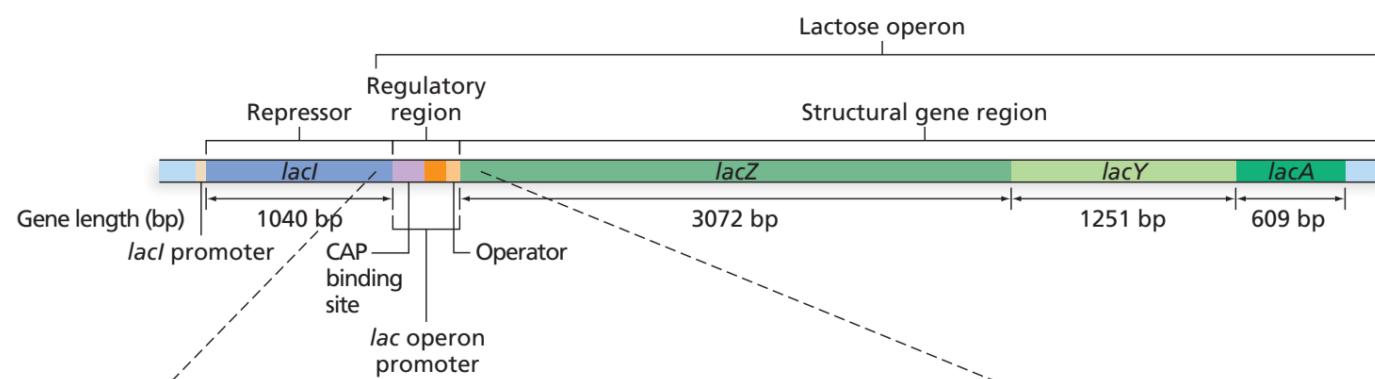
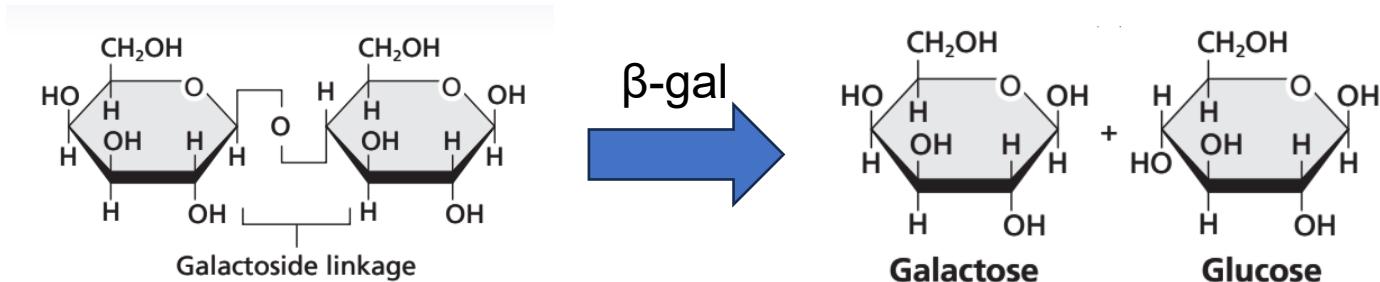
Operons

- Bacterial genomes are small and organized into groups of co-regulated genes
- These groups are under the control of the same regulatory regions
- Groups of genes and their shared regulatory regions are called operons
- These groups of genes are almost always involved in the same metabolic pathways
- Why are bacterial genomes so small and compact?
- A key to success as a bacterium is fast reproduction when environmental conditions are favorable
- Large genomes are metabolically costly and slower to replicate
- Operons have likely been favored by natural selection because these systems allow efficient transcriptional responses to the environment



Lactose metabolism and intro to lac operon

- Lactose is a disaccharide that is broken down into a molecule of glucose and a molecule of galactose
- Lac operon encodes three proteins:
- Permease (lacY) = Imports lactose into the cell
- Beta-galactosidase or β -gal (lacZ) = breaks down lactose into monosaccharides
- Transacetylase (lacA) = not required for lactose utilization
 - protects the cell from potentially damaging byproducts of lactose metabolism
- These three genes are transcribed together as a polycistronic mRNA



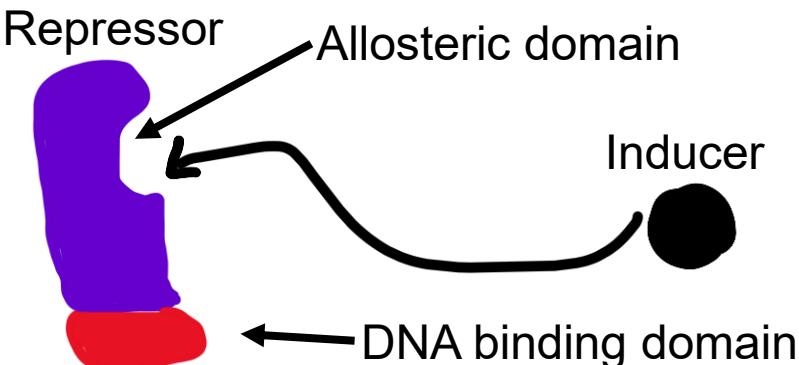
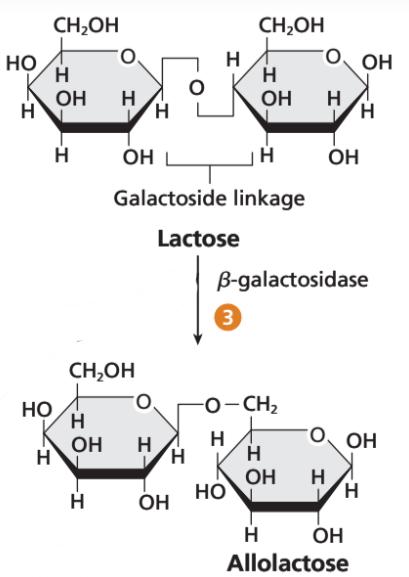
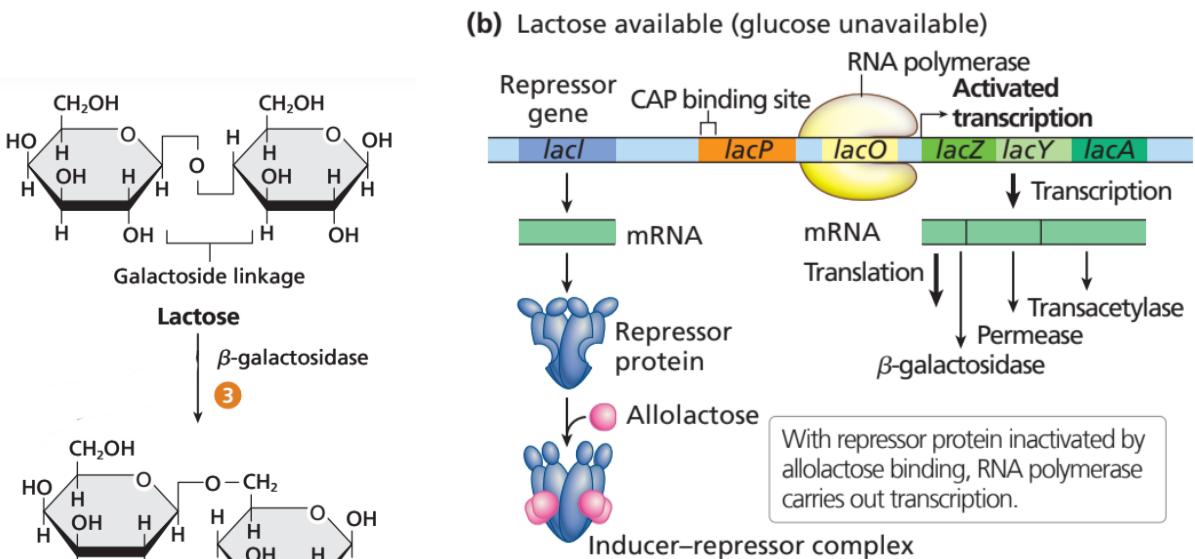
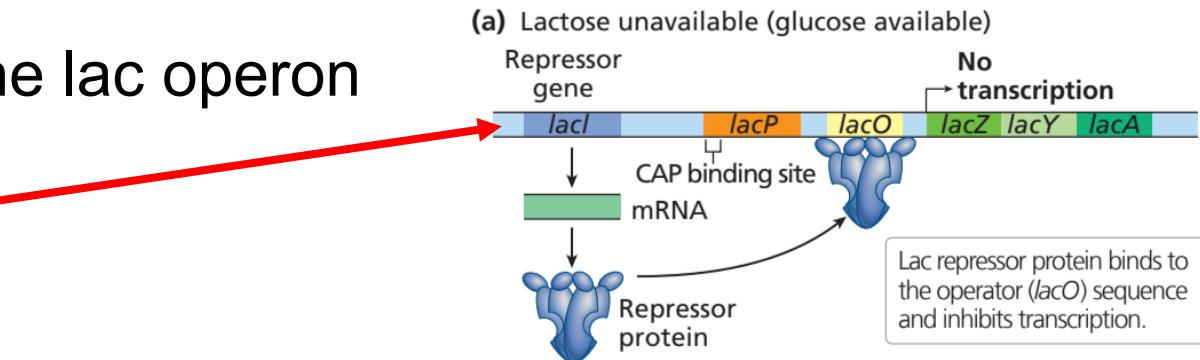
Lac operon: logic of the system

- Glucose is the preferred carbon source for most bacteria
 - Lactose requires breakdown
 - Galactose requires modification before it can enter glycolysis
- If glucose is present, operon off
- If lactose is absent, operon off
- If BOTH glucose is absent and lactose is present, operon on
- How is this accomplished?

