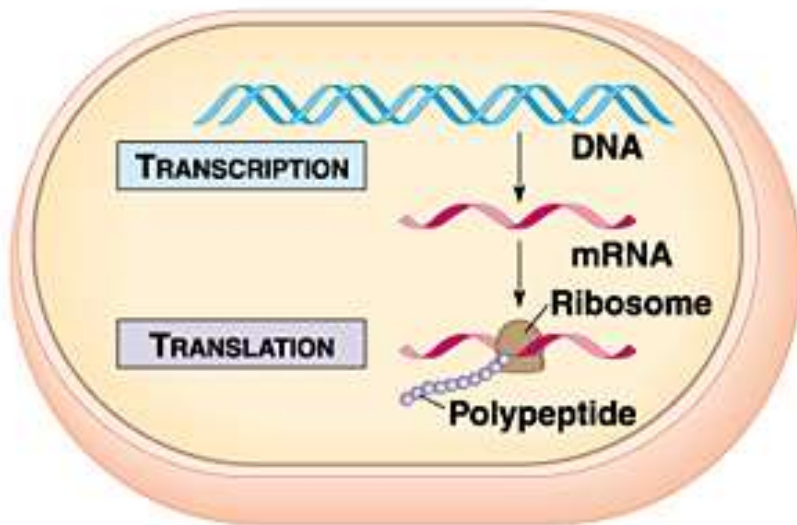


Peter Pristas

BNK1

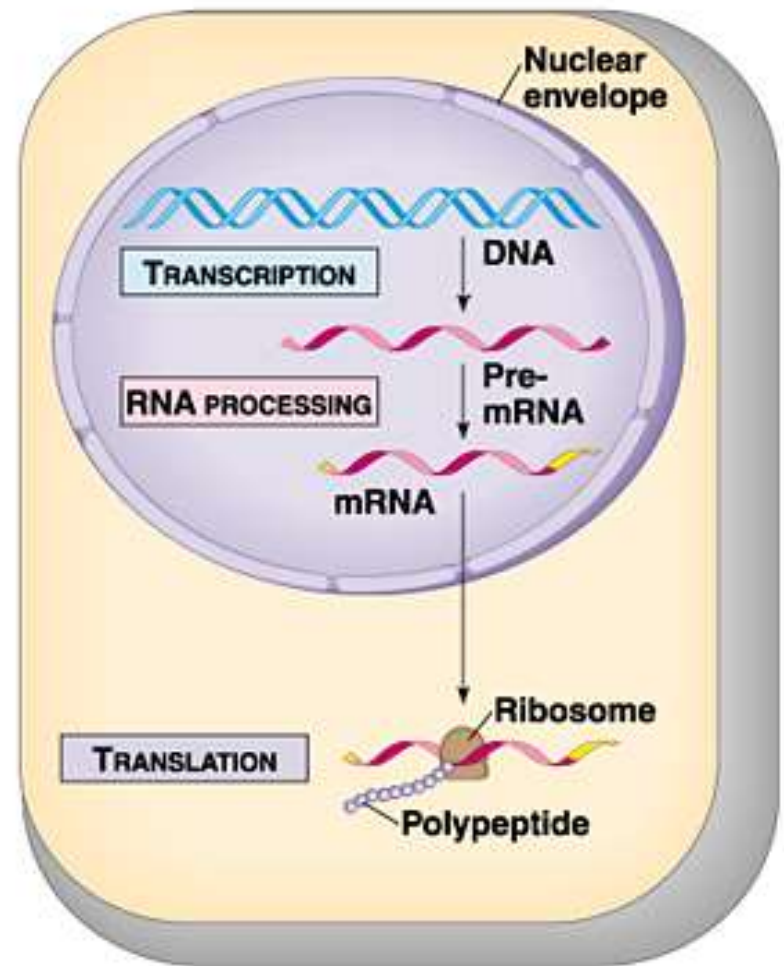
Gene regulation in prokaryotes

Prokaryotes and Eukaryotes



(a) Prokaryotic cell

- prokaryotes (bacteria) do not have nuclei
- eukaryotes segregate transcription in the nucleus. mRNA is also preprocessed prior to translation in eukaryotes



(b) Eukaryotic cell

Prokaryotic vs. Eukaryotic Gene Regulation

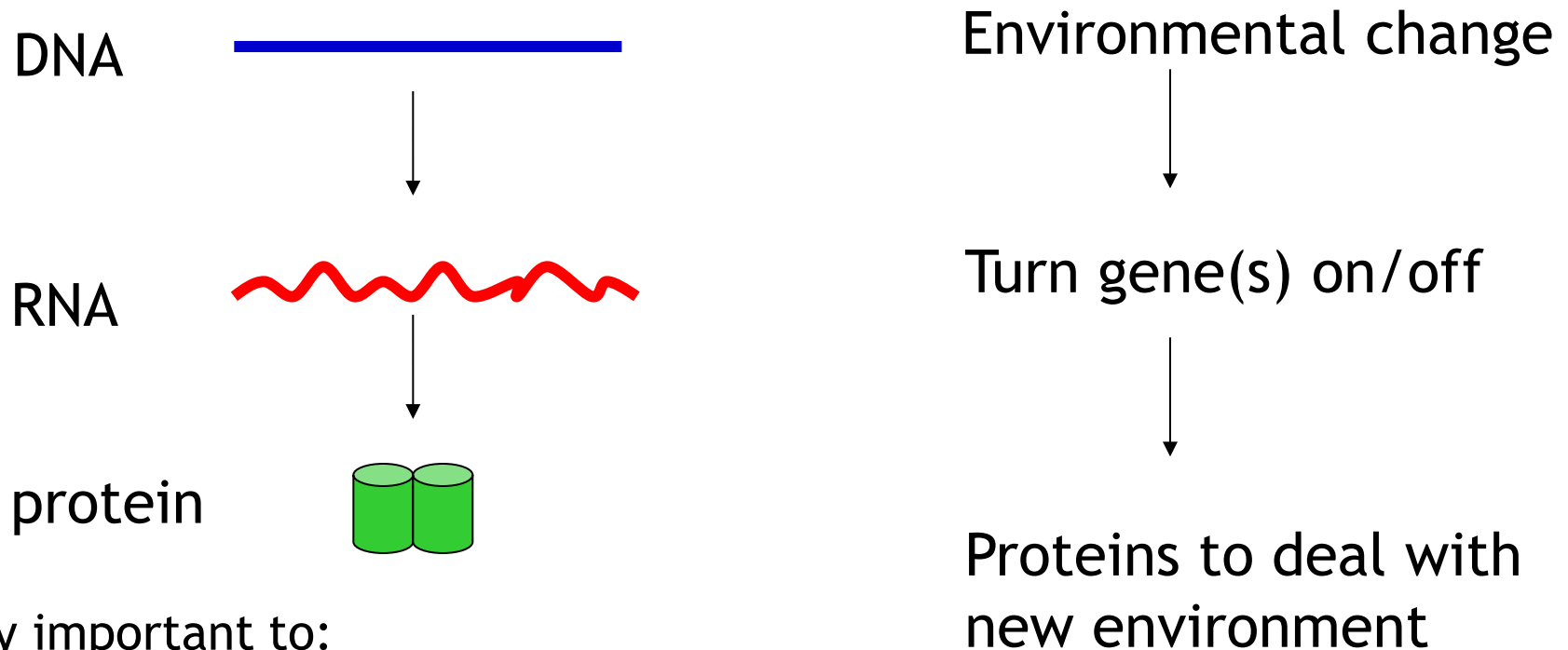
Prokaryotes

- Regulatory proteins
- Ground state = on
- No DNA/protein complexes
- Gene arranged in operons

Eukaryotes

- Regulatory proteins
- Ground state = off
- DNA/protein complexes = Chromatin
- No operons

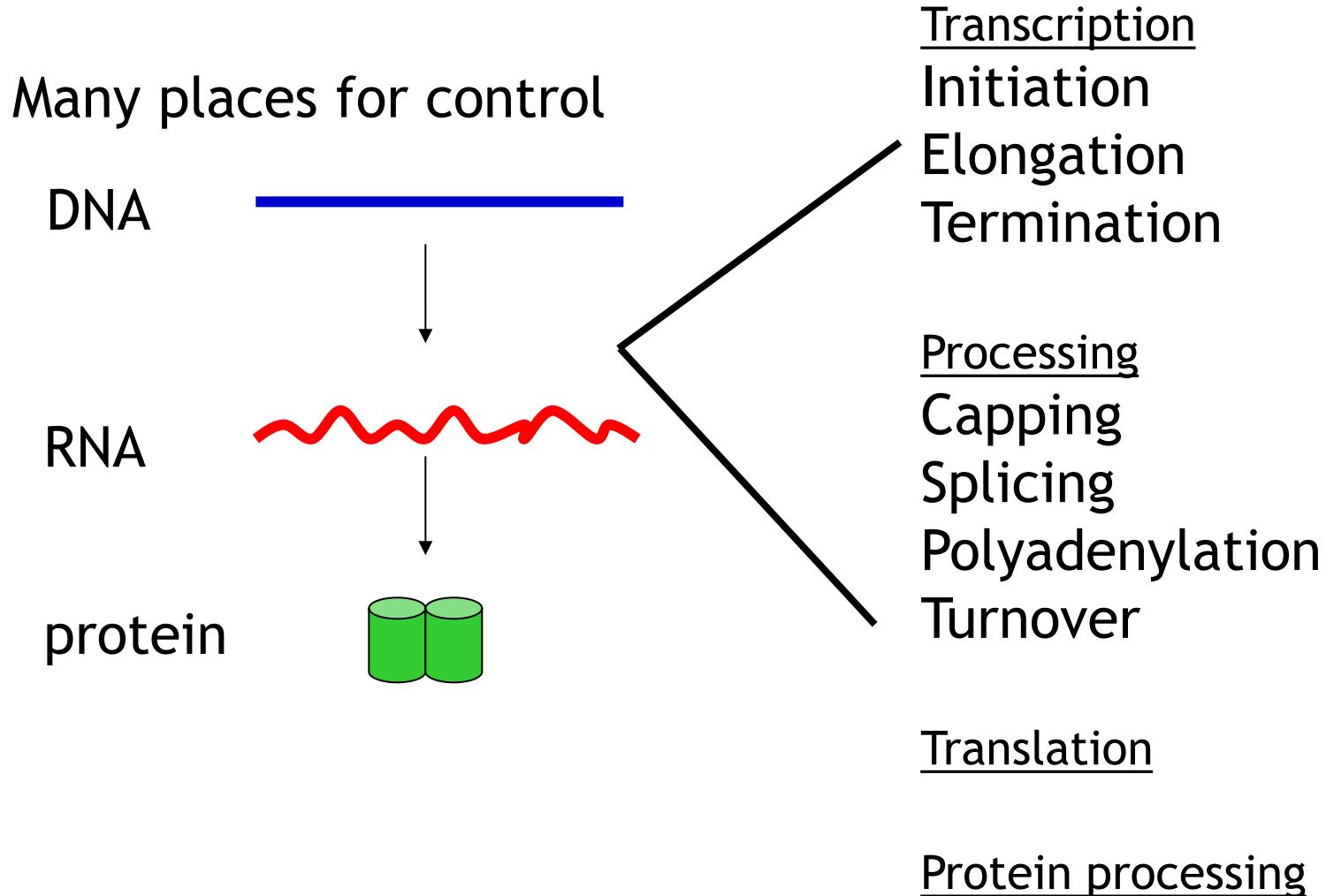
Gene expression regulation



Very important to:

1. express genes when needed
2. repress genes when not needed
3. Conserve energy resources; avoid expressing unnecessary/detrimental genes

Transcriptional Control



Expression of many genes in cells are regulated

Housekeeping genes: expressed constitutively, essential for basic processes involving in cell replication and growth.

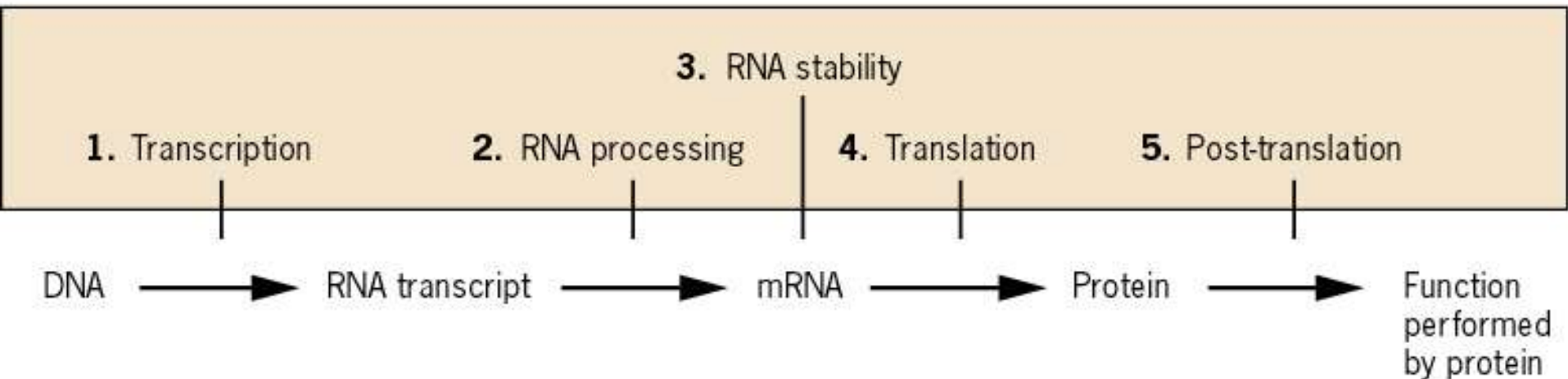
Inducible genes: expressed only when they are activated by inducers or cellular factors.

Expression of many genes in cells are regulated

- Gene expression is very often controlled by **extracellular signals**, which are communicated to genes by regulatory proteins:
- **Positive regulators** or activators INCREASE the transcription
- **Negative regulators** or repressors DECREASE or ELIMINATE the transcription

Levels of Regulation

Levels at which gene expression is regulated in prokaryotes

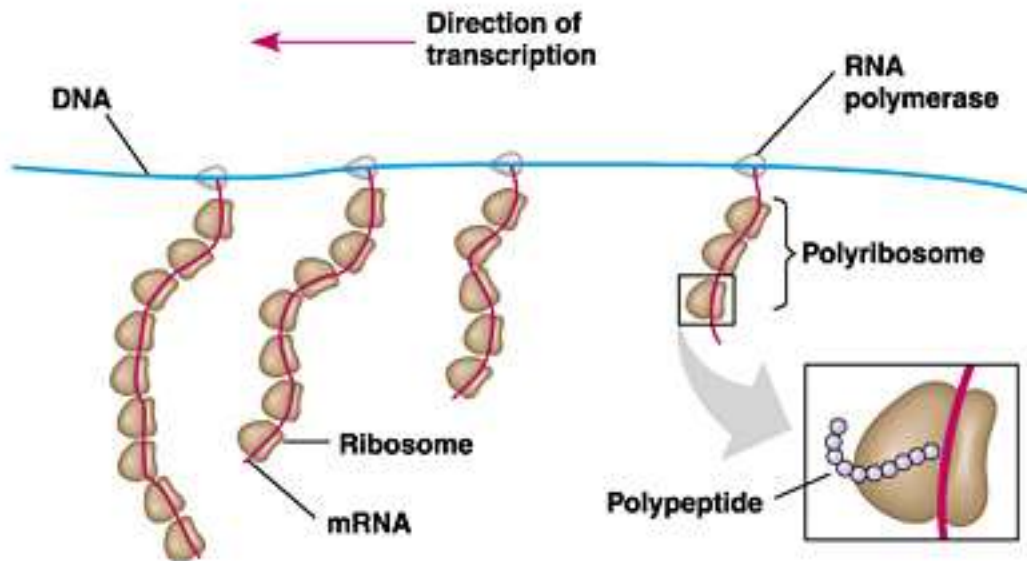
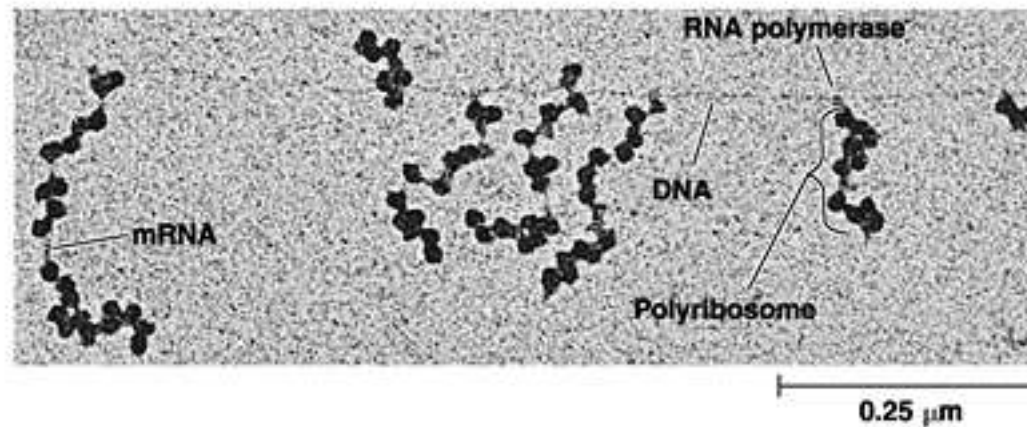