		Glucose absent	Glucose present
		OFF	OFF
Lactose absent	Glucose absent	OFF	OFF
	Glucose present	OFF	OFF
		ON	OFF
Lactose present		ON	OFF

The lac repressor (1) negative regulation of the lac operon

- Adjacent to, but not a part of the lac operon there is a gene called lacI
- This encodes the lac repressor and it is constitutively expressed
- The lac repressor is a homotetramer that has a DNA binding domain and an allosteric domain
- The DNA binding domain binds a sequence upstream of the operon called the operator (lacO), which includes the TSS
- The allosteric domain binds allolactose, if bound then the repressor releases DNA
- Allolactose is a disaccharide similar to lactose
 - It can be produced from β -gal acting on lactose and can also be broken down into glucose and galactose by β -gal
- If allolactose is not present, the repressor will bind the operator and block transcription initiation



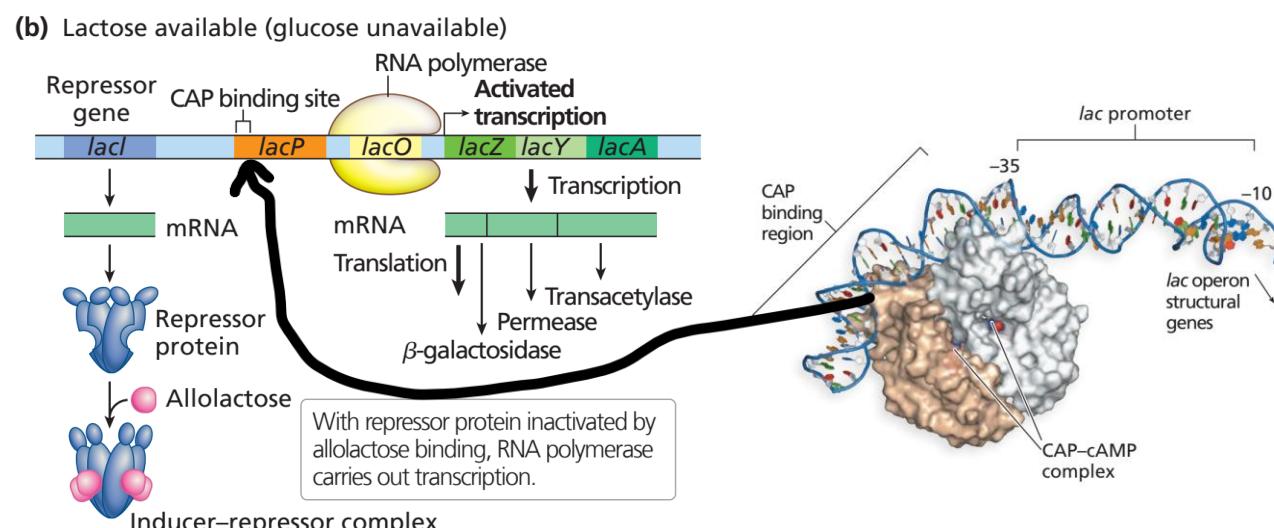
The lac repressor (2) How is allolactose in the cell if the operon is off?

- If the operon is off, shouldn't there be no β -gal in the cell, leading to no allolactose being produced?
- Further shouldn't there be no permease? No lactose in the cell?
- How does lactose even get into the cell?
- Gene expression is never truly completely zero!
- Gene expression is noisy, and repression is imperfect.
- Even when the operon is “off” there is leaky expression or basal expression of the genes
 - Repression is not 100% efficient
- There is always some low level of permease and β -gal present
- Without this, the system would not work!!

Positive regulation of the lac operon (cAMP and CAP)

- In the absence of glucose, the concentration in the cell of cyclic AMP or cAMP increases.
- This is caused by activation of adenylate cyclase, which converts ATP to cAMP
- When glucose is present, this enzyme does not catalyze this reaction
- cAMP binds a protein called CAP, causing it to bind DNA at the CAP binding site in the lac operon
- This facilitates RNA polymerase binding and activates transcription

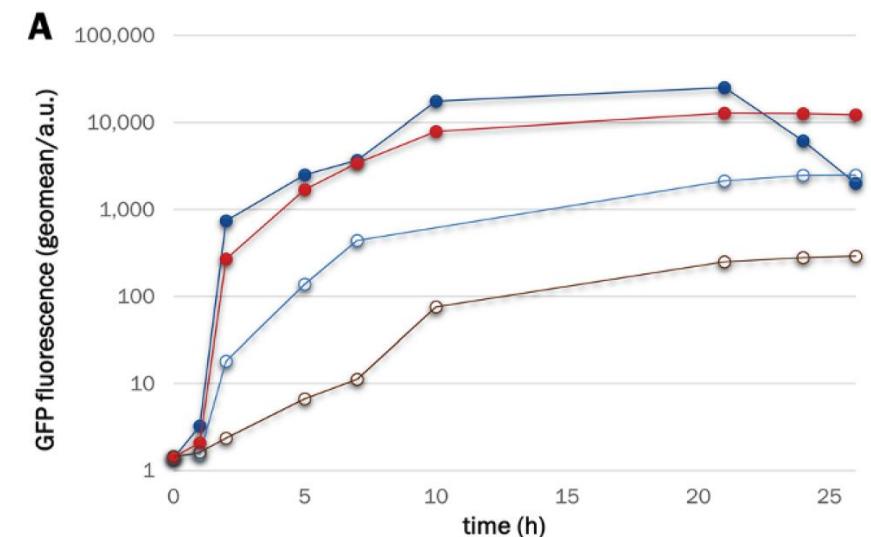
Glucose absent	Glucose present
High cAMP	Low cAMP
CAP bound to DNA	CAP not bound to DNA
Activation	No activation



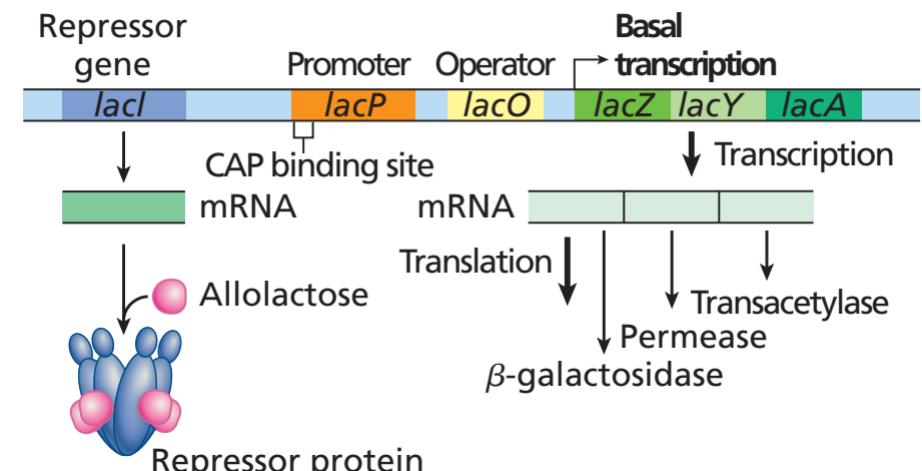
Leaky expression versus basal expression

- Leaky expression arises as a consequence of repressor binding being reversible and not 100% efficient
- In the absence of allolactose, there is still a very low amount expression
- RNA polymerase can only bind if the repressor spontaneously disassociates
- Basal expression arises as a consequence of only a lack of activation
- RNA polymerase can bind the promoter, but there is no activator to facilitate this

Closed circles = inducer present
Open circles = inducer absent
Blue = WT repressor
Red = mutant repressor (W220F)



(c) Lactose and glucose available



Normal Lac operon function summary

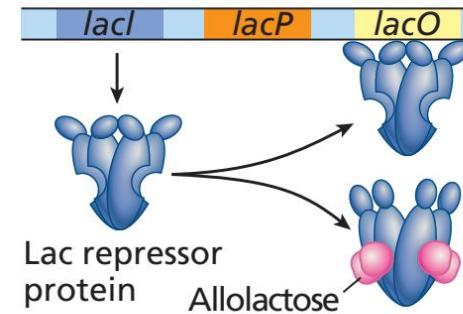
- Four possible states:
- Glucose present, lactose absent:
 - Low cAMP, no CAP binding, no activation
 - Low allolactose, repressor bound, active repression
 - Leaky expression due to repressor binding not being 100% efficient (reversible)
- Glucose present, lactose present
 - Low cAMP, no CAP binding, no activation
 - Allolactose present, repressor unbound
 - Basal expression due to lack of repression + lack of activation
- Glucose absent, lactose absent
 - High cAMP, binds CAP, activator present
 - Allolactose not present, repressor bound
 - leaky expression due to repressor not being 100% efficient.
- Glucose absent, lactose present
 - High cAMP, binds CAP, activator present
 - Allolactose is present, repressor unbound
 - High expression due to no repression + activation

		Glucose absent	Glucose present
Lactose absent	Glucose absent	OFF	OFF
	Glucose present	Leaky expression	Leaky expression
Lactose present	Glucose absent	ON	OFF
	Glucose present	Very high expression	Basal expression

Mutational analysis of the lac operon (1) Constitutive mutants in operator seq

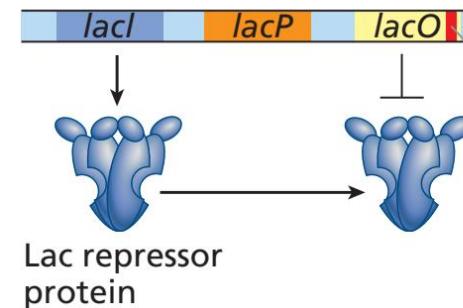
- Constitutive mutants express the operon irrespective of the presence or absence of lactose and glucose
- One way this can happen = Mutation in operator that makes it such that repressor can no longer recognize it
- Operator mutations are cis-acting,
 - Only influence transcription of genes on the same chromosome
- This can be illustrated by analyzing partial diploids (F' plasmids put to use!)
 - Note bacterial chromosome and F' plasmid are circular, linear depiction is for simplicity

(a) I^+ (wild type)



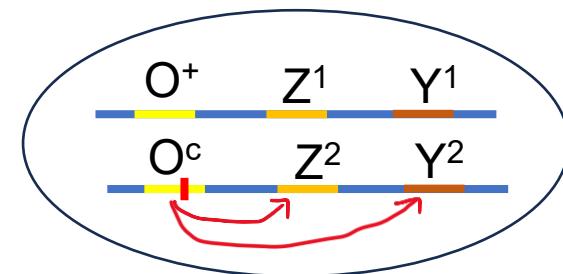
Repressor binds operator when the inducer is absent and forms an inducer-repressor complex when inducer is present.

(b) O^c (operator constitutive mutation)

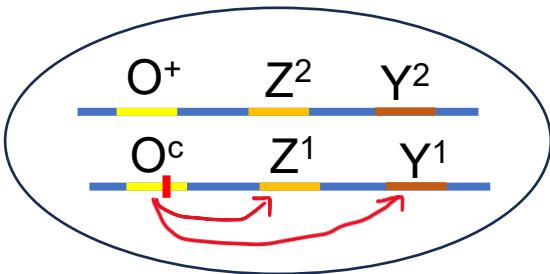


Operator-site mutation prevents repressor protein binding and leads to constitutive synthesis of the *lac* operon.

Z² and Y² constitutive

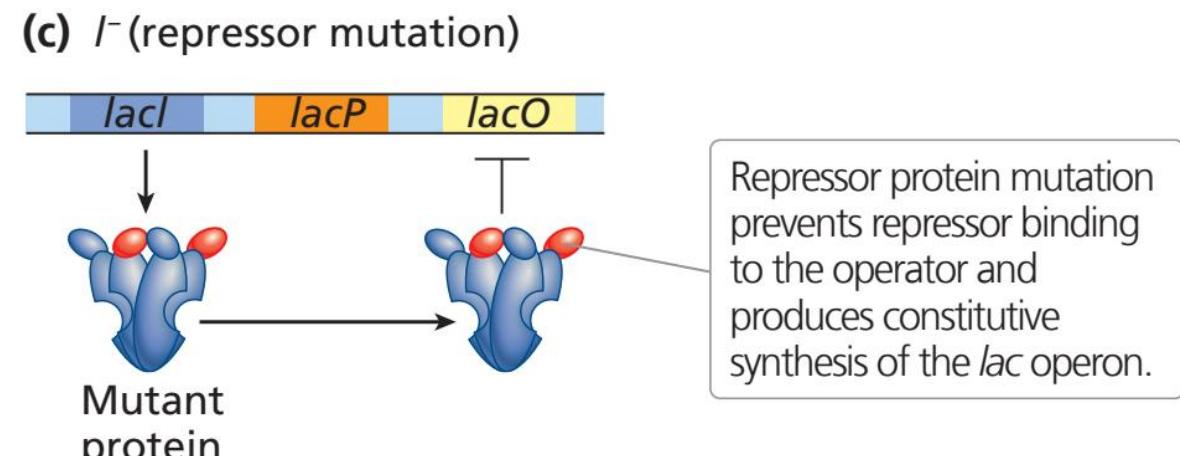


Z¹ and Y¹ constitutive



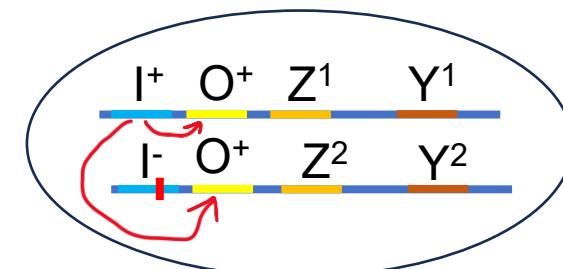
Mutational analysis of the lac operon (2) Constitutive mutants in repressor coding seq

- I⁻ mutations also yield constitutive expression
- I⁻ mutations alter the DNA binding domain of the repressor
- Mutant protein can no longer recognize and bind the operator
- This leads to constitutive expression
- Coding changes in DNA binding proteins act in-trans
- The I⁺ allele is trans-acting because it is capable of altering expression of genes on other chromosomes

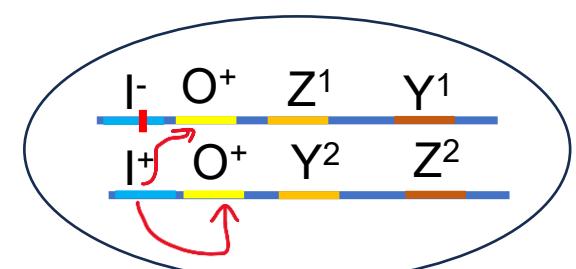


Does not matter which chromosome carries the mutation, all alleles inducible because the I⁺ allele acts in-trans

All alleles inducible



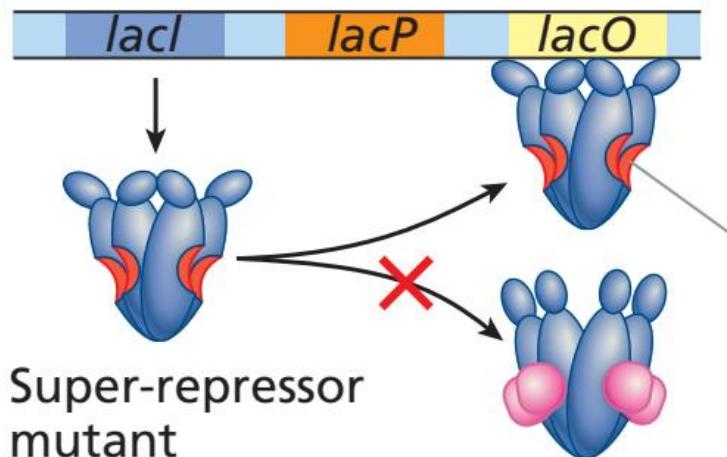
All alleles inducible



Mutational analysis of the lac operon (3) Super repressor mutations in lacI

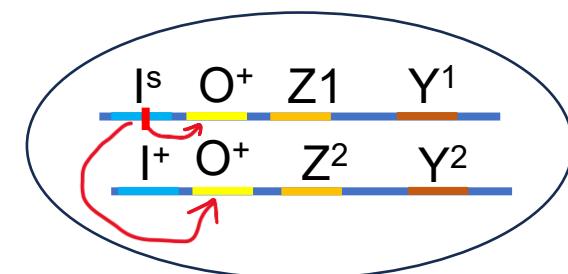
- Super repressor mutations make the operon unresponsive to induction by allolactose
- Mutations in I that alter its allosteric domain without altering its DNA binding properties
- Even when allolactose is present, the protein cannot release the DNA
- I^s mutations act in trans

(d) I^s (super-repressor mutation)

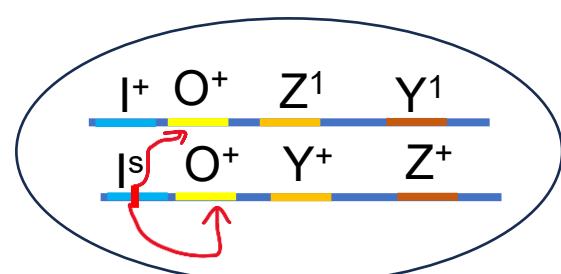


Repressor protein mutation blocks binding to the inducer, preventing formation of the inducer-repressor complex. Mutant repressor protein binds to the operator, preventing transcription.