- Transcriptional regulation plays the largest role

Typical half-lives of mRNA molecules

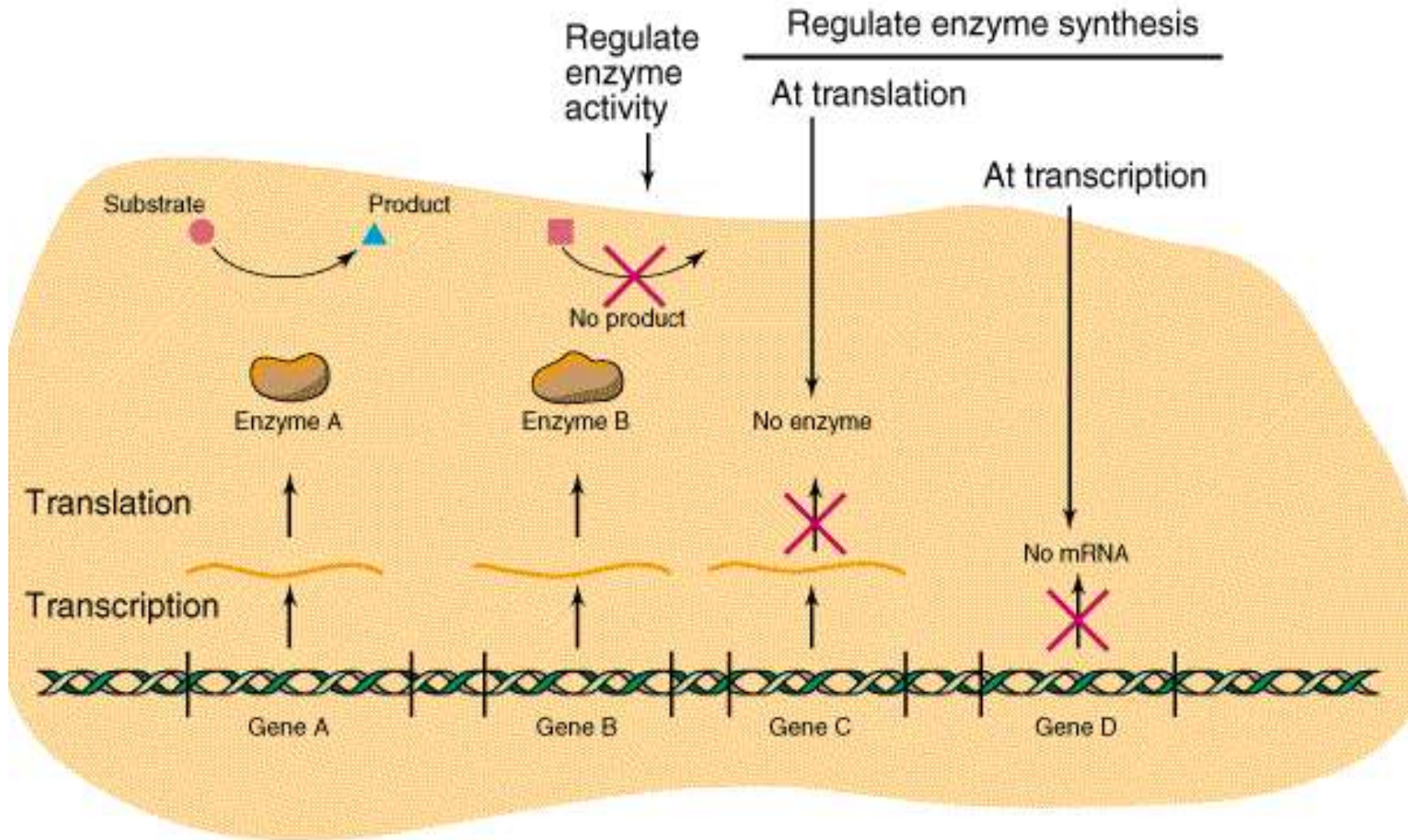
Cell	Generation time of cell	mRNA Half-Lives	
		Average	Range
<i>Escherichia coli</i>	20 - 60 min	3 - 5 min	2 - 10 min
<i>Saccharomyces cerevisiae</i> (yeast)	3 hr	22 min	4 - 40 min
Cultured human or rodent cells (histone,	16 - 24 hr	10 hrs	30 min or less <i>c-myc</i> mRNAs) 0.3 - 24 hr (specific mRNAs)

Transcription and translation are coupled in prokaryotes



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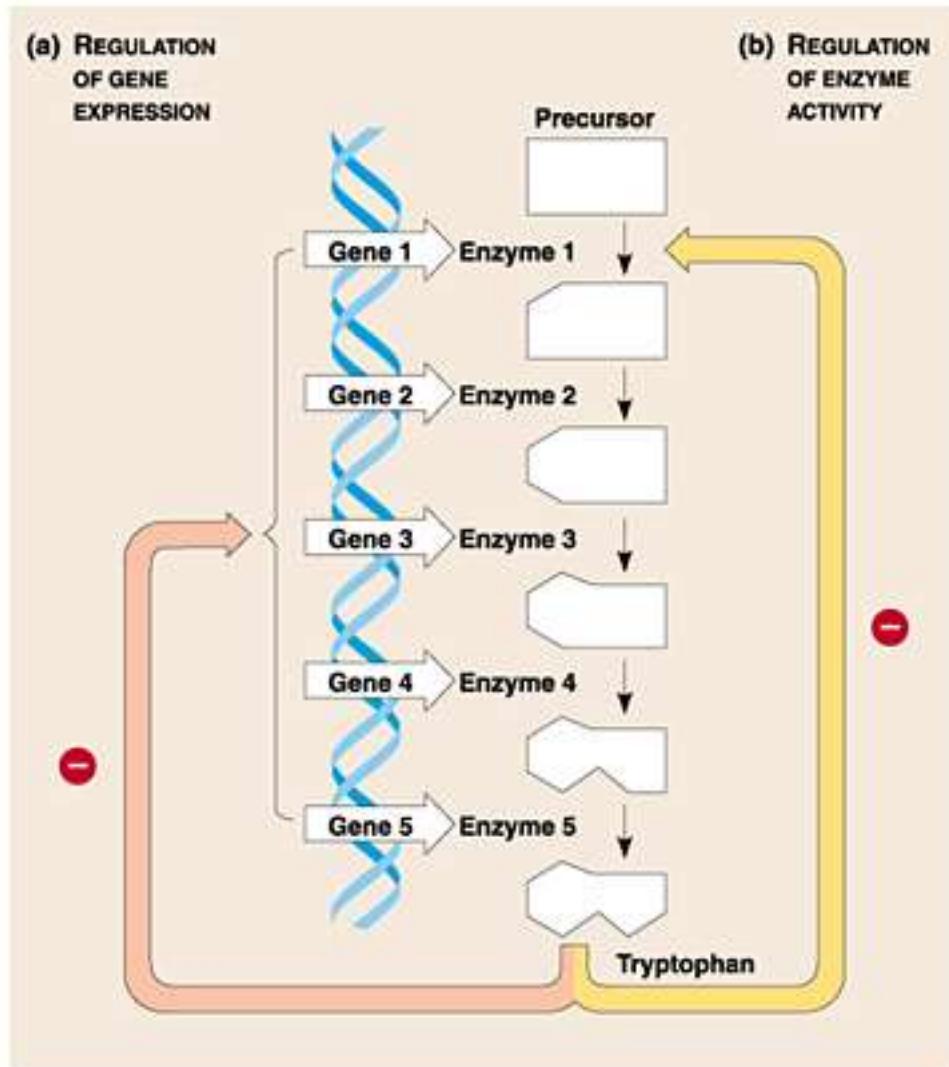
Regulatory pathways in prokaryotes



Coordinate Regulation - operons

- Expression of several or numerous genes can be controlled simultaneously.
- Operon: a set of genes that are transcribed from the same promoter and controlled by the same regulatory sites.
- Regulon: a set of genes (and/or operons) expressed from separate promoter sites, but controlled by the same regulatory molecule. Global regulons may coordinate expression of many genes and operons, and may induce some, but repress others.

Operons



In addition to regulating enzyme activity through feedback inhibition, cells can also regulate the production of one enzyme.

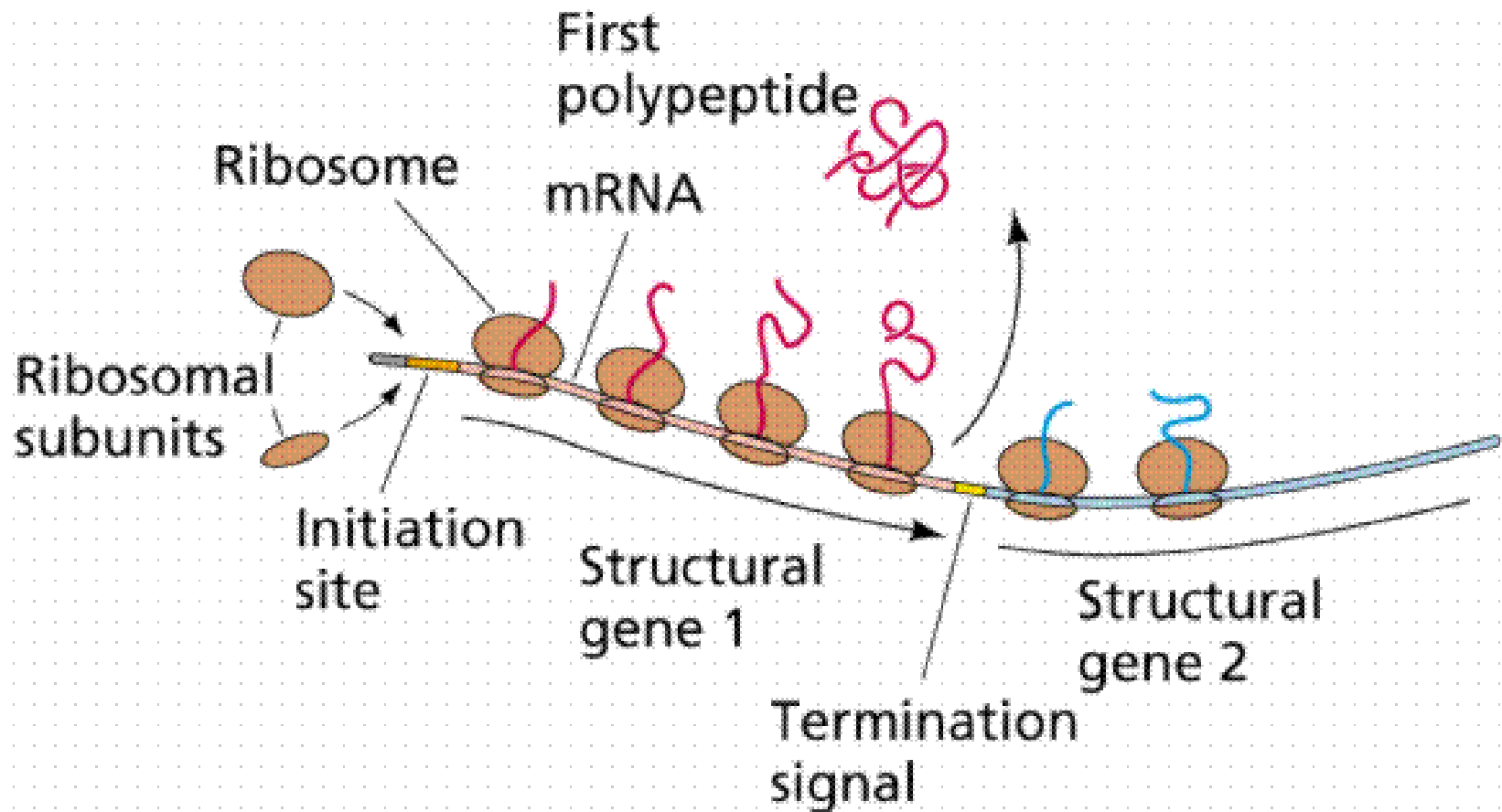
Entire pathways are transcribed and translated using operons.

Operons are a set of genes that are common to a specific metabolic event all regulated by an operator sequence which controls their ensemble expression.

Examples

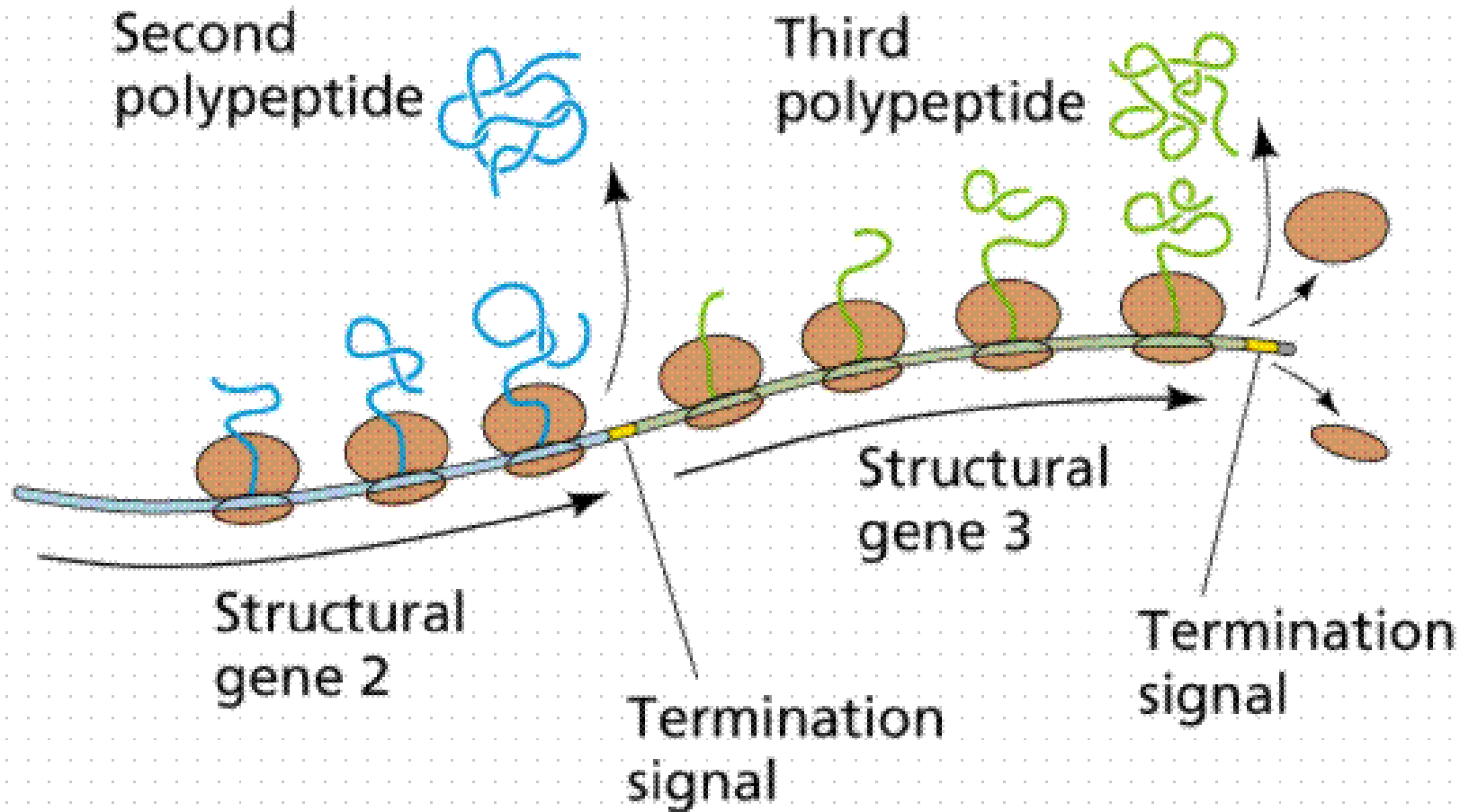
- trp operon
- lac operon

Operons



Once the mRNA is transcribed, many ribosomes translate all of the proteins (enzymes) in the operon at once.

Operons



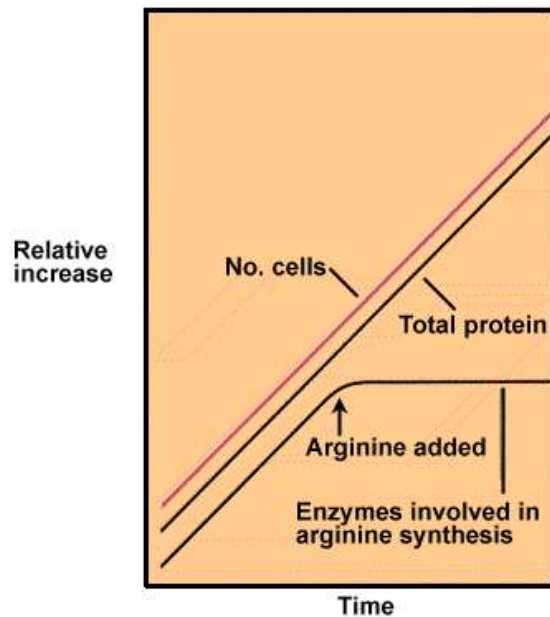
Translation continues until the end of the message (mRNA).

Two major steps to regulated gene expression

1. Regulatory proteins – usually DNA binding proteins
 - a. Repressors – inhibit transcription
 - b. Activators – elevate transcription
 - c. function regulators – may do either - conditional
2. Choice of sigma factors – dictate which promoters are on and which are off.

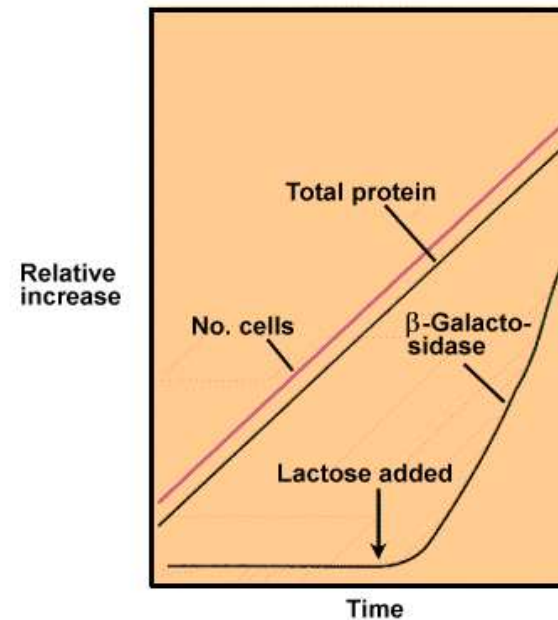
Repressors can impart different regulatory patterns