All alleles non-inducible



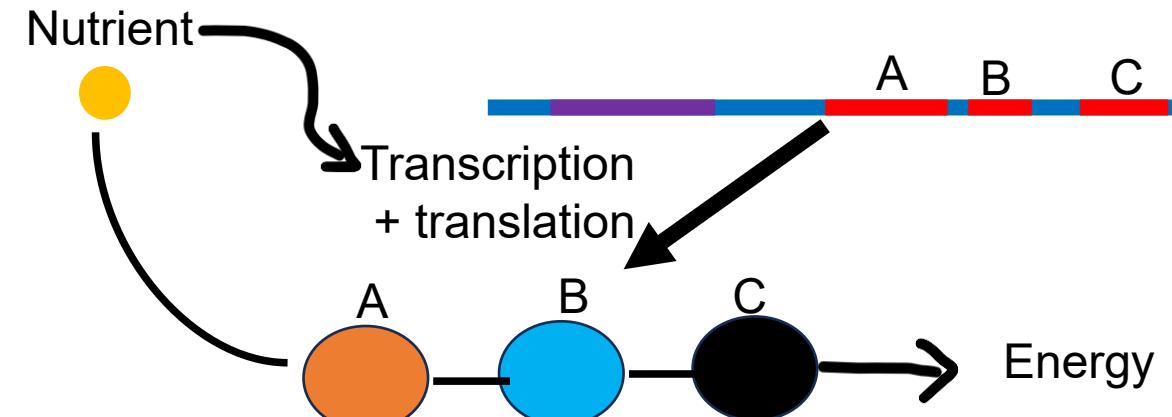
All alleles non-inducible



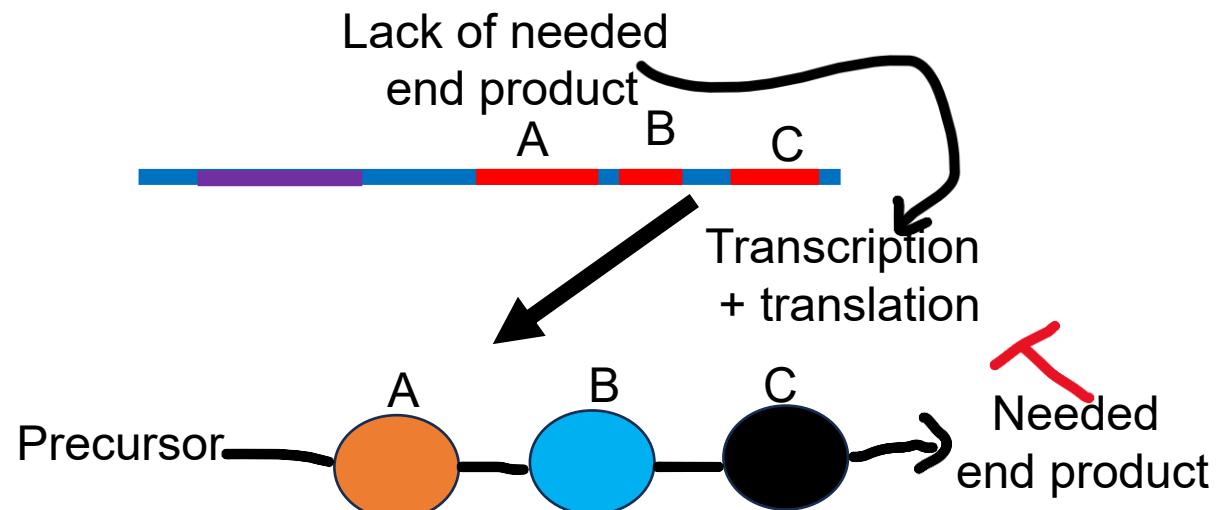
Catabolic versus anabolic operons

- Catabolism = breaking down large molecules into smaller component parts (yields energy)
- Anabolism = constructing large molecules from smaller component parts (costs energy)
- Catabolic operons like the lac operon are often inducible by the presence of a particular nutrient
- Anabolic operons are typically repressible by the end product (negative feedback)
 - Why make more if we already have some?
- Attenuation is a property of some repressible operons allowing the magnitude of expression to be tightly controlled in response to various concentrations of the end product
- Attenuation acts like a dimmer rather than an on/off switch
- This property is often displayed by operons involved in the production of amino acids
- Cells have evolved to maintain near constant concentrations of amino acids

Catabolic operon (inducible)

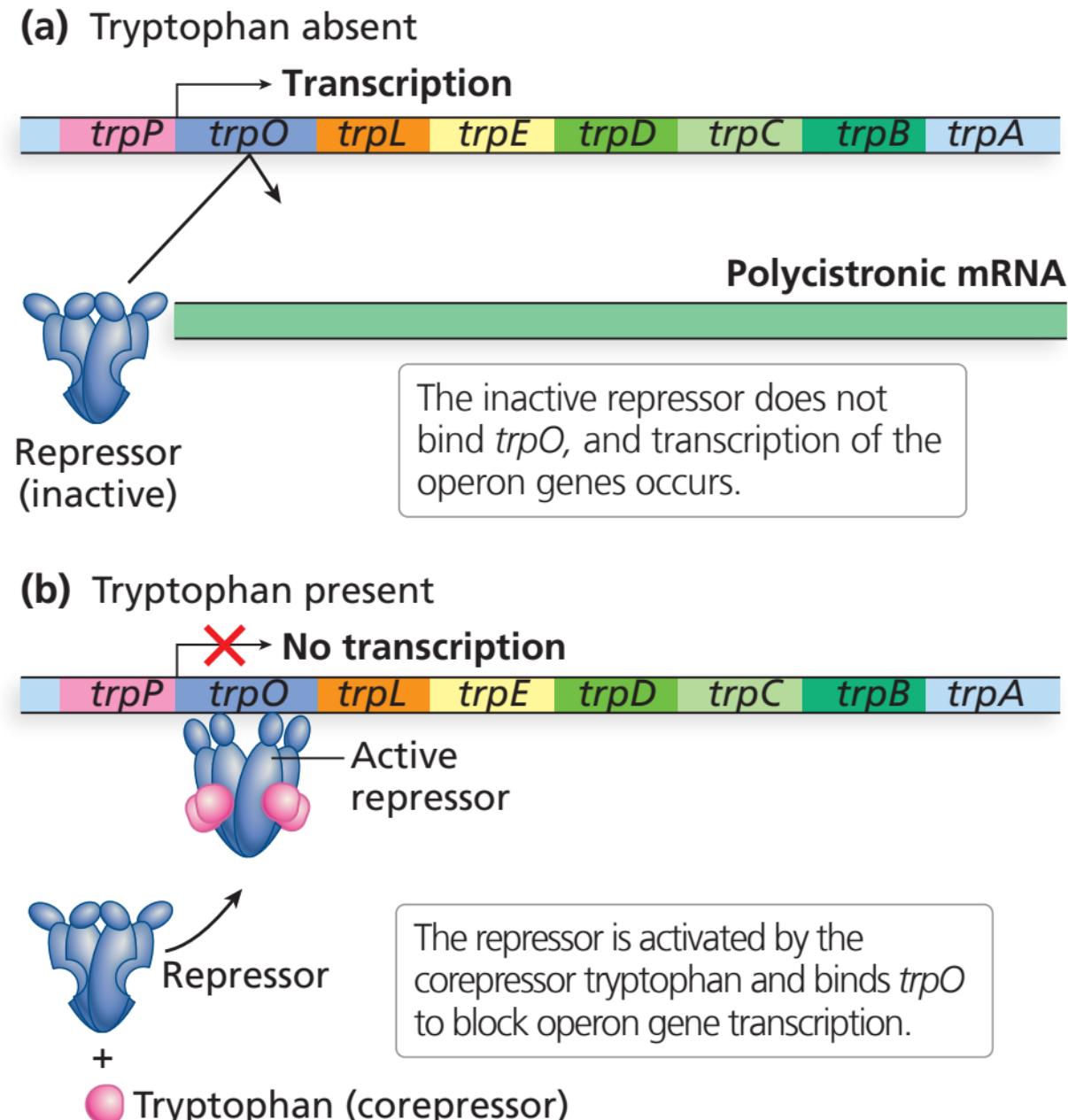


Anabolic operon (repressible by negative feedback)



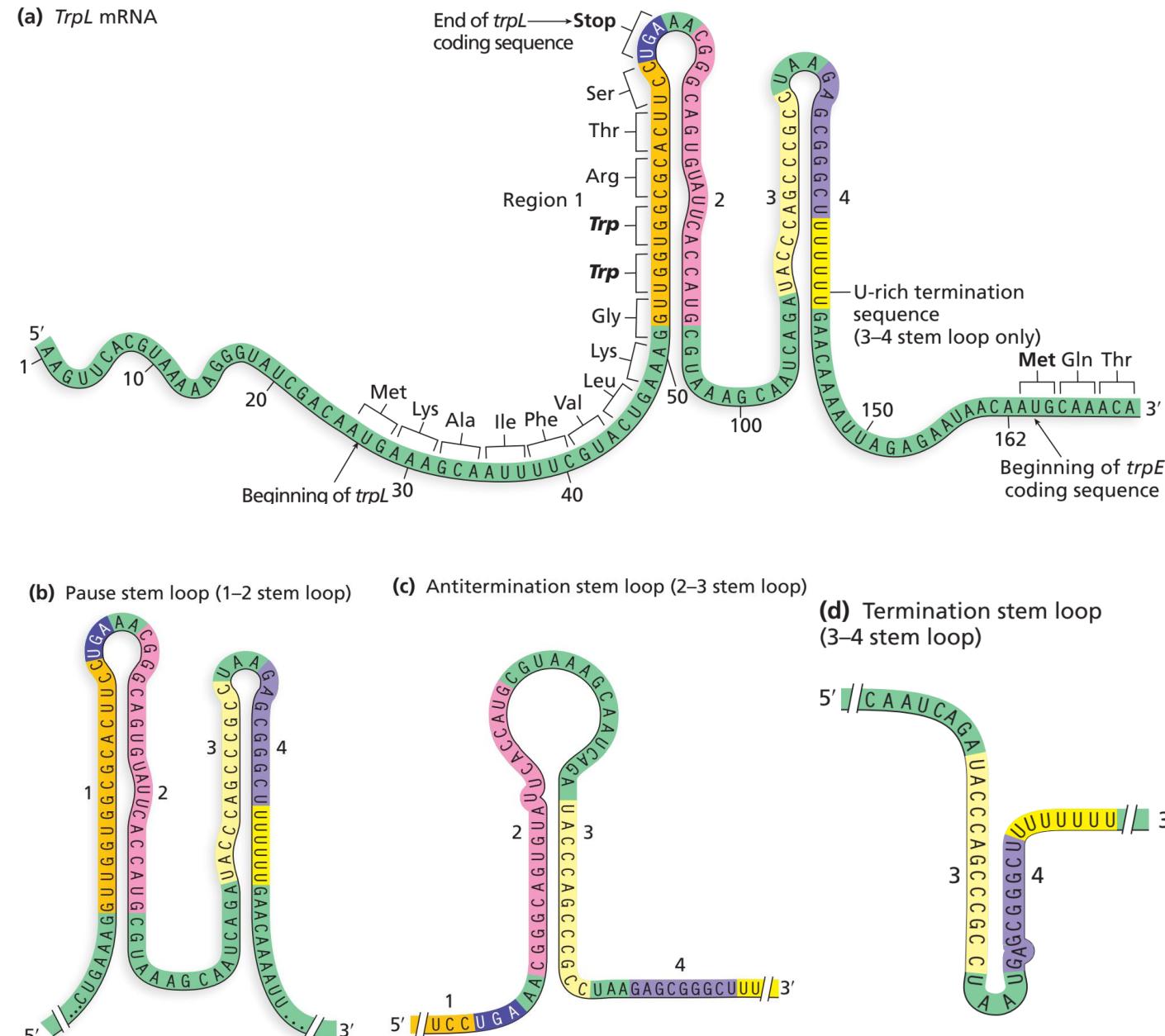
Trp operon cast of characters and repression

- The trp operon contains 5 genes that share three key regulatory regions
 - Promoter
 - Operator
 - Leader region –upstream of operon and is transcribed
- The 5 genes are trpE, trpD, trpC, trpB, and trpA all needed for tryptophan synthesis
- Another gene trpR outside the operon encodes the trp repressor
- The repressor is activated by tryptophan
- Tryptophan is thus a co-repressor
- Trp present, operon off
- Trp absent, operon on
- These sorts of systems have evolved due to their metabolic efficiency
- Energy is not wasted transcribing these genes when trp is present
- There is also a second layer of regulation...



Attenuation (1) introduction to the leader region

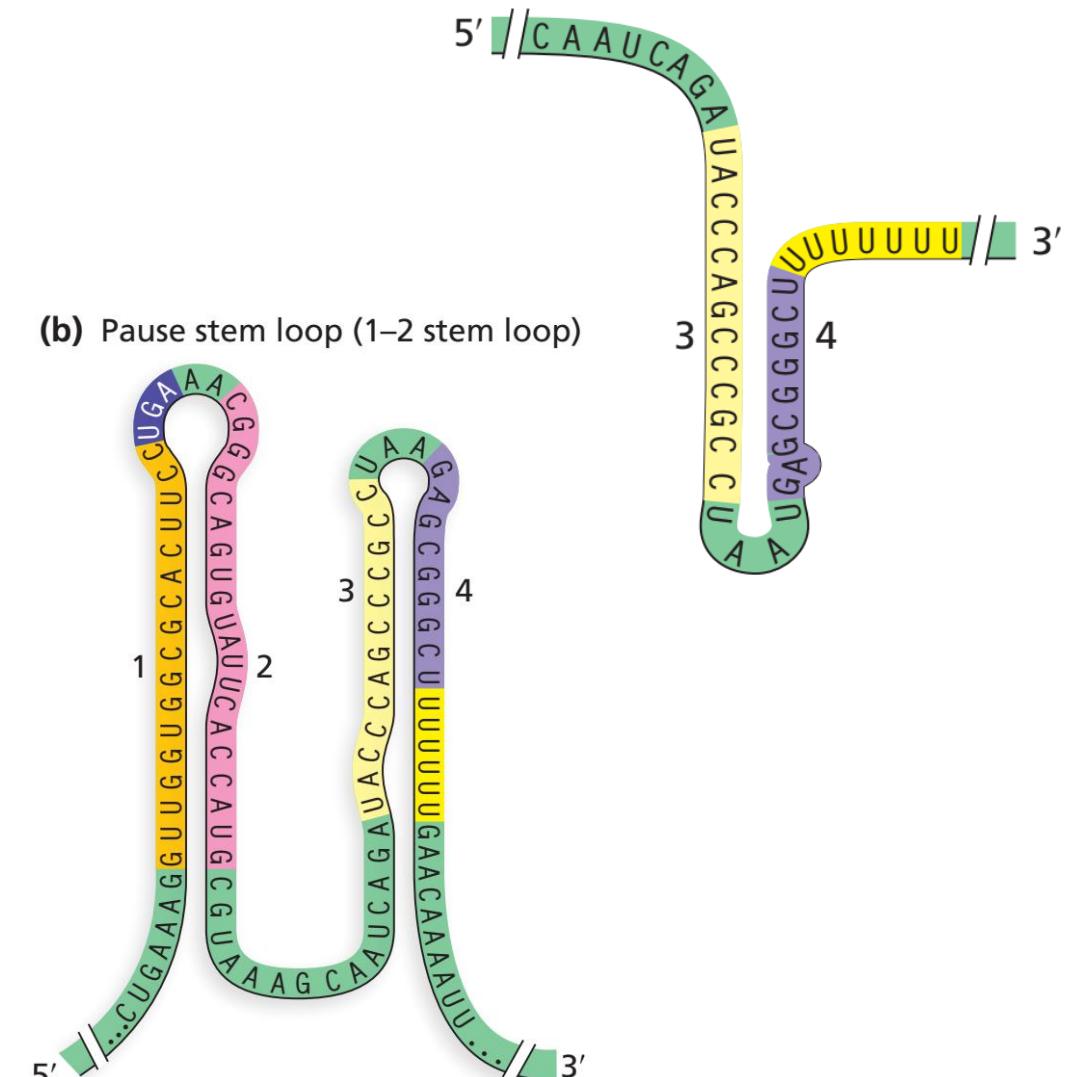
- The second layer of regulation has to do with two alternative types of folding that can take place in the mRNA
- Specifically, in the 162bp leader region
- The leader region has two key properties:
 - 1. It contains 4 regions that can form self complimentary stem loop structures
 - 2. it encodes a 14 AA peptide with its own start and stop codon
- There are three stem loop structures that can be formed:
 - 1-2 stem loop
 - 2-3 stem loop
 - 3-4 stem loop
- 2-3 and 3-4 are the most important for attenuation
- 1-2 and 3-4 can co-occur
- 2-3 is mutually exclusive with the other two