Arginine biosynthesis



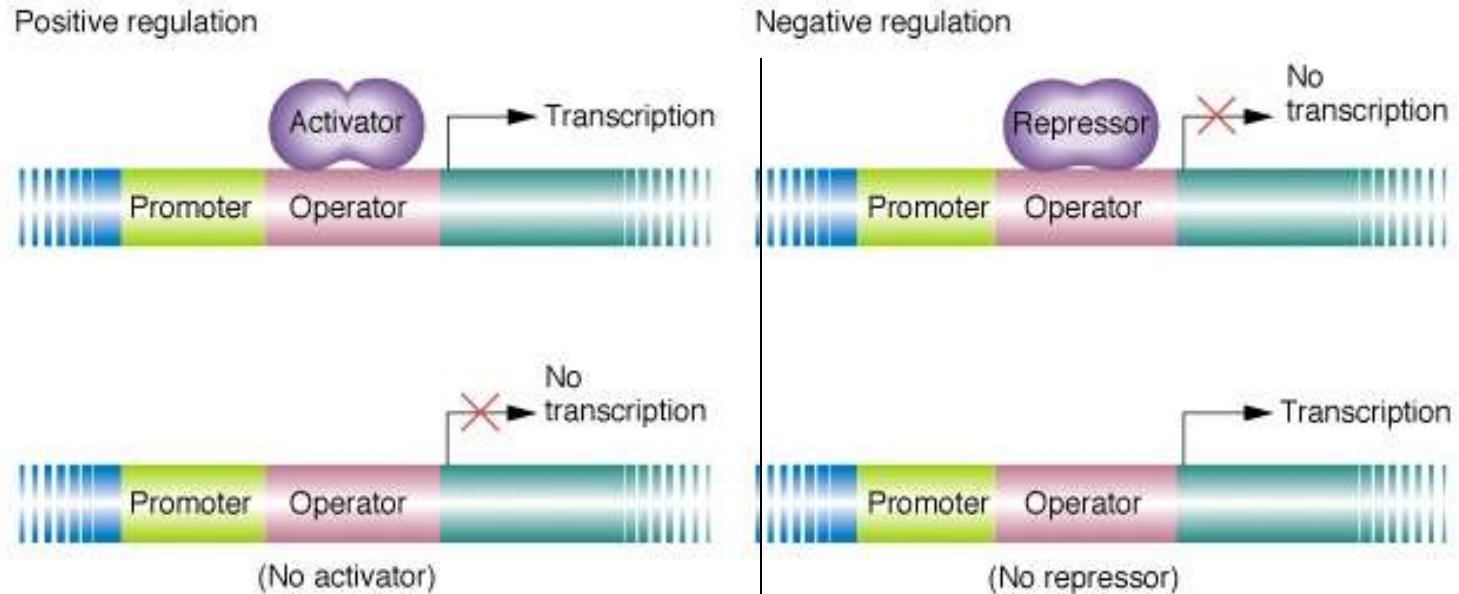
Repression

Lactose degradation



Induction

Regulatory Components



cis regulatory elements only affect the same DNA molecule

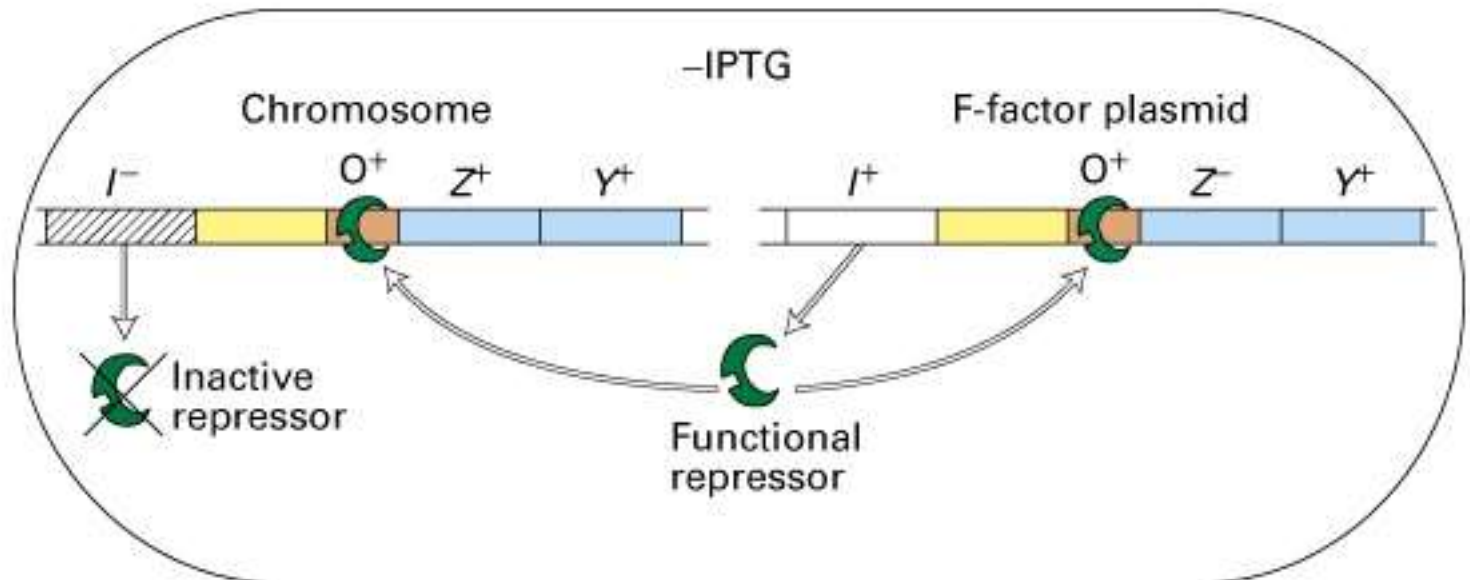
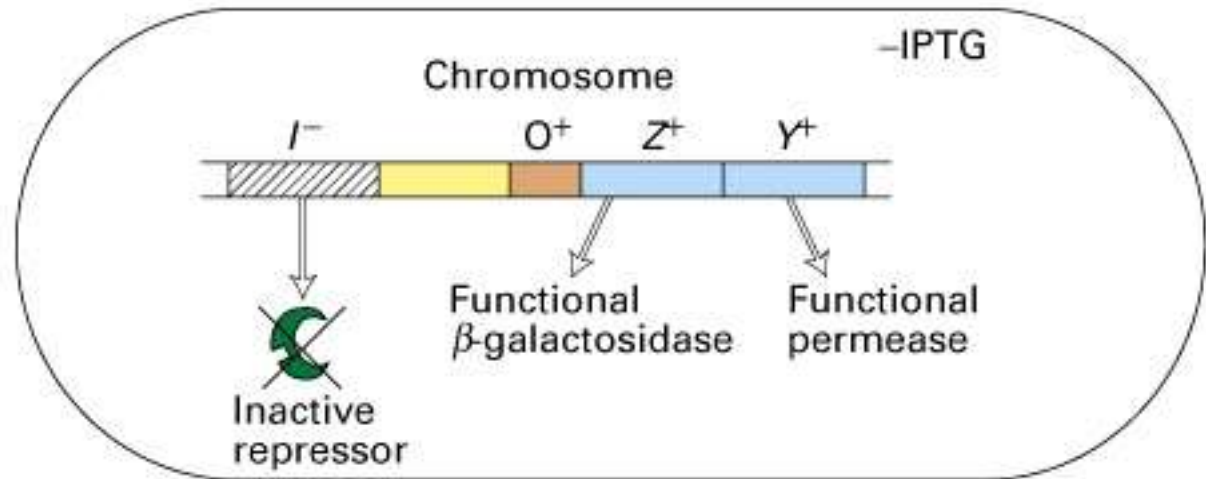
- promoters, operators, enhancers

trans regulatory elements will affect any DNA molecule

- activators, repressors, transcription factors

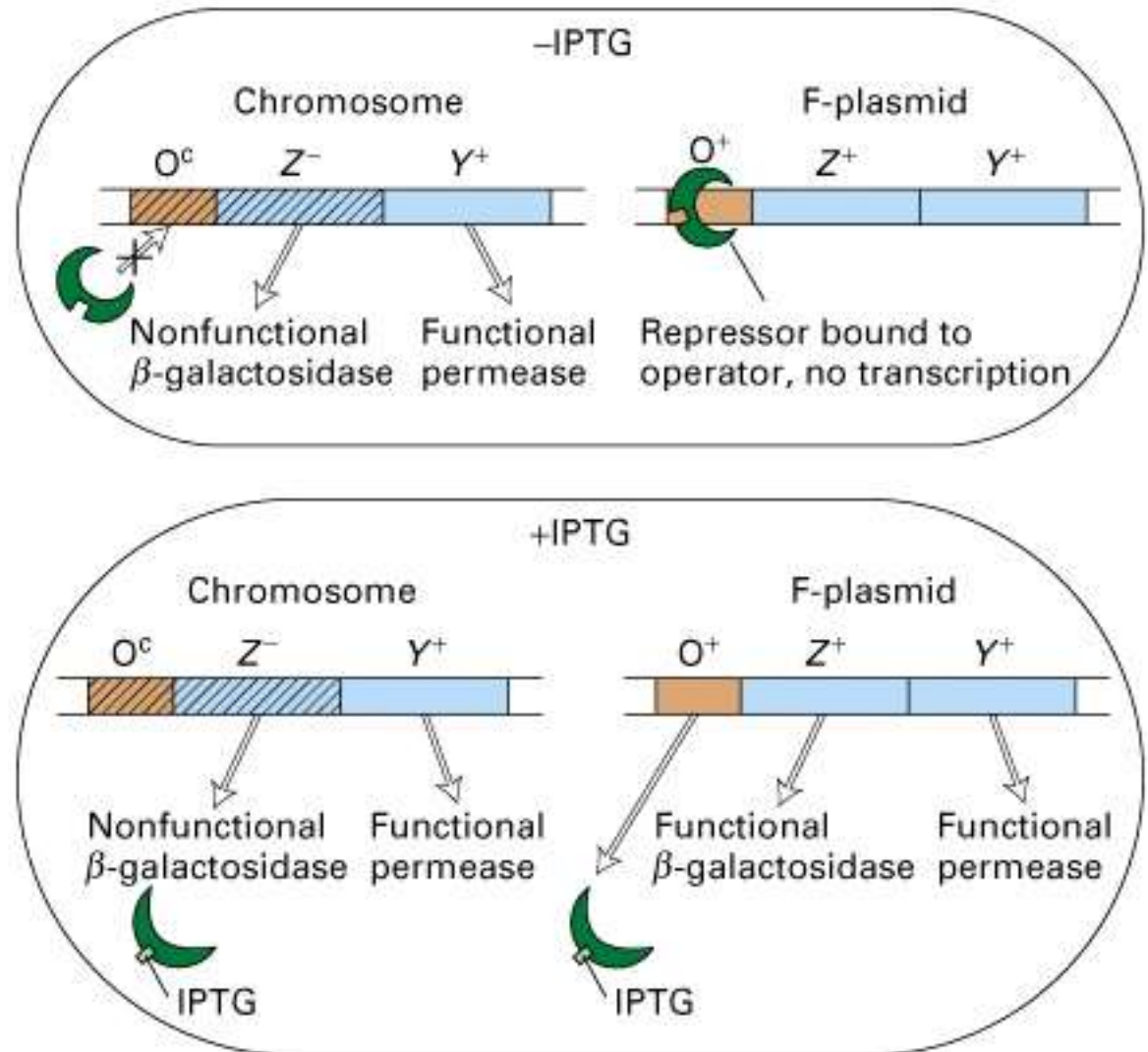
Trans-acting elements

lacI is
trans-
acting



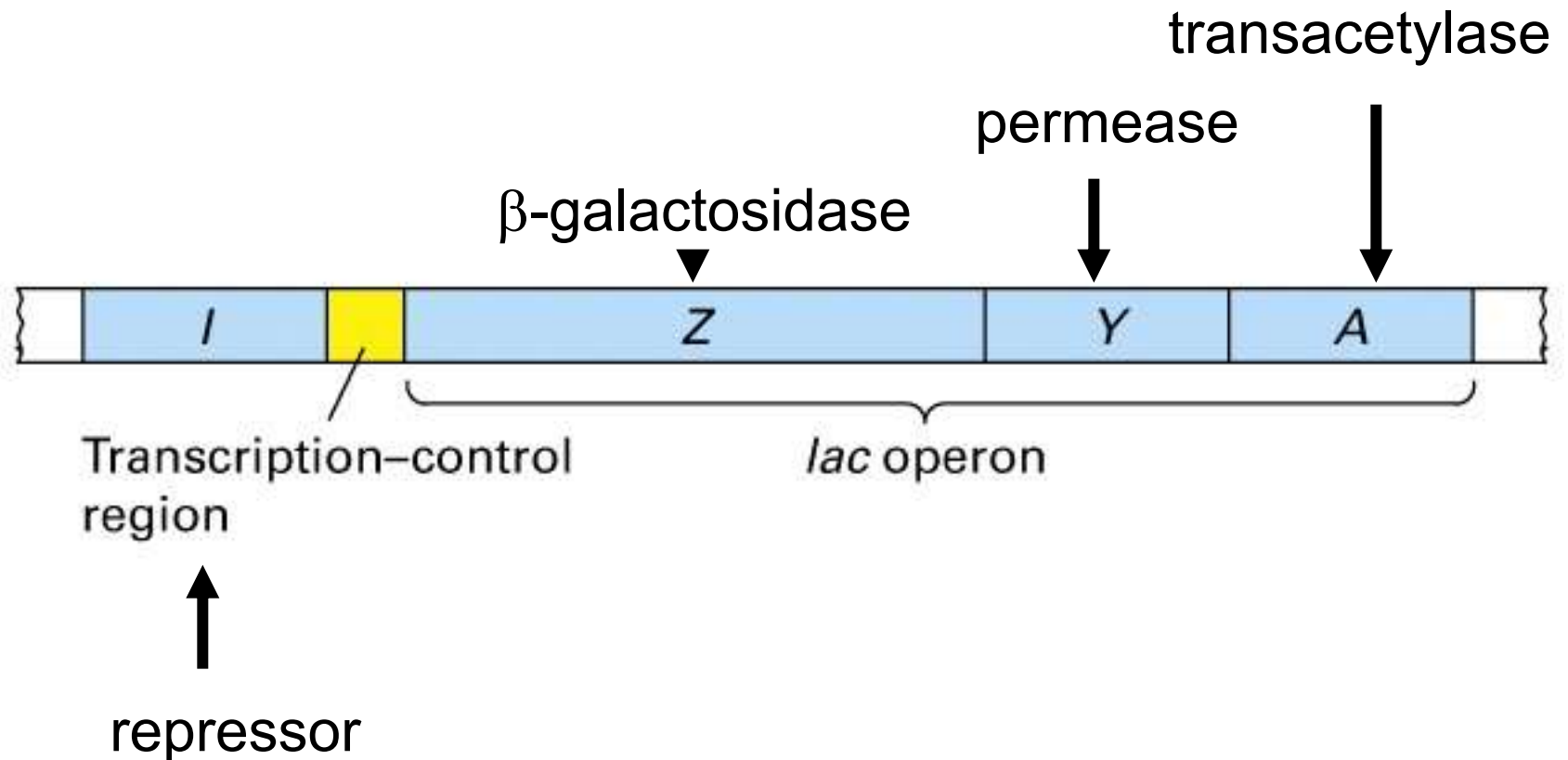
Cis-acting elements

O^c is *cis*-acting



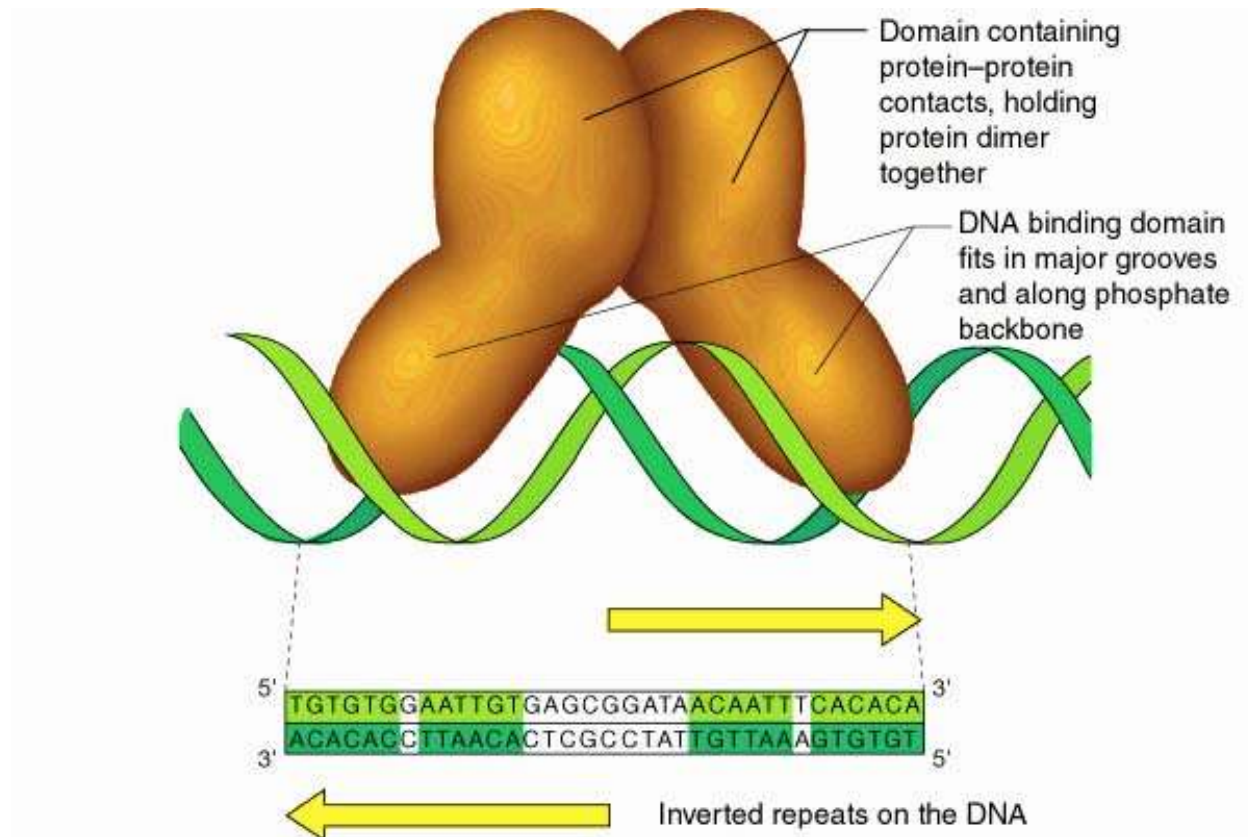
Where to regulatory proteins bind to promoters?

Activators almost always bind upstream of the -30 position, while many repressors bind downstream, as well as upstream of -30

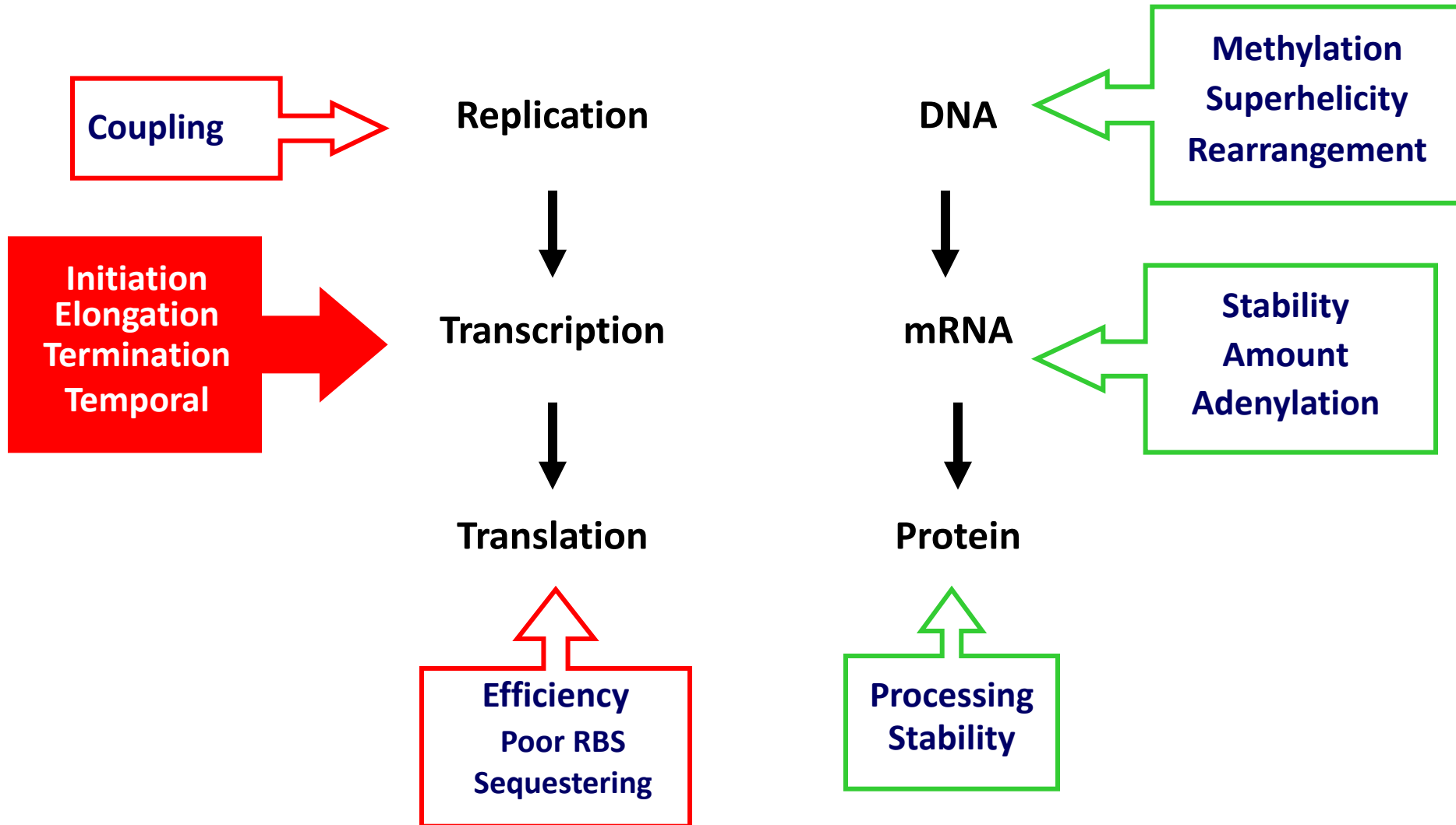


Regulatory proteins

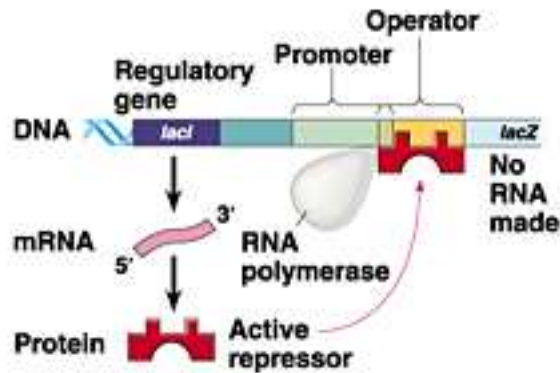
Typically DNA binding proteins that associate with the regulated promoter and either decrease or increase the efficiency of transcription, repressors and activators, respectively - A significant number of regulators do either one depending on conditions



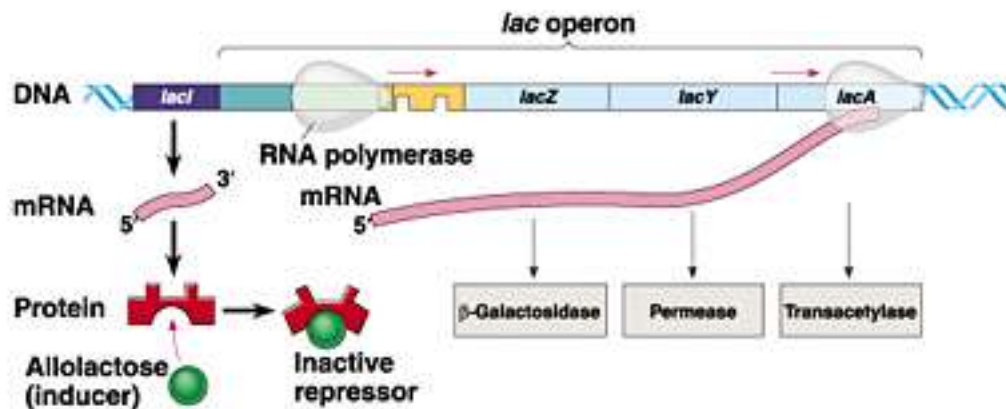
Regulatory pathways in prokaryotes



The *lac* - induction



(a) Lactose absent, repressor active, operon off

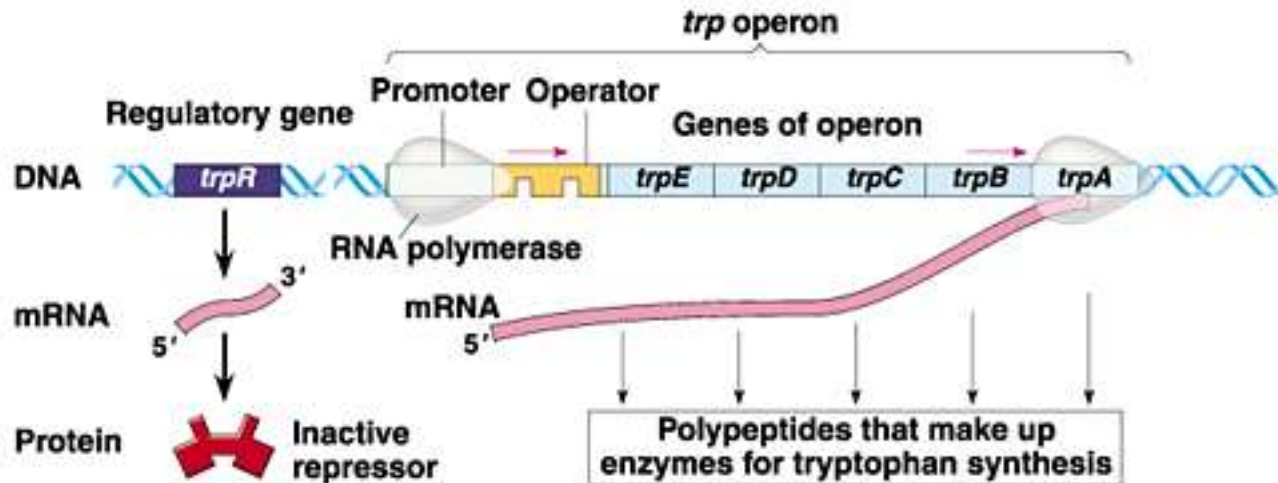


(b) Lactose present, repressor inactive, operon on

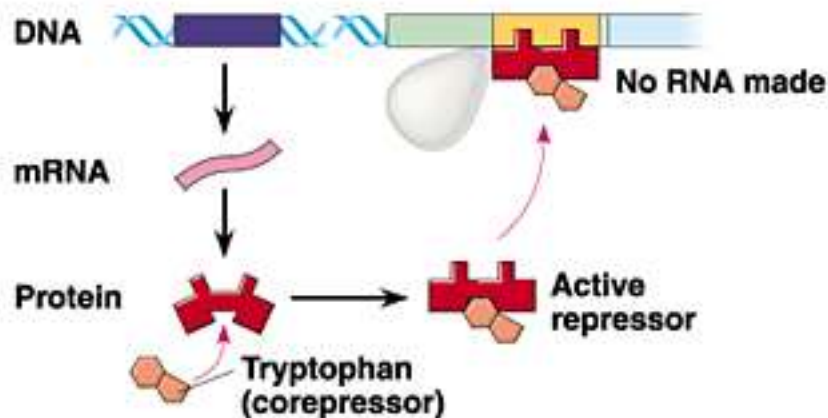
Lactose metabolism is regulated by three genes. Normally, a cell wants to grow in glucose. But in the presence of low glucose and high lactose, the cell will induce the production of lactose specific enzymes

Lactose = glucose + galactose

The *trp* operon - repression



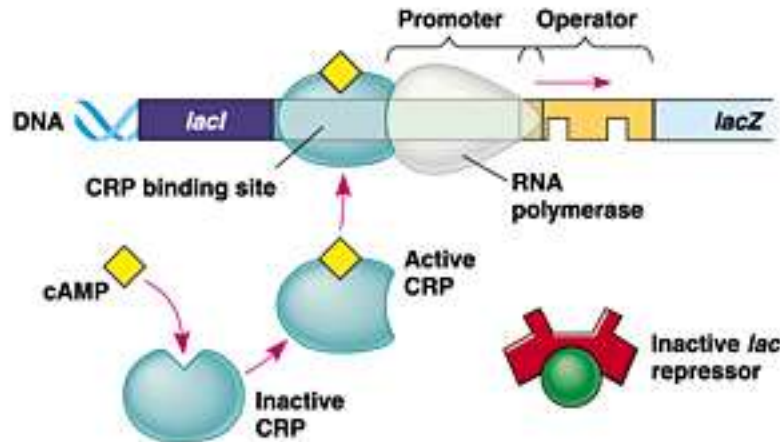
(a) Tryptophan absent, repressor inactive, operon on



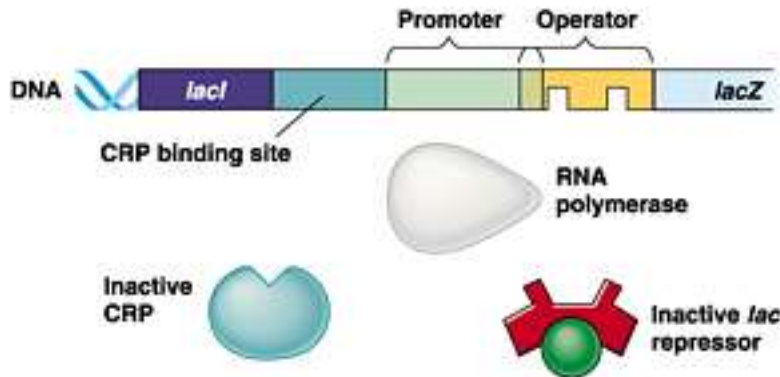
(b) Tryptophan present, repressor active, operon off

In a type of feedback inhibition, the product determines the binding of a repressor protein which blocks transcription

The *lac* - catabolic repression



(a) Lactose present, glucose absent (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

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Lactose metabolism is regulated by three genes. Normally, a cell wants to grow in glucose. But in the presence of low glucose and high lactose, the cell will induce the production of lactose specific enzymes

Lactose = glucose + galactose

Since the cell prefers glucose, it has a secondary mechanism to ensure that lactose metabolism occurs only when the concentration of glucose is low

The *CAP* activator and the *lac* operon

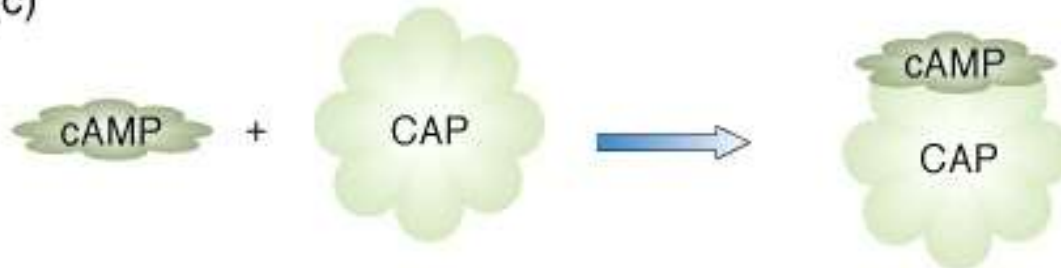
(a) High glucose  Inactive adenylate cyclase

ATP  No cAMP

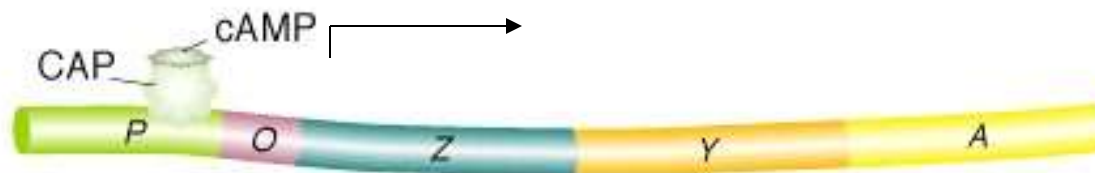
(b) Low glucose  Active adenylate cyclase

ATP  cAMP

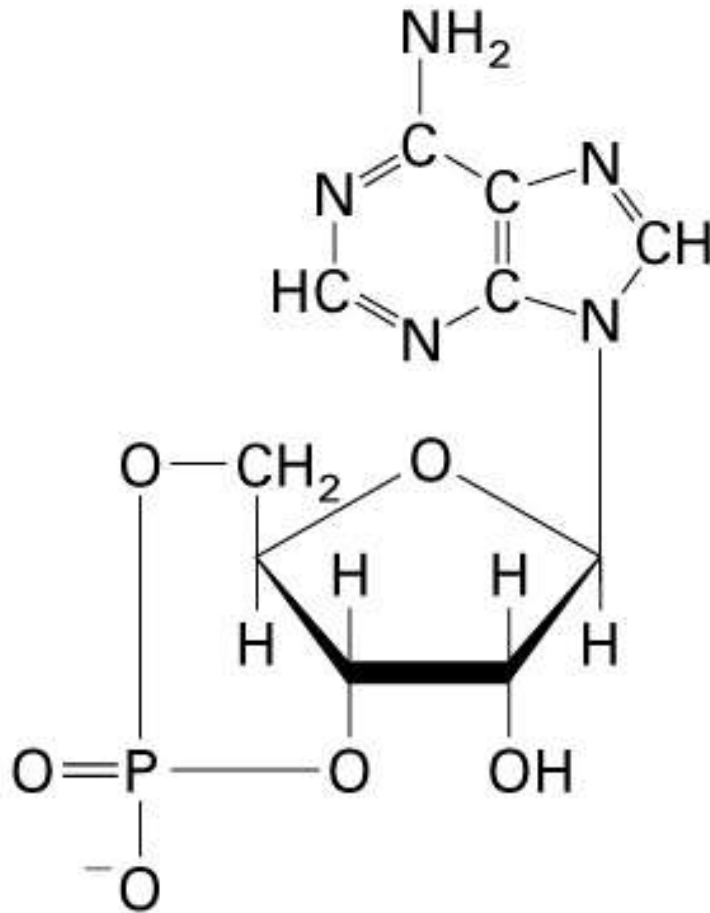
(c)



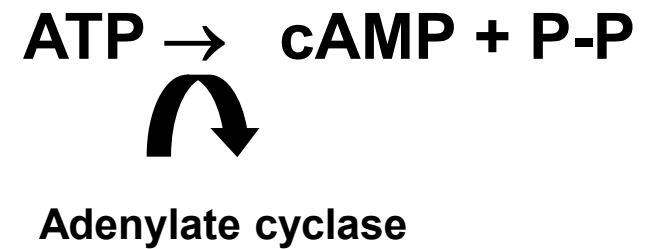
(d)