Attenuation (2) the 3-4 stem loop and the 1-2 stem loop

- The 3-4 stem loop signals transcription termination
- This stem loop is followed by a poly-U sequence, identical to what causes intrinsic termination
- If this 3-4 loop forms, the RNA polymerase will be destabilized
- transcription will stop before the 5 genes are transcribed
- The 1-2 stem loop forms when a ribosome does not quickly associate with the nascent trpL mRNA
- The 1-2 stem loop slows transcription to allow for this binding
- If a ribosome binds to the nascent mRNA, translation initiation causes the 1-2 stem loop to come apart and transcription resumes
- If a ribosome does not bind, then the 1-2 and 3-4 stem loops can co-occur and transcription will terminate

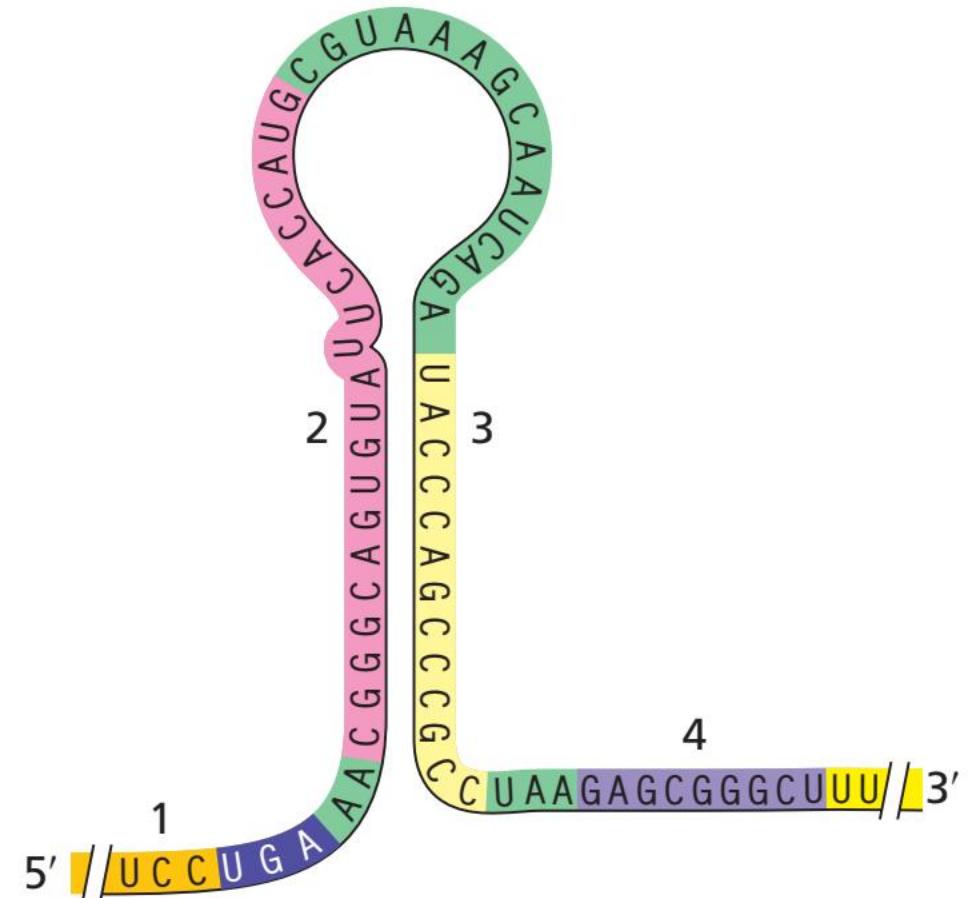
(d) Termination stem loop (3-4 stem loop)



Attenuation (3) the 2-3 stem loop

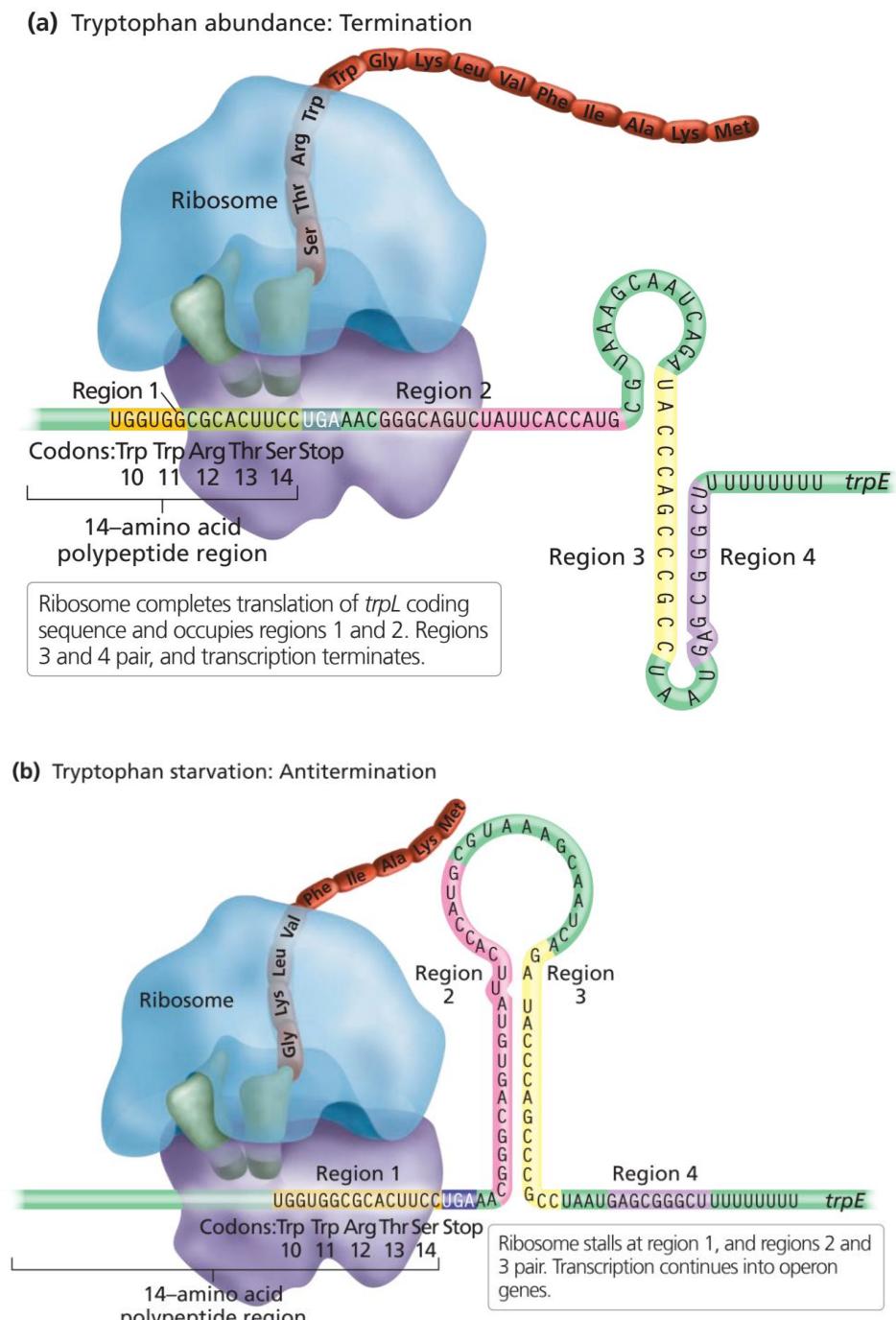
- The 2-3 stem loop is the antitermination stem loop
- No poly U seq following the loop
- Without the 3-4 stem loop transcription does not terminate
- Polycistronic mRNA of the 5 genes is made
- Each mRNA either forms a 2-3 or a 3-4 stem loop
- Coupling of transcription and translation decides the outcome

(c) Antitermination stem loop (2-3 stem loop)