Cyclic AMP

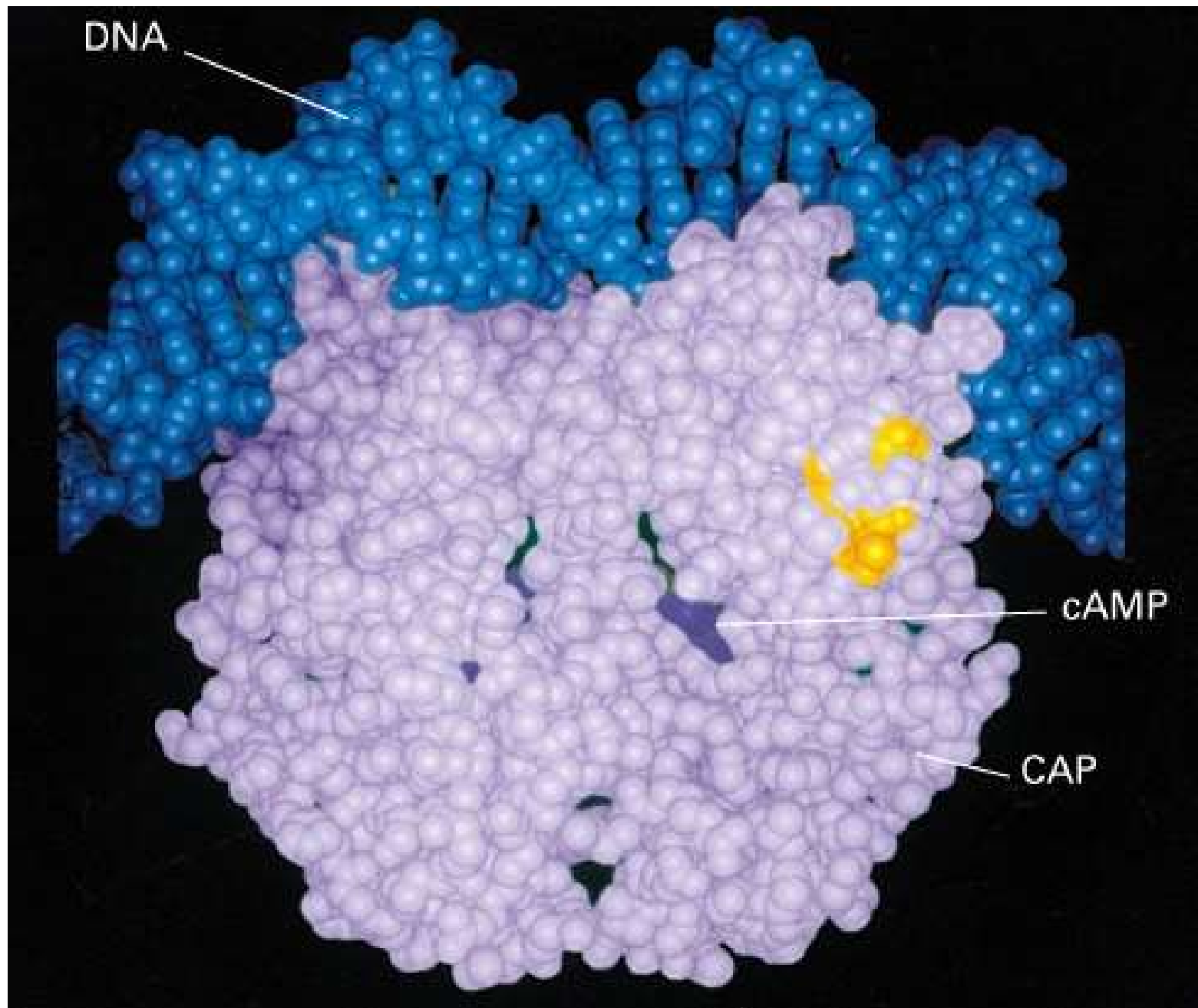


Cyclic AMP

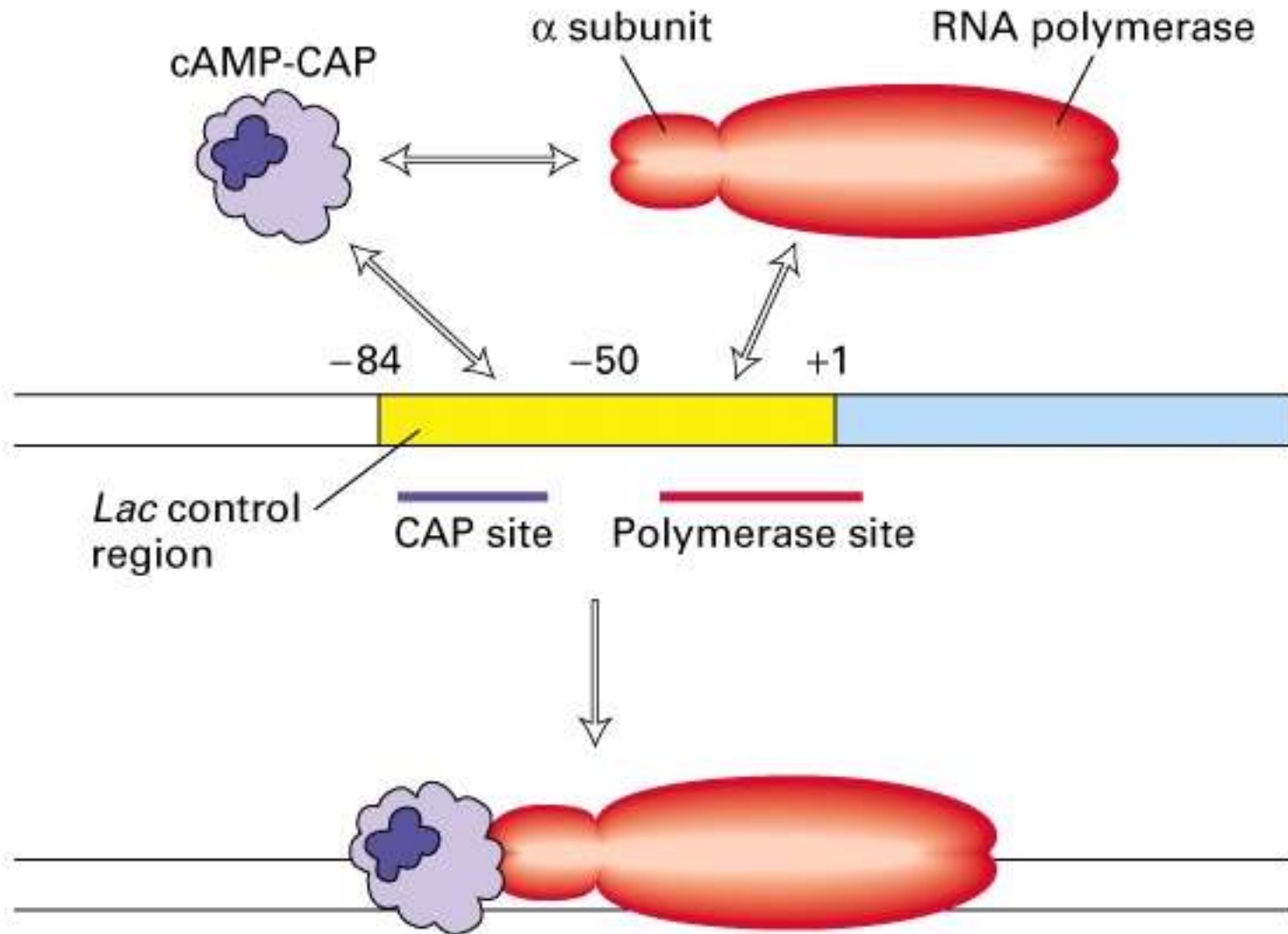


CAP binding alters the DNA secondary structure

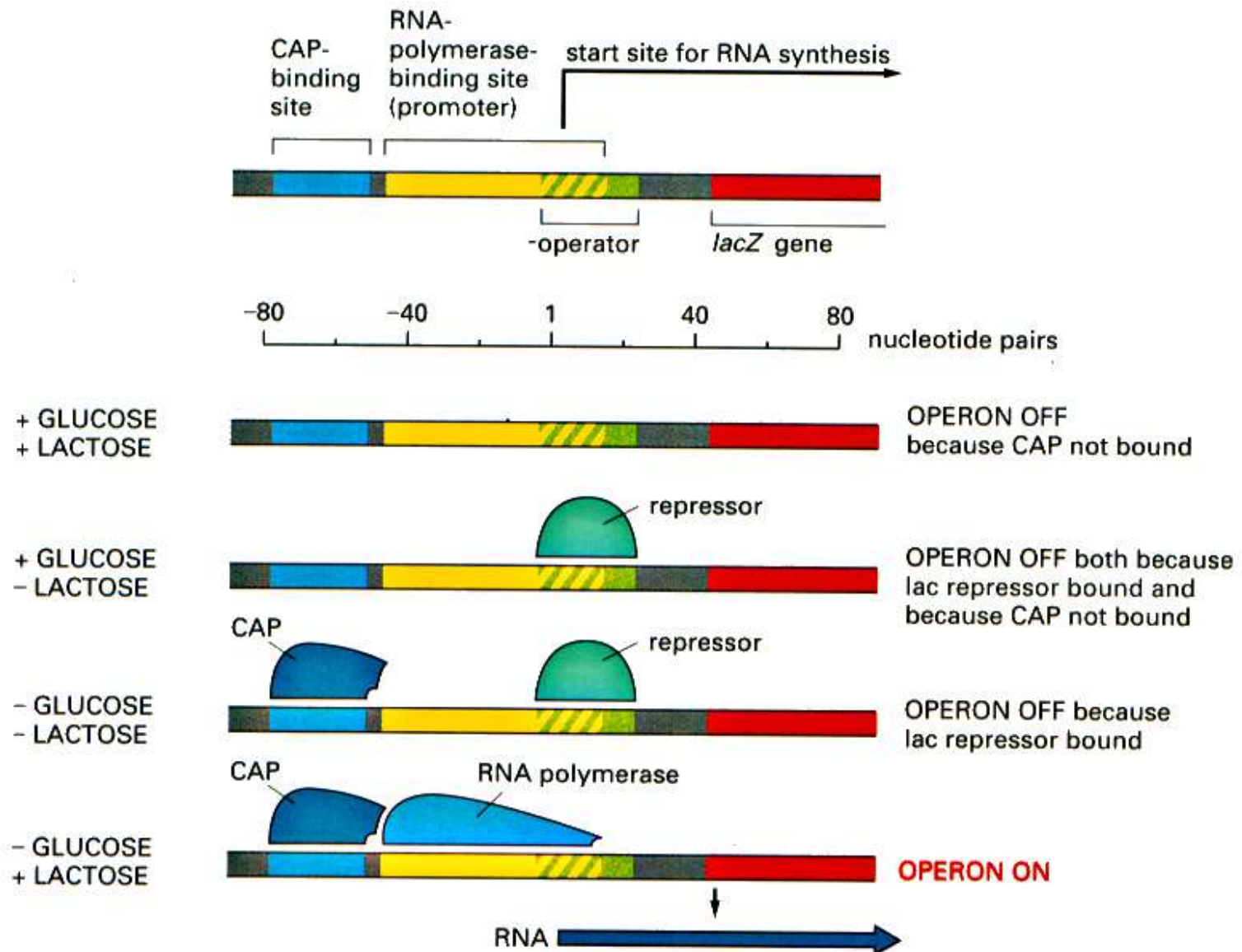
- DNA bending makes the promoter more accessible to RNA polymerase
- increased initiation frequency



Cyclic AMP interacts with RNA polymerase

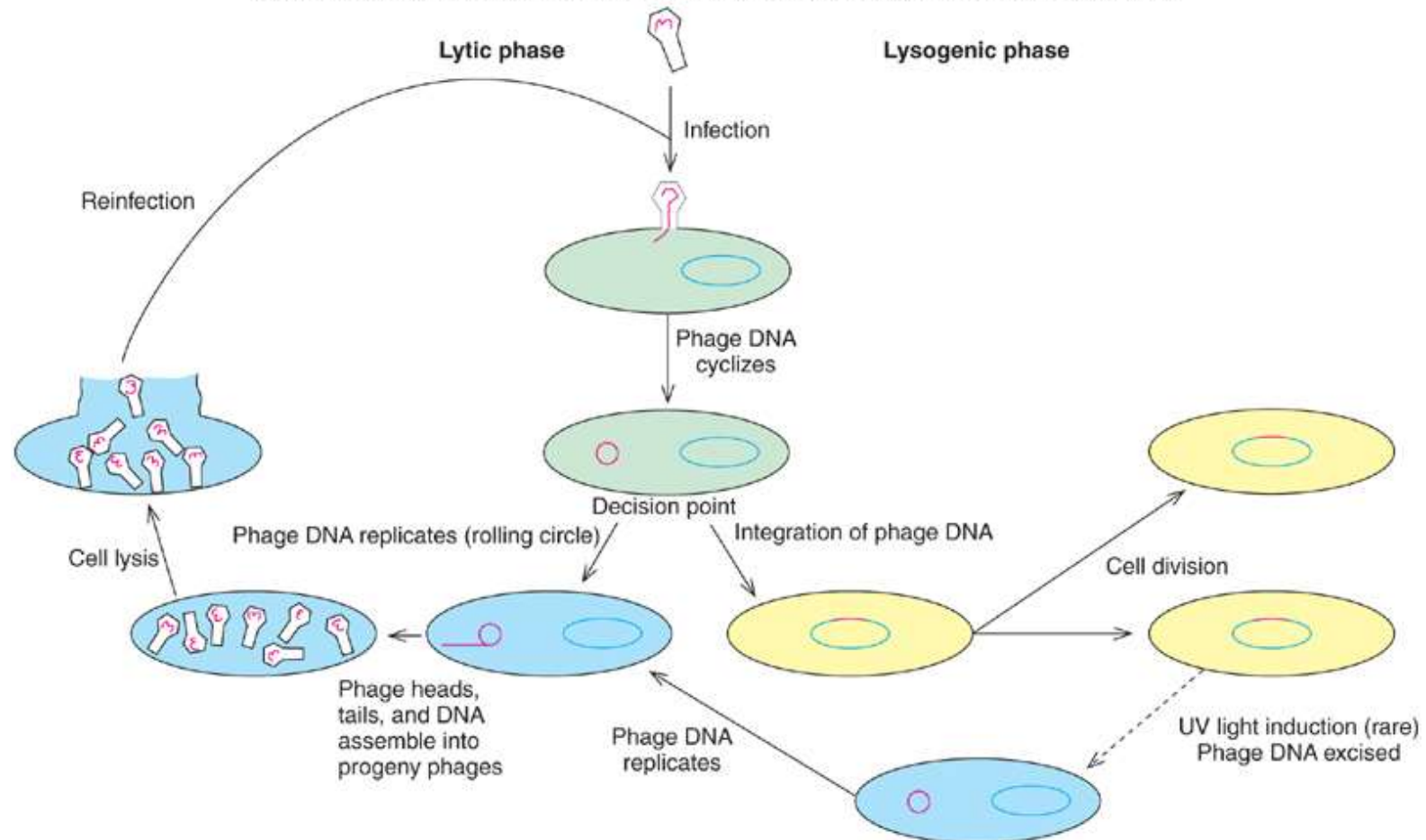


Dual control of the lactose operon



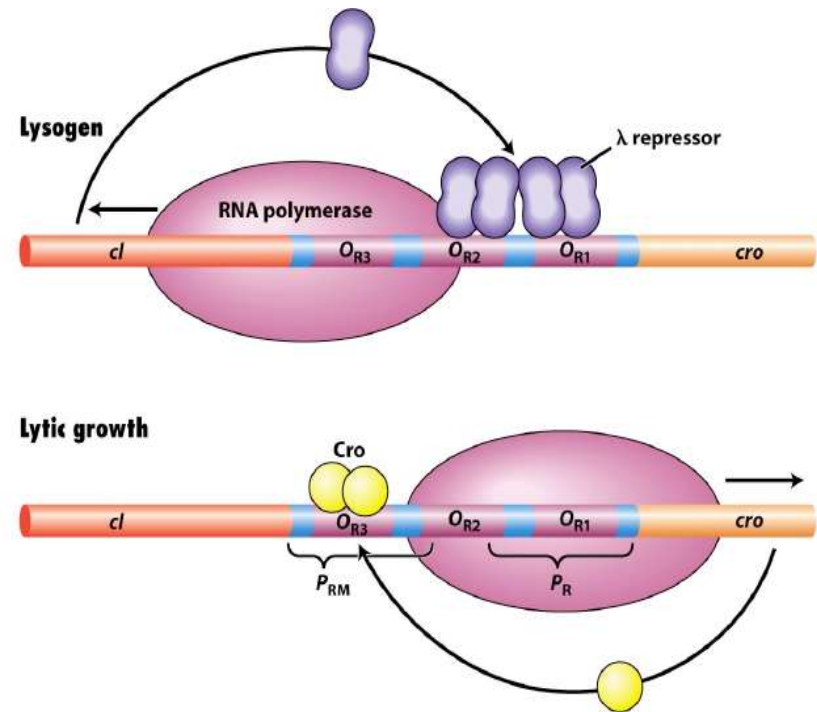
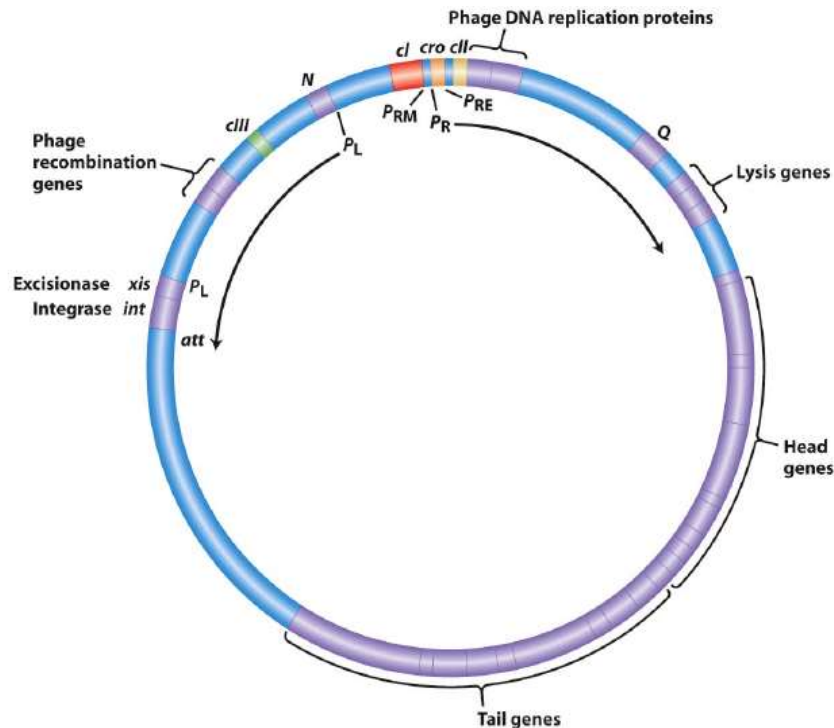
Two Paths of Phage Reproduction

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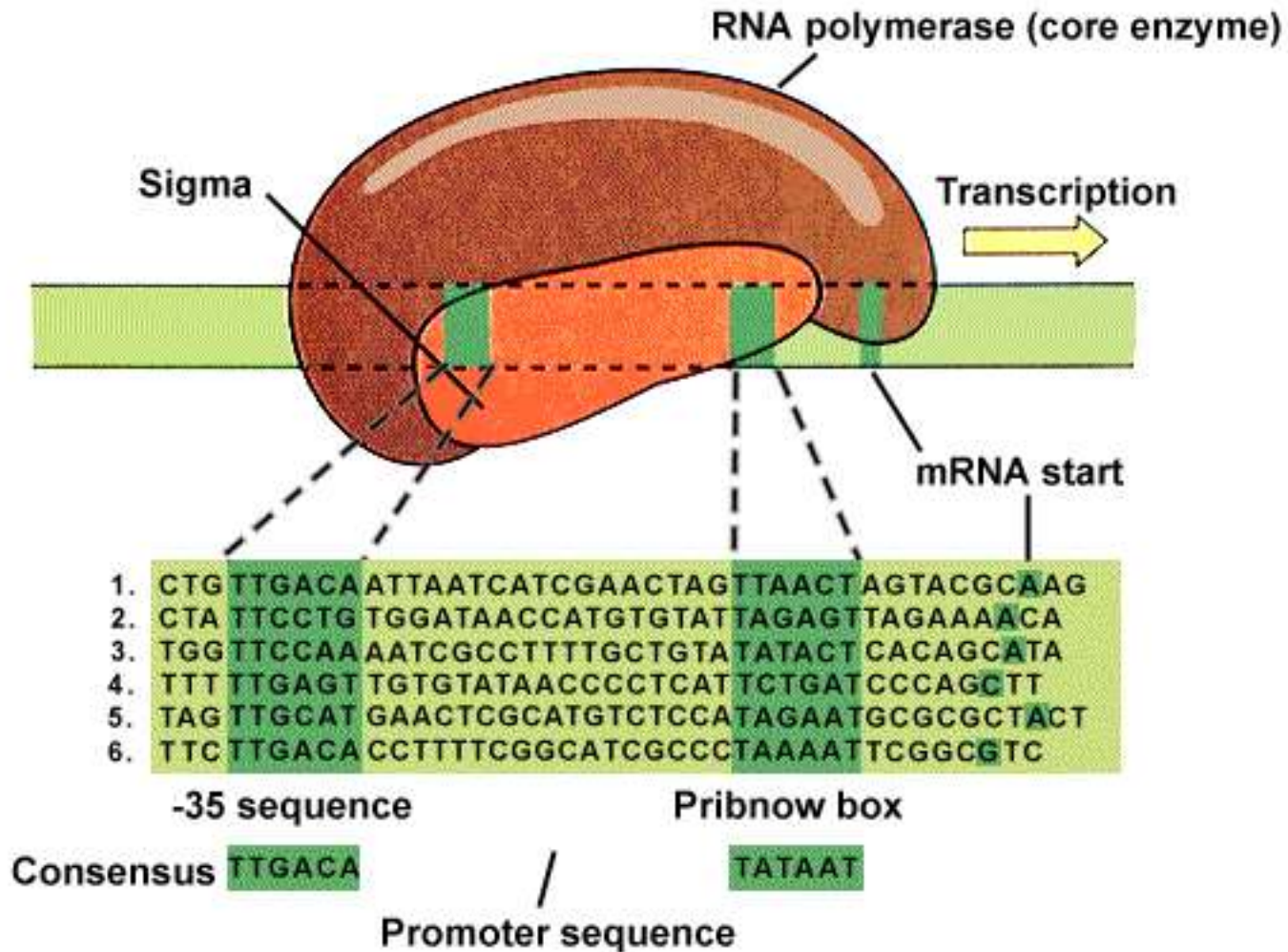
Control of the two modes of growth of phage λ in *E. coli*

This is an example of a genetic switch, a stable situation that is passed on to progeny cells. This type of switch is used in eukaryotic cell differentiation.



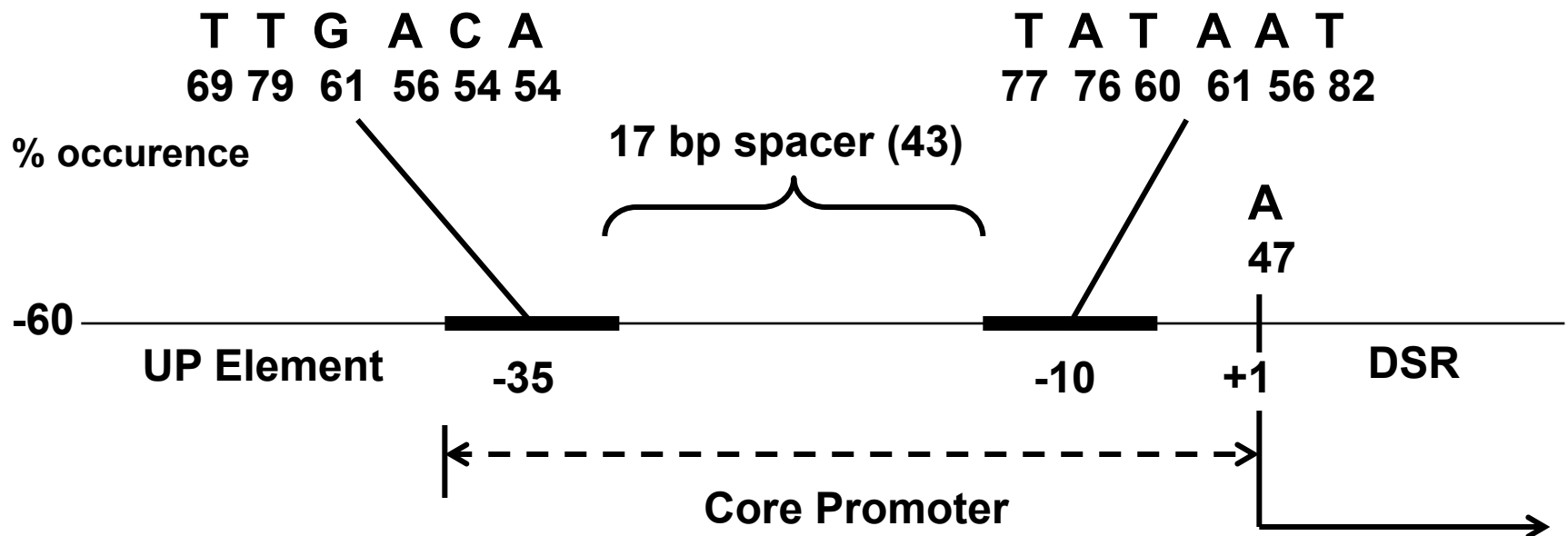
Initiation of transcription in prokaryotes

holoenzyme = RNAP core + Sigma



Architecture of a vegetative (σ^{70}) promoter

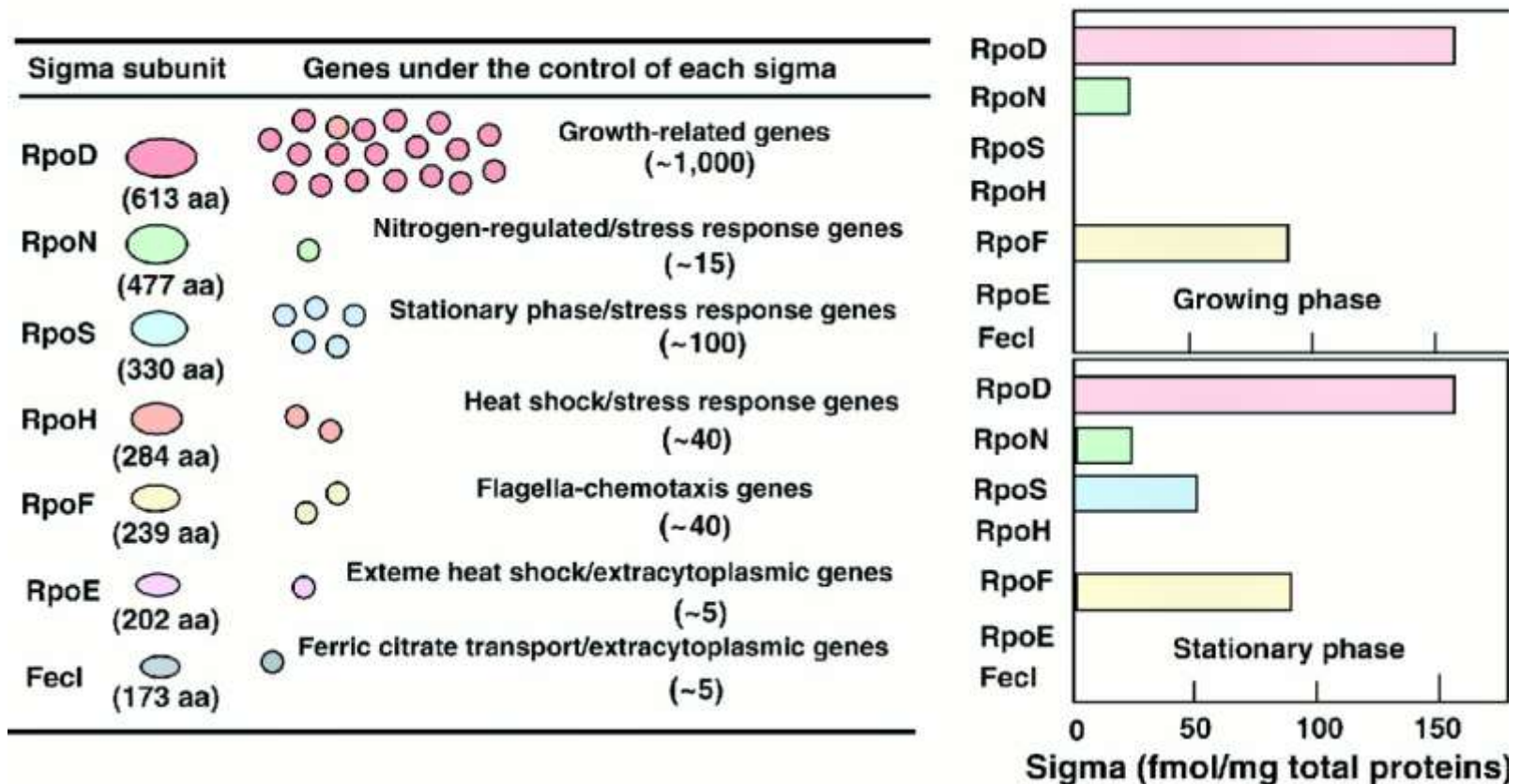
-core promoter recognized by sigma factor



Alternate Sigma Factors

recognize promoters of different architecture –
different regulons of genes

Intracellular Concentrations of RNA Polymerase Sigma Subunits in *Escherichia coli* W3110



Promoters regulated by alternate sigma factors may have completely different consensus sequences

σ^{70} TTGACA – 17 bp – TATAATN₃₋₆-A
-35 -10

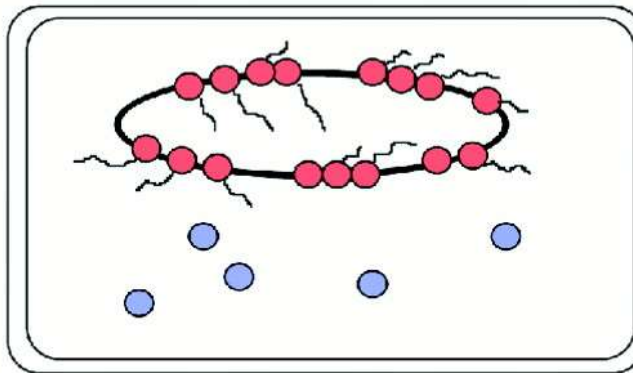
σ^{32} CTTGAAA – 16 bp – CCCCATNTN₃₋₁₀-T/A
-35 -10

$$\sigma^{54} \quad \text{GG} - \text{N}_{12} - \text{GC/T} - 12\text{bp} - \text{A}$$

-24
-12

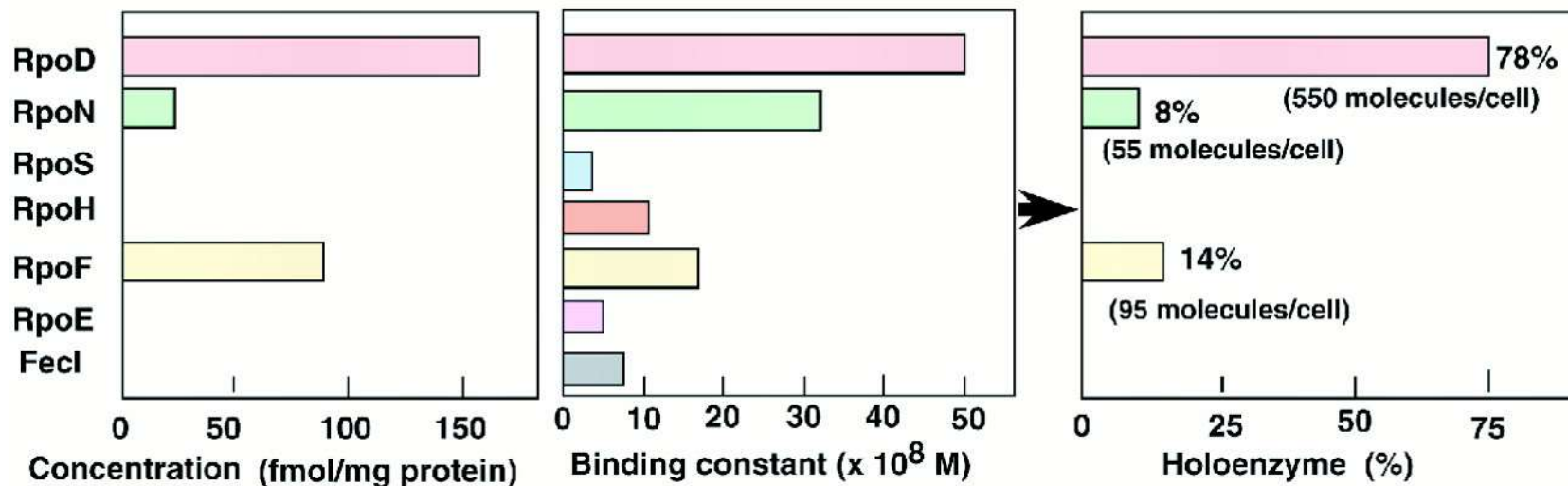
Intracellular concentration of different RNA polymerases

Intracellular Concentrations of RNA Polymerase Holoenzymes in Growing *Escherichia coli* Cells



Total number of RNA polymerase
2000 molecules / cell

- RNA polymerase involved in transcription, 1300 molecules
- RNA polymerase not involved in transcription, 700 molecules

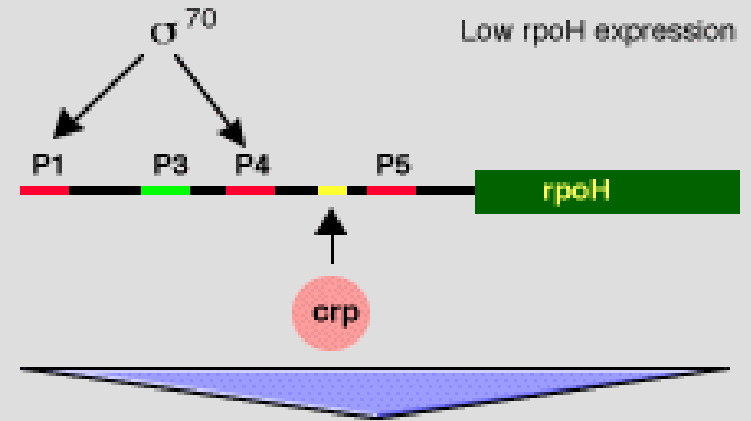


Escherichia coli rpoH transcription

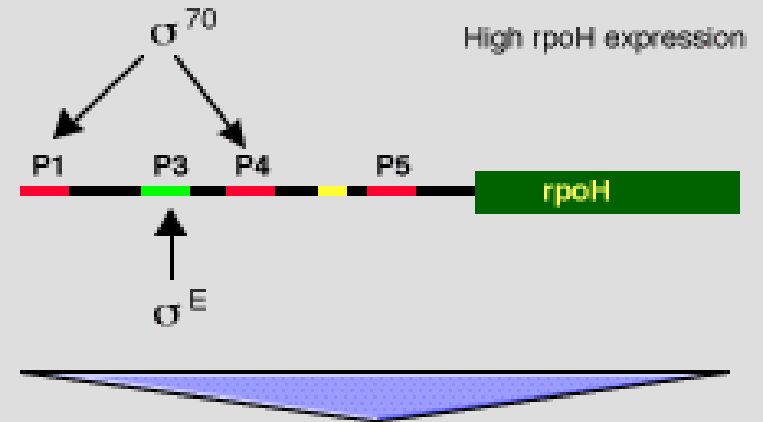
Translation of mRNA is increased

Stabilization of protein at 42°C

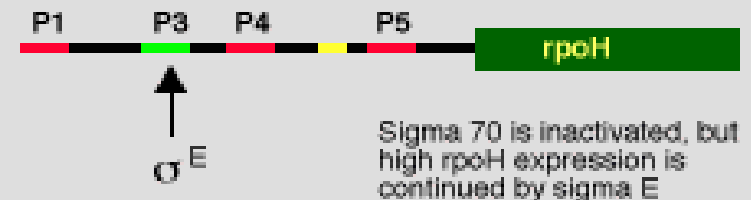
Normal Conditions (32° C)



Heat Shock (42° C)

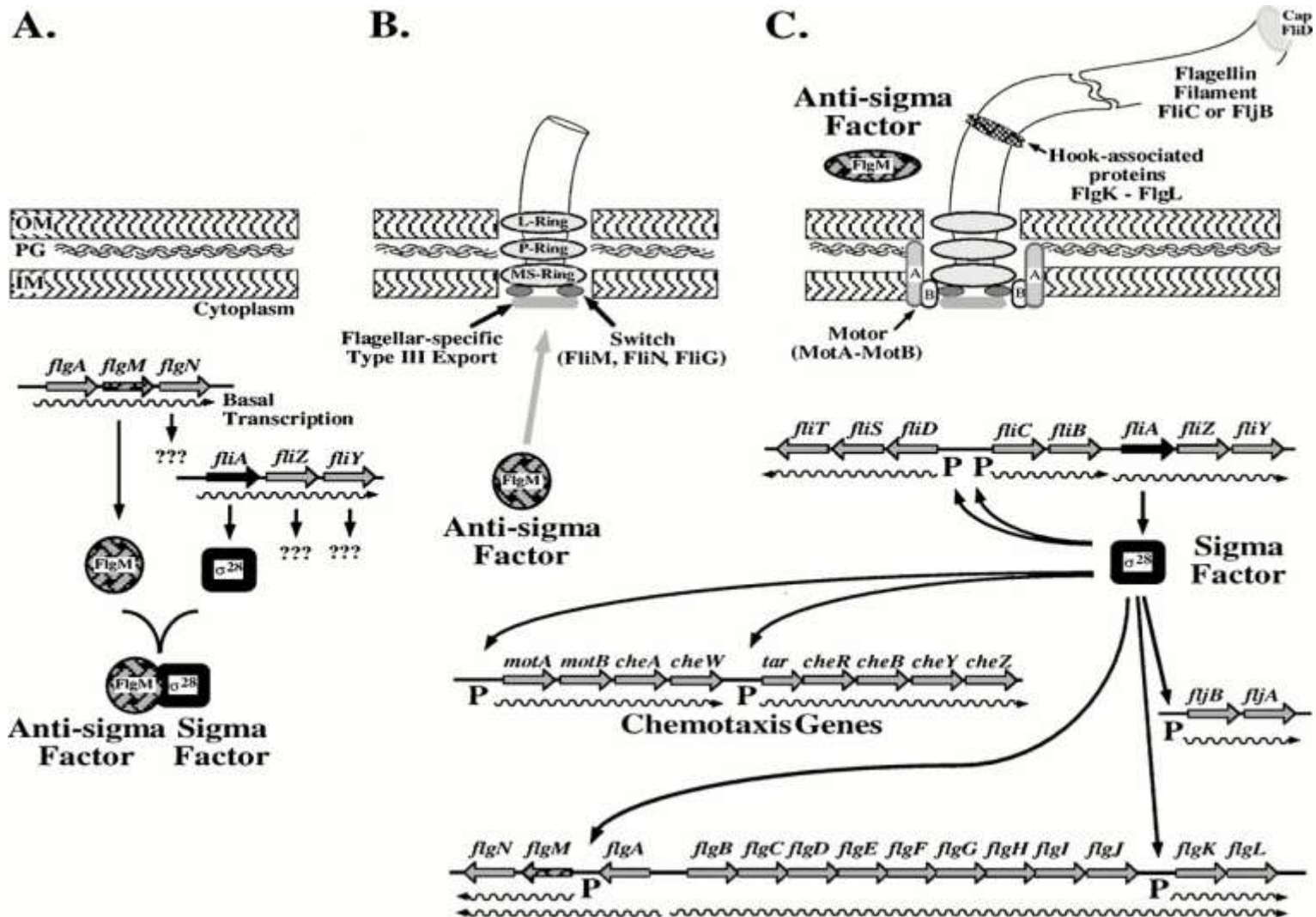


Severe Heat Shock (50° C)



How might a sigma factor provide differential gene regulation?

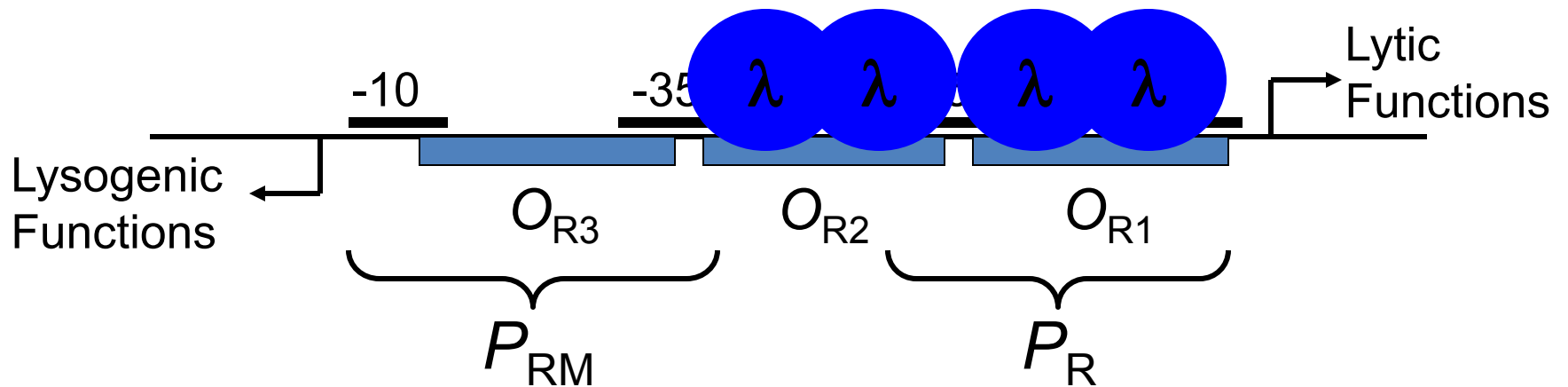
One example: an anti-sigma factor (FlgM – σ^{28})



Mechanisms of repression

1. Steric hindrance – binding site overlaps with promoter and repressor has a higher binding affinity than RNAP (K_I)
2. Protein-protein interaction – repressor prevents subsequent steps following binding (k_{II} and k_{IV})
3. RNA Polymerase Caging – repressor affects local DNA structure limiting productive interaction with bound RNAP (k_{II} and k_{IV})
4. Multipartite promoters and DNA looping – multiple repressors bound at different sites change DNA conformation and affect RNAP binding (K_I)