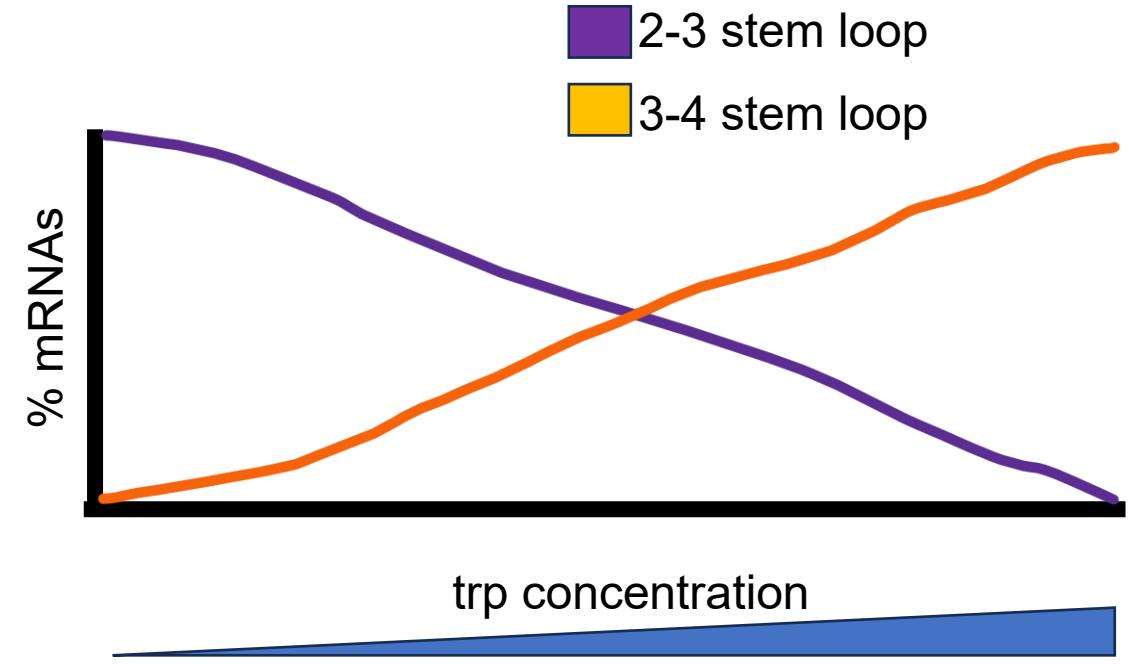
Attenuation (4) determining 2-3 or 3-4 stem loop formation

- Region 1 encodes a 14 AA peptide that includes 2 consecutive trp codons
- In the presence of trp the ribosome efficiently translates the peptide
- When the ribosome progresses quickly, region 2 is occupied by the ribosome and the 2-3 loop cannot form but the 3-4 loop does (termination)
- In the absence of trp, the ribosome stalls on these codons
- When the ribosome stalls, the 2-3 stem loop is formed, precluding the 3-4 stem loop leading to transcription of the operon (antitermination)
- region 2 availability and ribosome occupancy is the key!



Trp operon concepts

- Each trpL mRNA makes a molecularly based “decision”
- Form 2-3 stem loop or form 3-4 stem loop
- The percentage of each outcome will vary with trp concentration
- At any given moment there is a ratio of 2-3 and 3-4 stem loops forming, giving rise to very precise increments of transcriptional control
- The cell can respond to varying concentration in a more nuanced manner compared to on vs off
- Other amino acid operons work in a very similar manner



his operon:

— Met The Arg Val Gln Phe Lys His His His His His His Pro Asp //

leu operon:

— Met Ser His Ile Val Arg Phe Thr Gly Leu Leu Leu Leu Asn Ala Phe //

pheA operon:

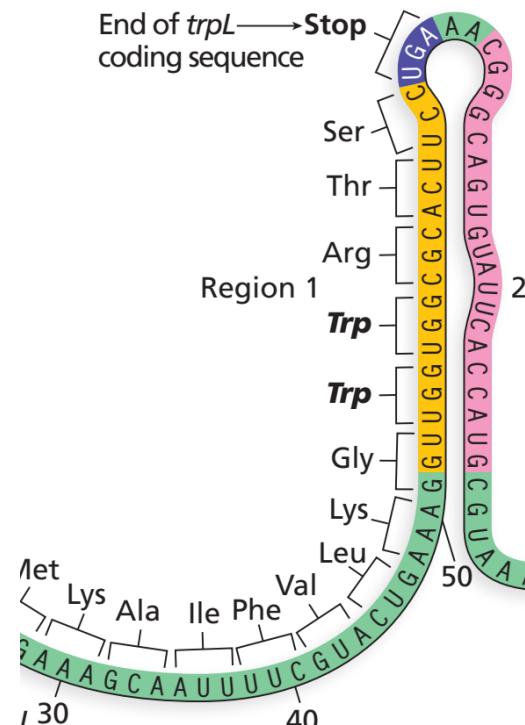
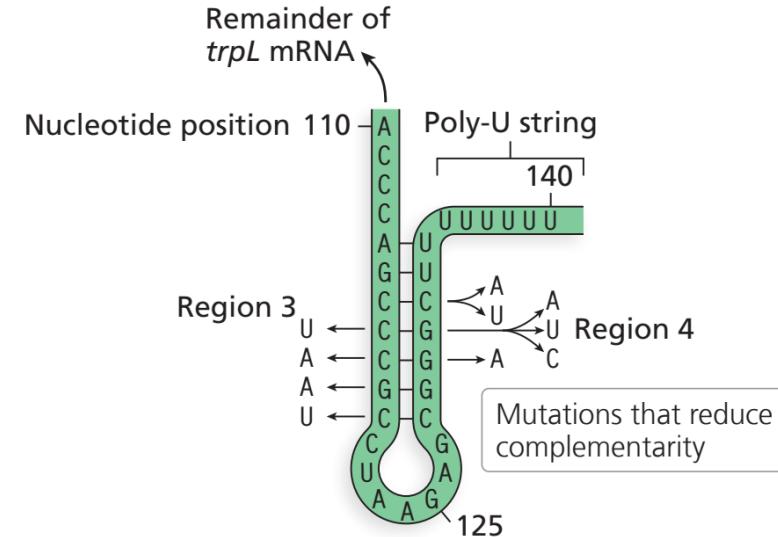
— Met Lys His Ile Pro Phe Phe Phe Ala Phe Phe Phe Thr Phe Pro //

thr operon:

— Met Lys Arg Ile Ser Thr Thr Ile Thr Thr Thr Ile Thr Thr Gly //

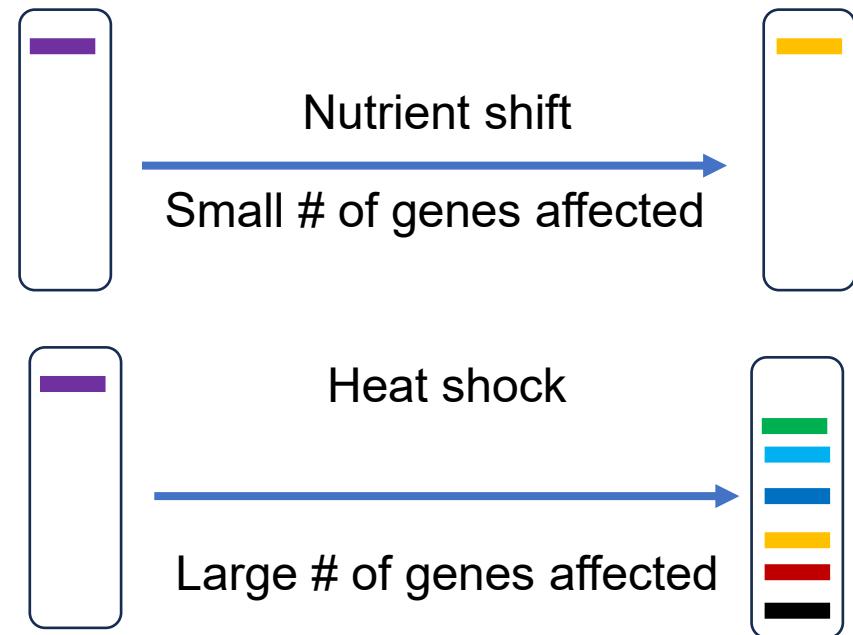
Trp operon mutations

- Mutations that reduce complementarity in the 3-4 stem loop can destabilize this structure leading in reduced effectiveness of repression
- Mutations in the 2 trp codons can place the operon under the control of the availability of different amino acids
- Practice question, suppose the attenuator codons are mutated to:
 - UUG UUG (leucine)
- Trp operon will respond to changes in concentrations of leucine!

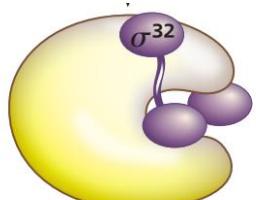
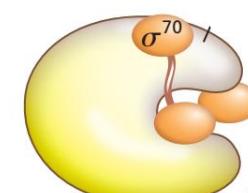
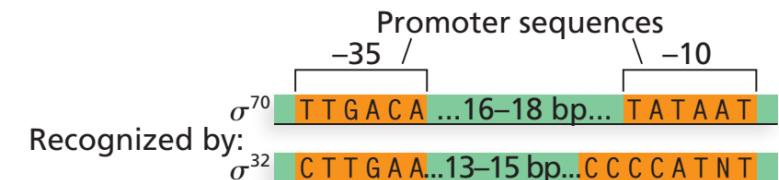


The transcriptional response to stress is different than that for operons

- Changes in the availability of nutrients are routine changes and involve a few genes per nutrient
- Stressful changes such as heat shock are more rare
- Many genes affected
- *E. coli* grows optimally at 37°C
- 45°C is near its upper thermal limit and temps this high activate the heat shock response
- Many genes involved in combating heat induced damage are all simultaneously activated and expressed
- Similar responses exist in other microorganisms, fruit flies, and humans
- How do we alter expression of many genes at once?
- Express alternative sigma subunit!
- σ^{32} recognizes the promoters of genes involved in heat shock