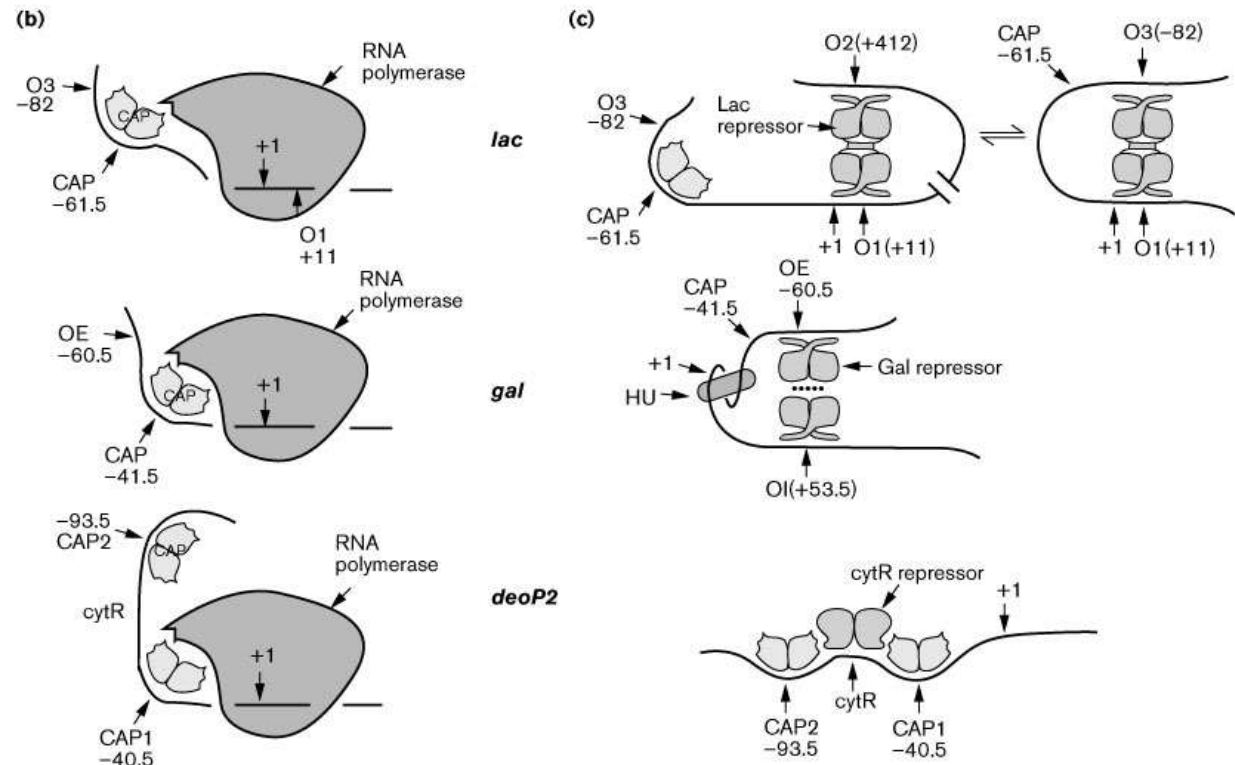
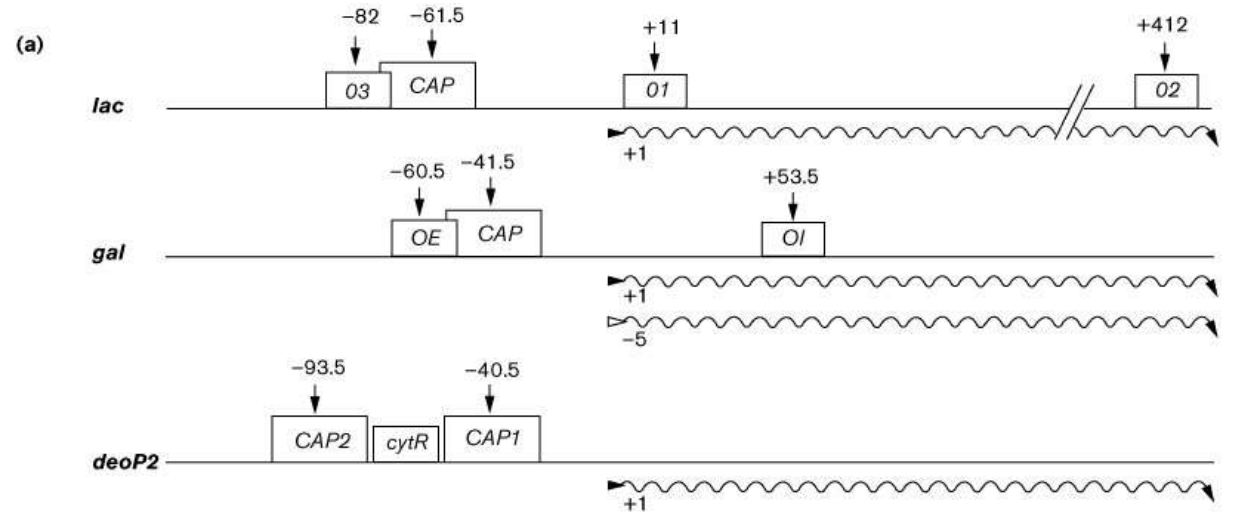
λ Repressor prevents P_R activity by steric hindrance



Most repressors are much more complicated – including the LacI repressor

Multipartite operators and looping is common

Additional proteins (such as CAP and CytR) are often involved

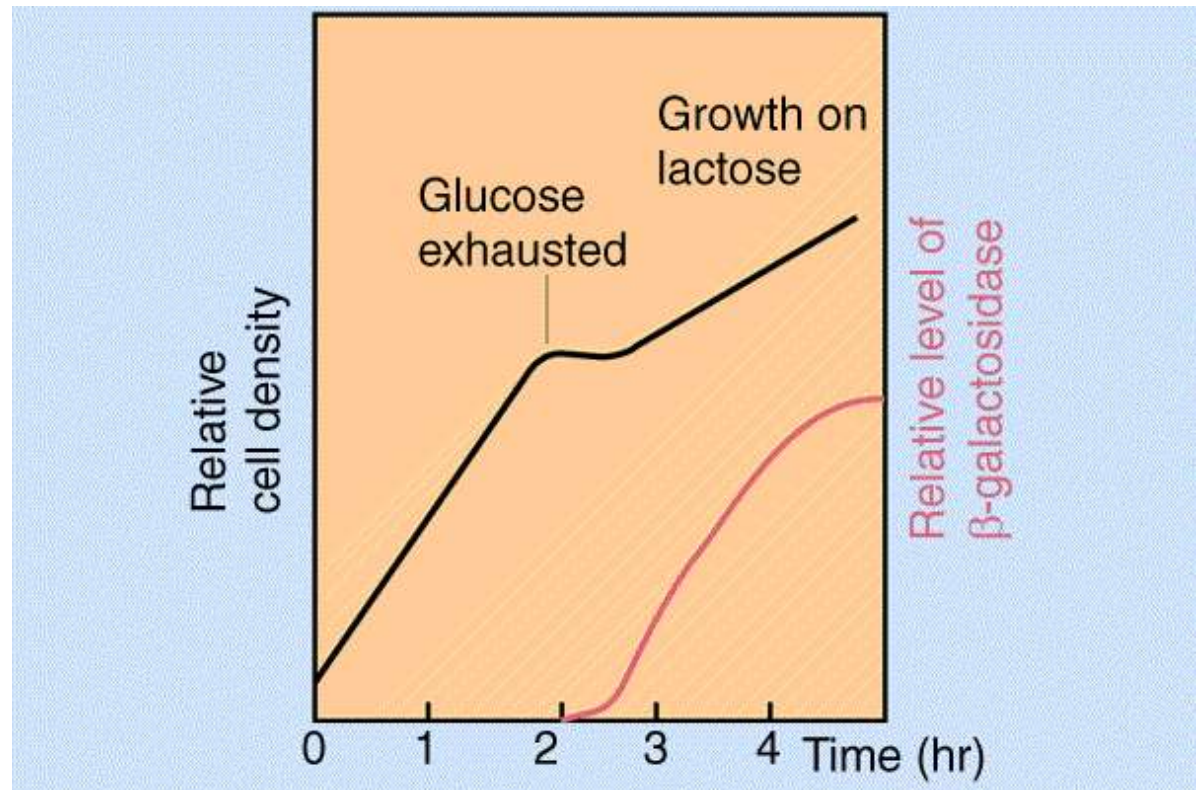


Transcriptional activation can occur via several different mechanisms

Almost always involves contacts with RNAP

An excellent example regulates catabolite repression – catabolite activator protein (CAP)

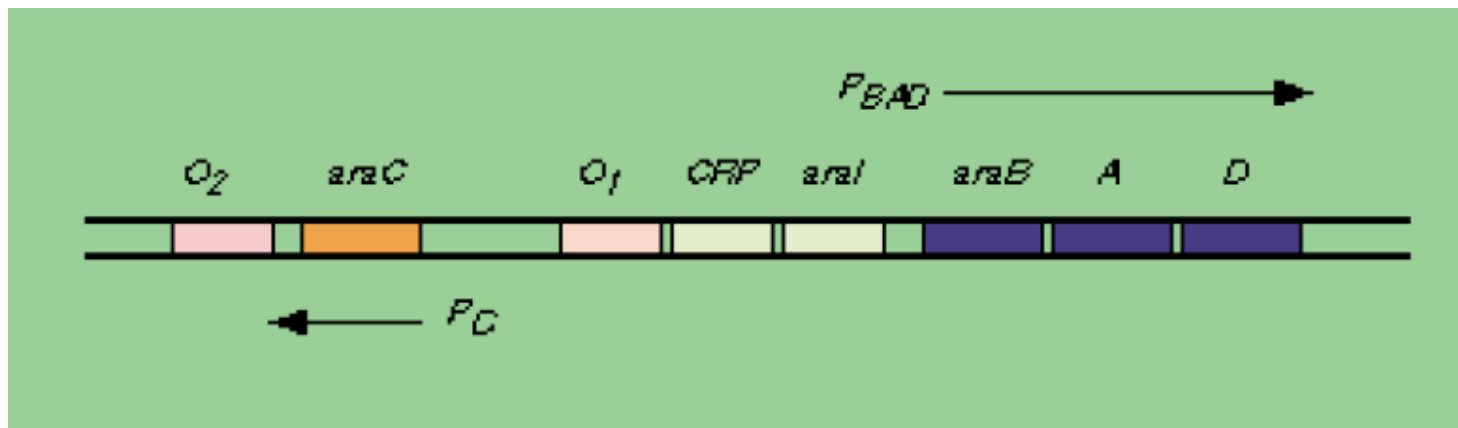
A “global” regulator – controlling > 100 promoters



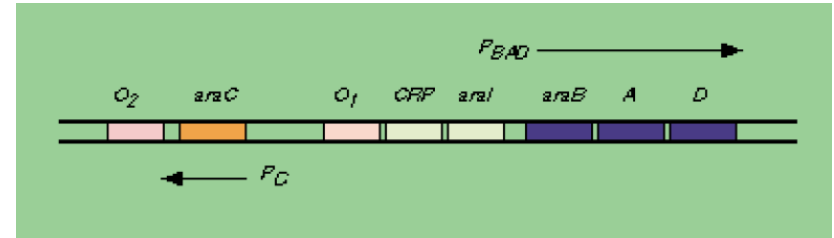
Regulators that both activate and repress

Regulator of arabinose (a sugar) degradation,
AraC is an excellent example

Many genes are involved in uptake and catabolism

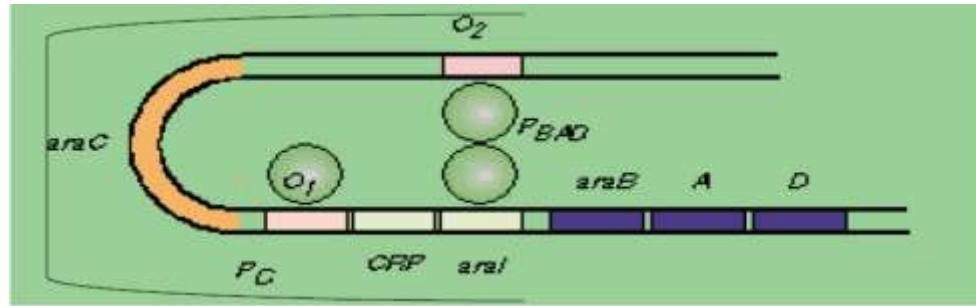


Arabinose operon



- The three pBAD structural genes are arranged in an operon that is regulated by the *araC* gene product (a regulator protein). There are four important regulatory sites:
 - *araO1* is an operator site. AraC binds to this site and represses its own transcription from the PC promoter. In the presence of arabinose, however, AraC bound at this site helps to activate expression of the PBAD promoter.
 - *araO2* is also an operator site. AraC bound at this site can simultaneously bind to the *araI* site to repress transcription from the PBAD promoter
 - *araI* is also the inducer site. AraC bound at this site can simultaneously bind to the *araO2* site to repress transcription from the PBAD promoter. In the presence of arabinose, however, AraC bound at this site helps to activate expression of the PBAD promoter.
 - CRP binds to the CRP binding site. It does not directly assist RNA polymerase to bind to the promoter in this case. Instead, in the presence of arabinose, it promotes the rearrangement of AraC when arabinose is present from a state in which it represses transcription of the PBAD promoter to one in which it activates transcription of the PBAD promoter

Arabinose operon – arabinose absent



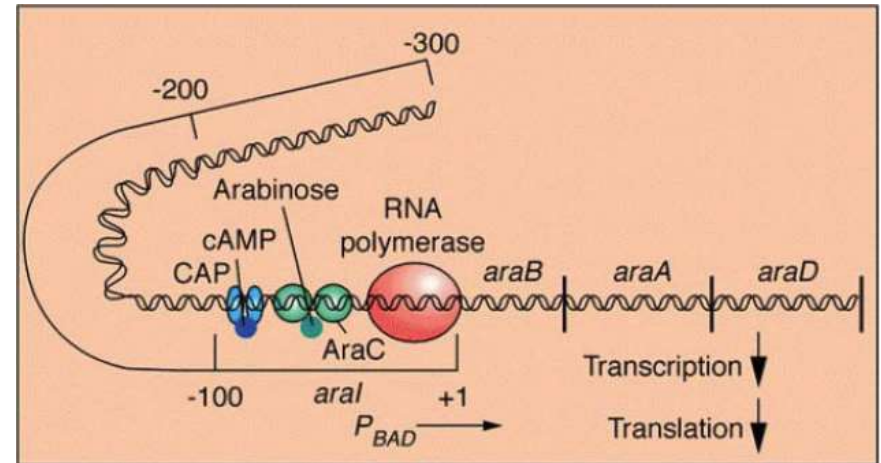
When arabinose is absent, there is no need to express the structural genes (negative regulation)

AraC (dimer) does this by binding simultaneously to both *araI* and *araO₂*. As a result the intervening DNA is looped. These two events block access to the *P_{BAD}* promoter which is, in any case, a very weak promoter (unlike the *lac* promoter).

AraC also prevents its own expression. Thus, it is an autoregulator of its own expression. This makes sense; there is no need to over-express AraC. If the concentration falls too low then transcription of *araC* resumes until the amount of AraC is sufficient to prevent more transcription again.

Arabinose operon

- arabinose present



In the presence of arabinose, AraC specificity is changed by an allosteric transition induced by binding of arabinose. The AraC duplex-arabinose complex binds preferentially to *araI*, not *araO2*, activating transcription. Structural genes are expressed.

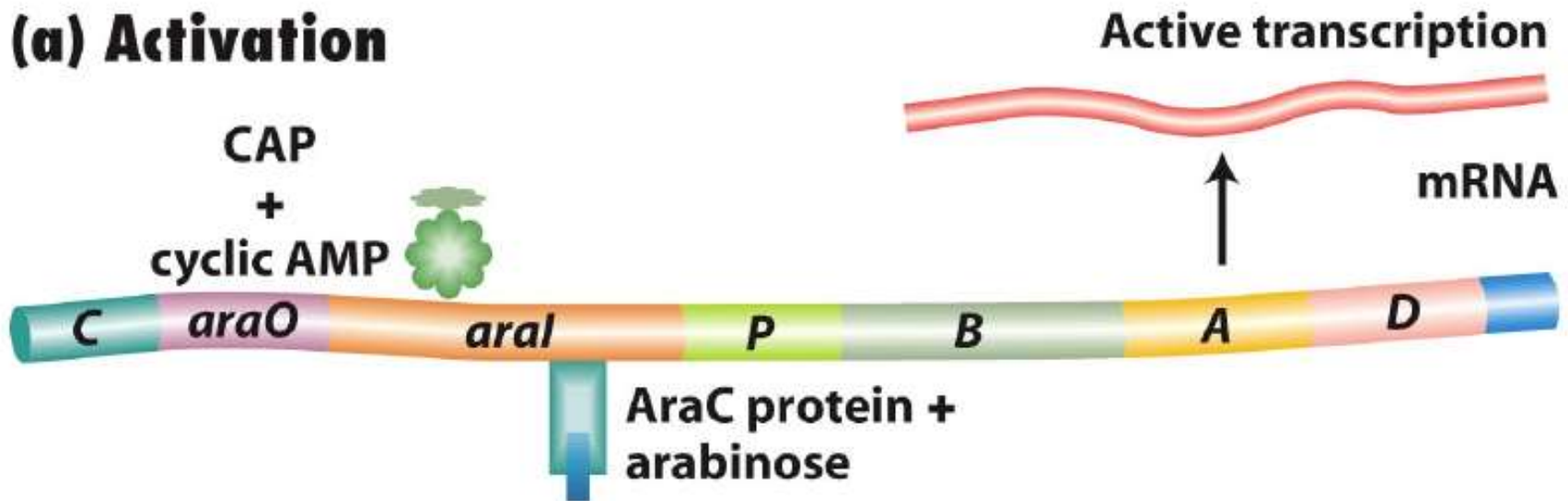
□ This is positive regulation – induction.

Regulators that both activate and repress

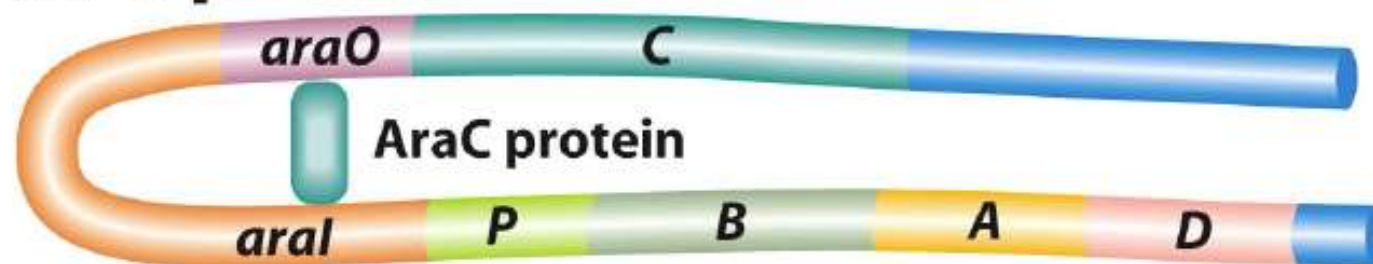
Regulator of arabinose (a sugar) degradation, AraC is an excellent example

Many genes are involved in uptake and catabolism

(a) Activation



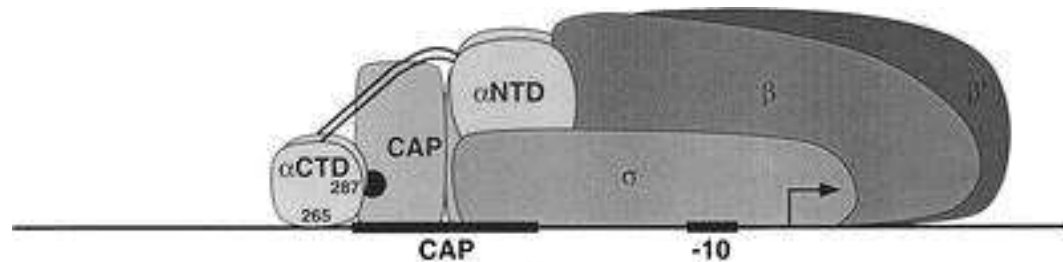
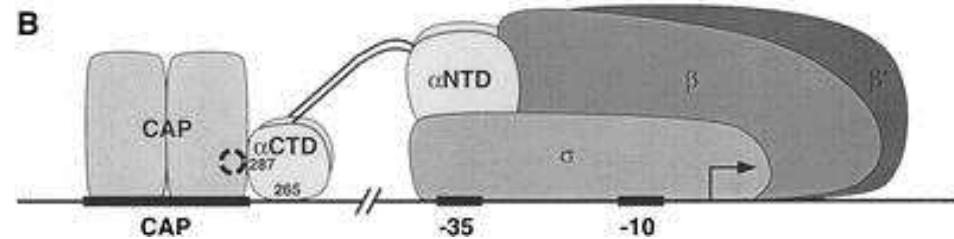
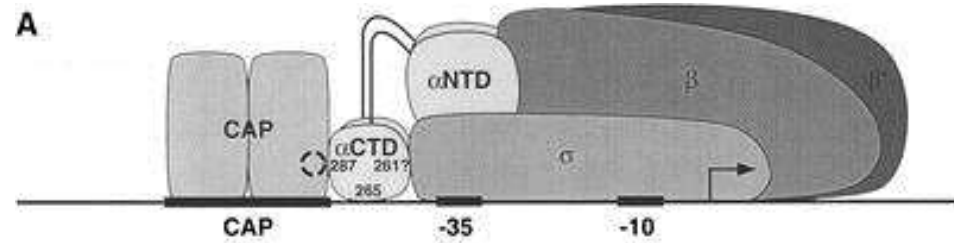
(b) Repression