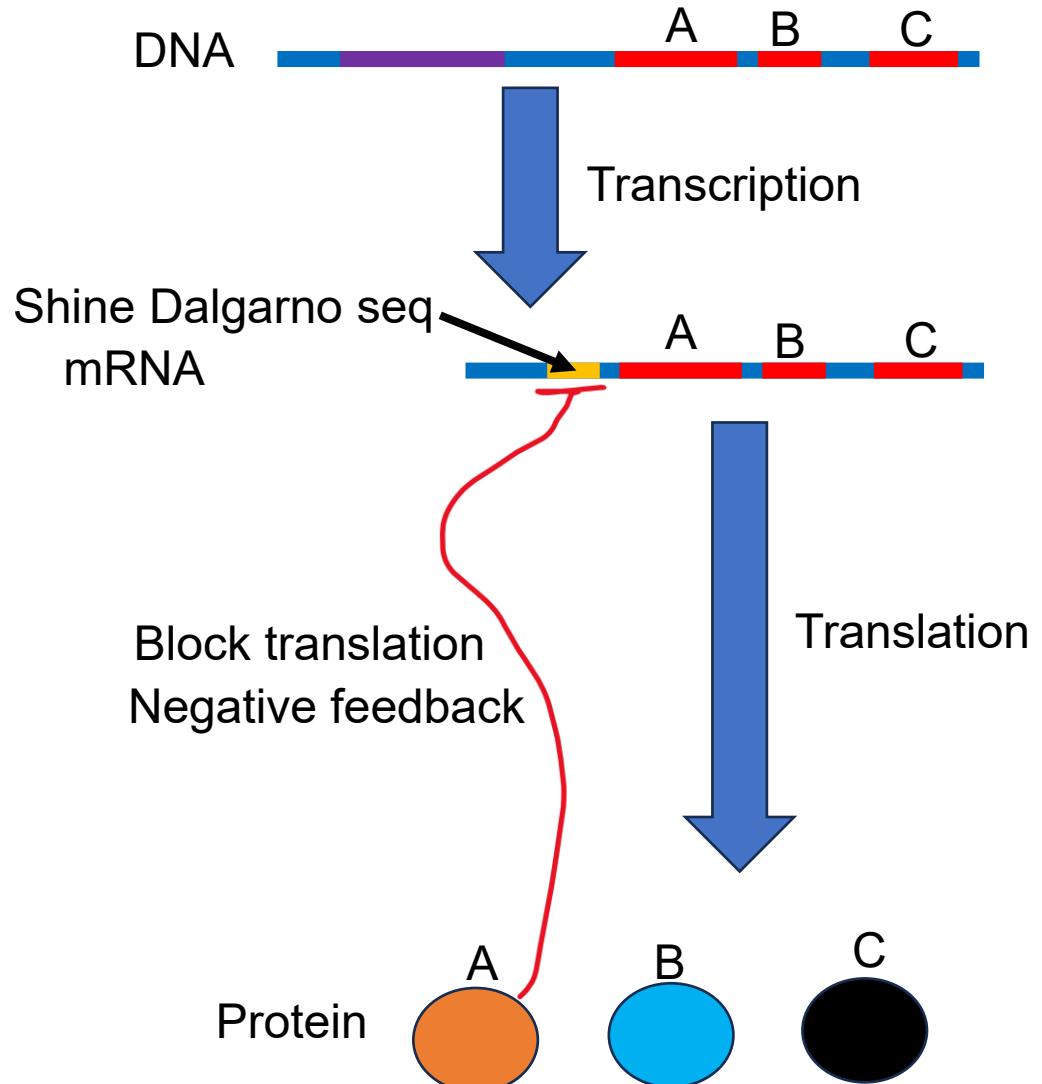


(a) Promoter sequences recognized by different sigma factors



Translational regulation in bacteria (1)

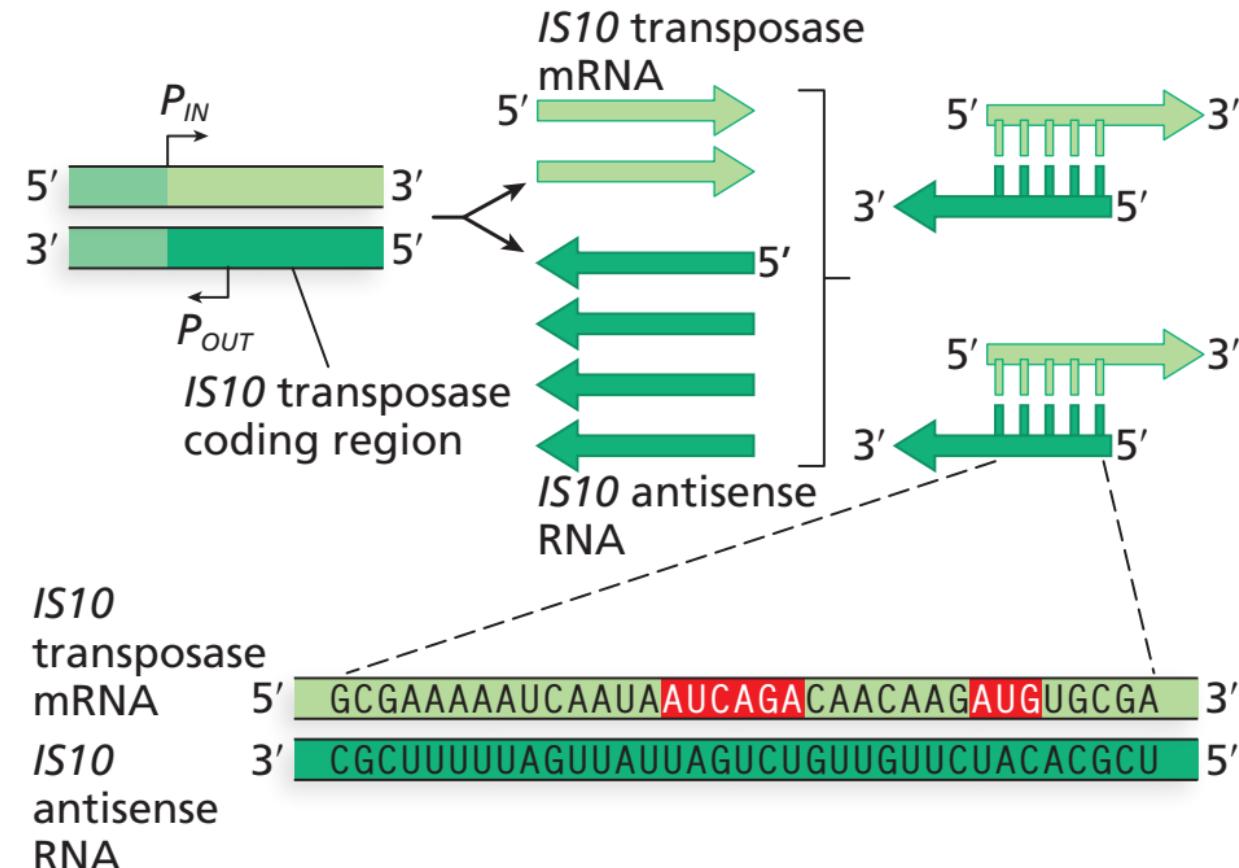
- Most gene regulation in bacteria occurs at the level of transcription
- Regulation at the level of translation can occur in two main ways:
- 1. Protein binding to mRNA
 - Translation repressor proteins
 - Example: Ribosomal proteins are transcribed in operons
 - One of the products of each operon can bind their own mRNAs at the Shine-Dalgarno seq preventing their translation
 - This provides negative feedback and is the main way production of these proteins is regulated



Translational regulation in bacteria (2)

• 2. Antisense RNA

- If antisense RNA binds to an mRNA it can prevent its translation
- A well characterized example of this is IS10 which encodes a transposase
- Bacteria can tolerate low expression, but high expression can cause problems if genes are getting disrupted by the transposon
- The gene has two promoters oriented in opposite directions
- P-in is a weak promoter, this drives expression of the transposase
- P-out is a strong promoter and drives expression of an overlapping antisense RNA
- When these RNAs base pair the Shine-Dalgarno seq and the start codon are sequestered and cannot bind the ribosome
- System is imperfect and there is occasional escape by a transposase mRNA leading to occasional transposition.



Riboswitches Regulation of mRNA stability

- Riboswitch mechanisms involve binding a segment of mRNA to a regulatory molecule
- Example in *B. subtilis* affects mRNA stability of the glmS gene
- glmS catalyzes the formation of a sugar called GlcN6P
- If GlcN6P concentration is low, riboswitch inactive, mRNA is translated
- If GlcN6P concentration is high, riboswitch is active, and a portion of the 5' UTR catalyzes cleavage of the mRNA (ribozyme)
- This leads to degradation of the 3' cleavage product by RNase J1
- This is also negative feedback