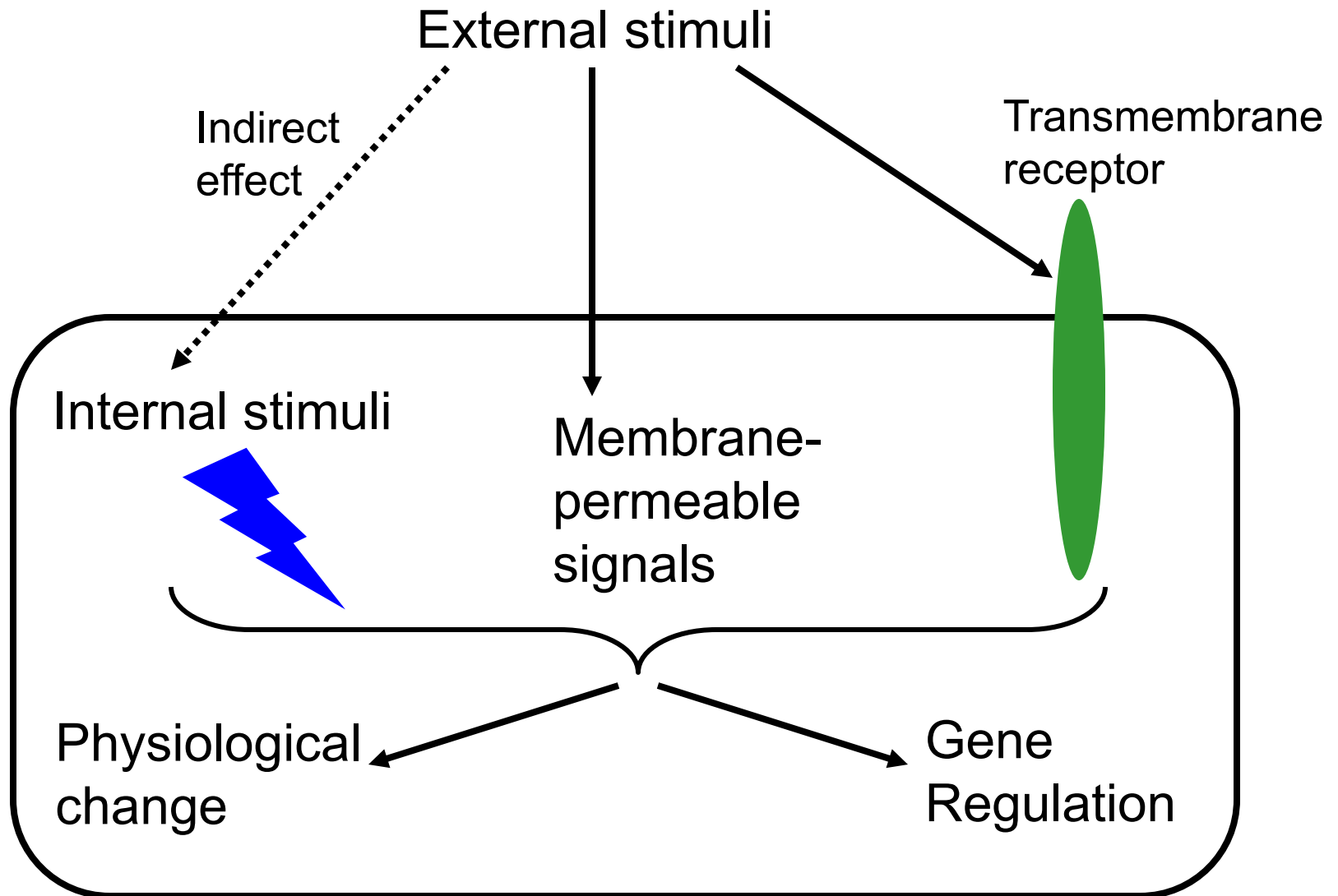
Models for Class I and Class II promoter activation

Class I CAP binding sites can be from -62 to -103 . CAP interact with the carboxy terminus of the RNAP α -subunit (α CTD)

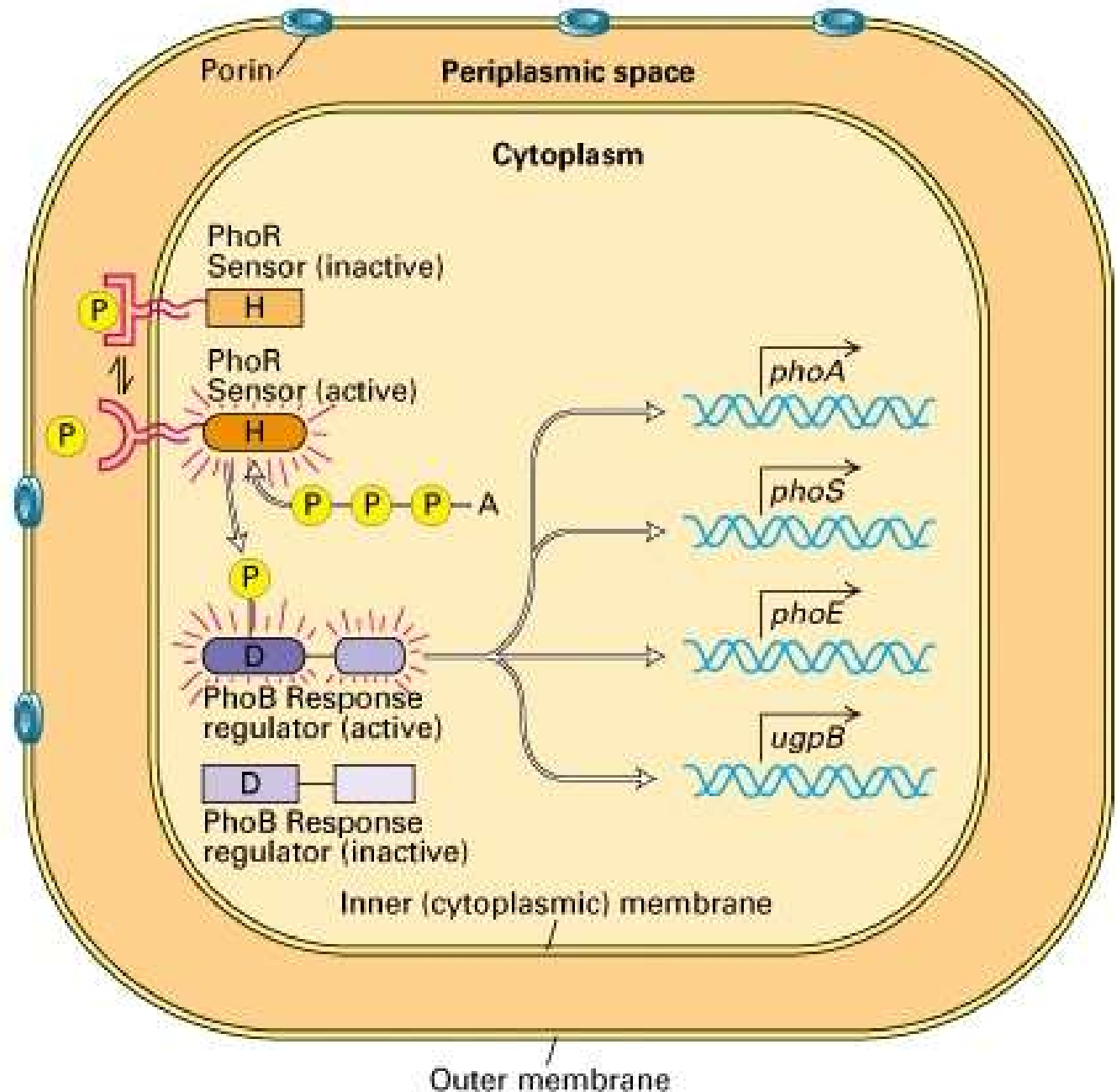
Class II CAP binding sites usually overlap the -35 . CAP interact with the α CTD, α NTD, and the σ factor



Environmentally-responsive adaptation

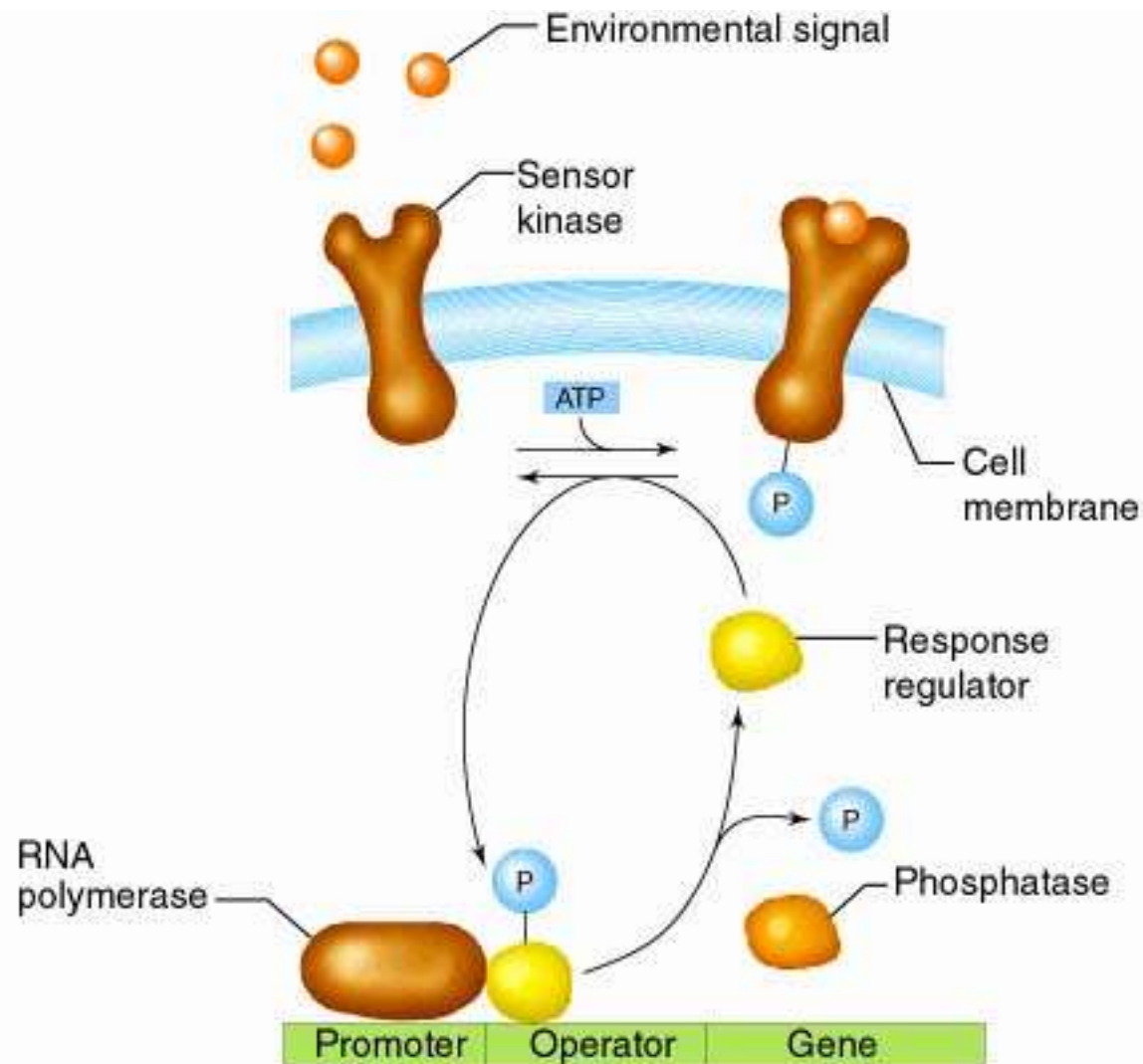


Two component systems



Simple paradigm for environmental signalling – the two-component system

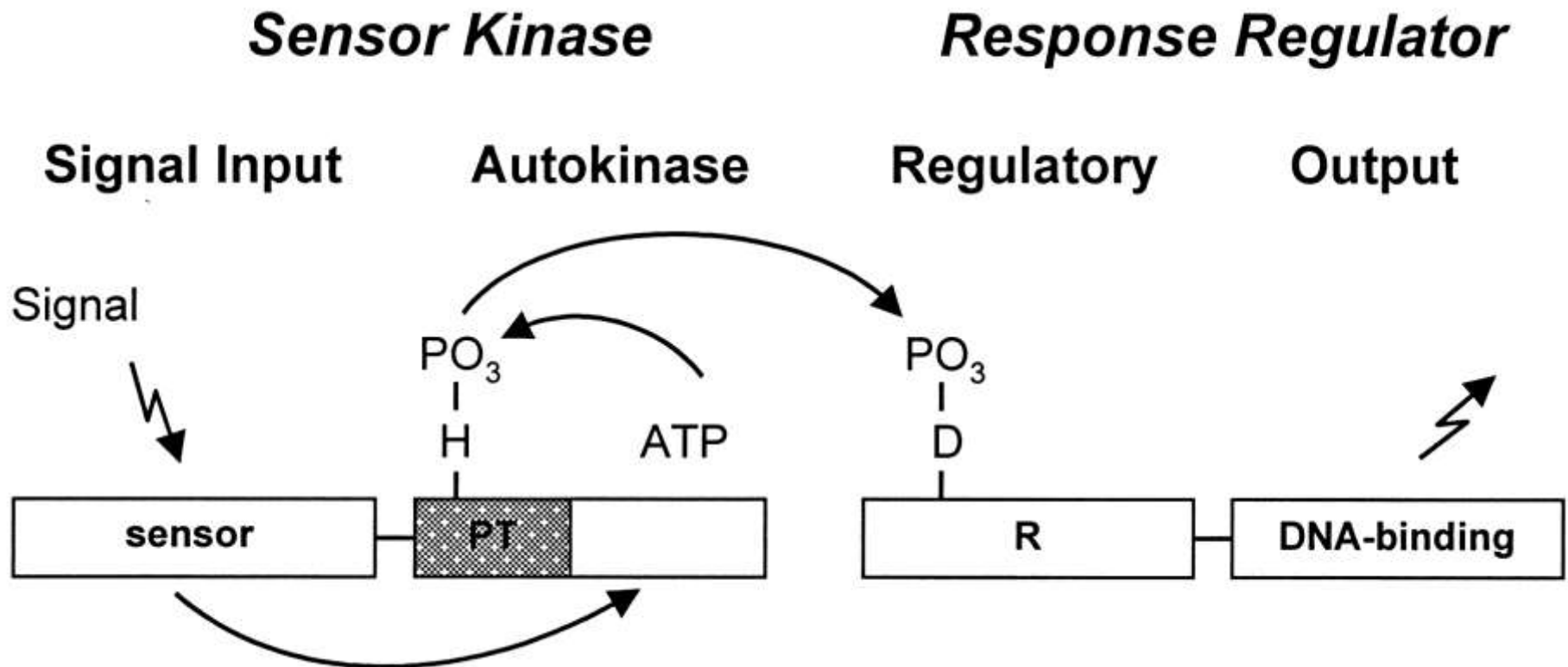
> 30 such systems in *E. coli* – also found in plants and fungi



Basic model for a two component-regulatory system

Sensor histidine kinase (HK) – may or may not be transmembrane – phosphorylates itself

Response regulator (RR) – often, but not always affects gene expression – phosphorylated by HK



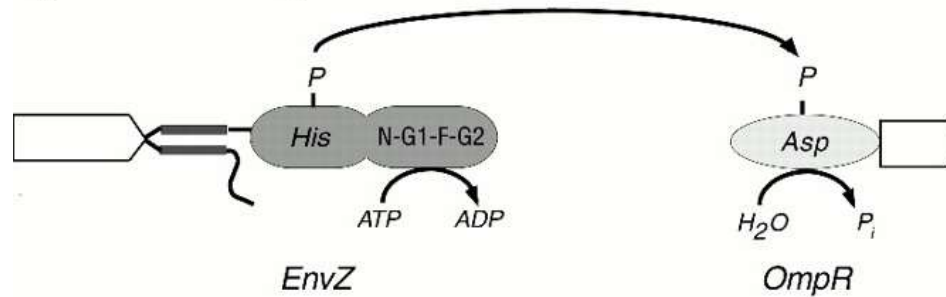
Allow response to wide range of chemical and physical stimuli

Many variations on the basic theme exist and the more they are studied the more permutations are observed

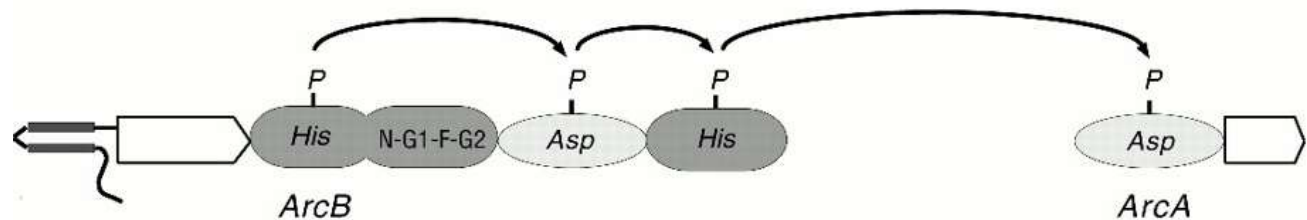
Histidine Kinase

Response Regulator

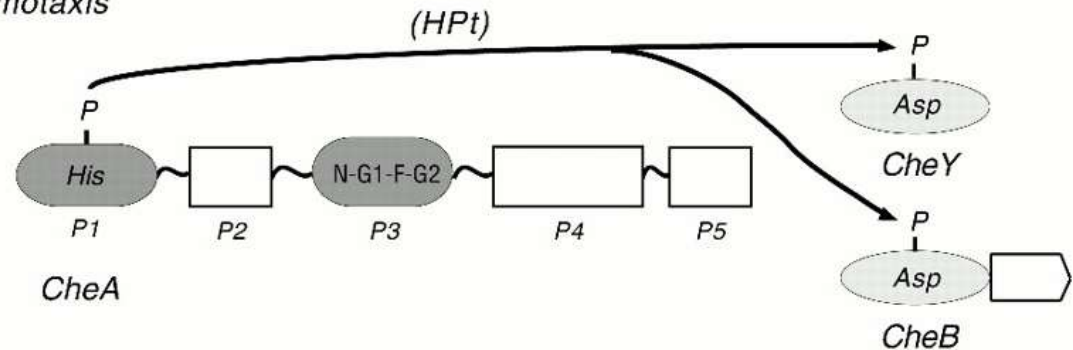
a) *E. coli* Osmoregulation



b) *E. coli* Anoxic Redox Control



c) *E. coli* Chemotaxis

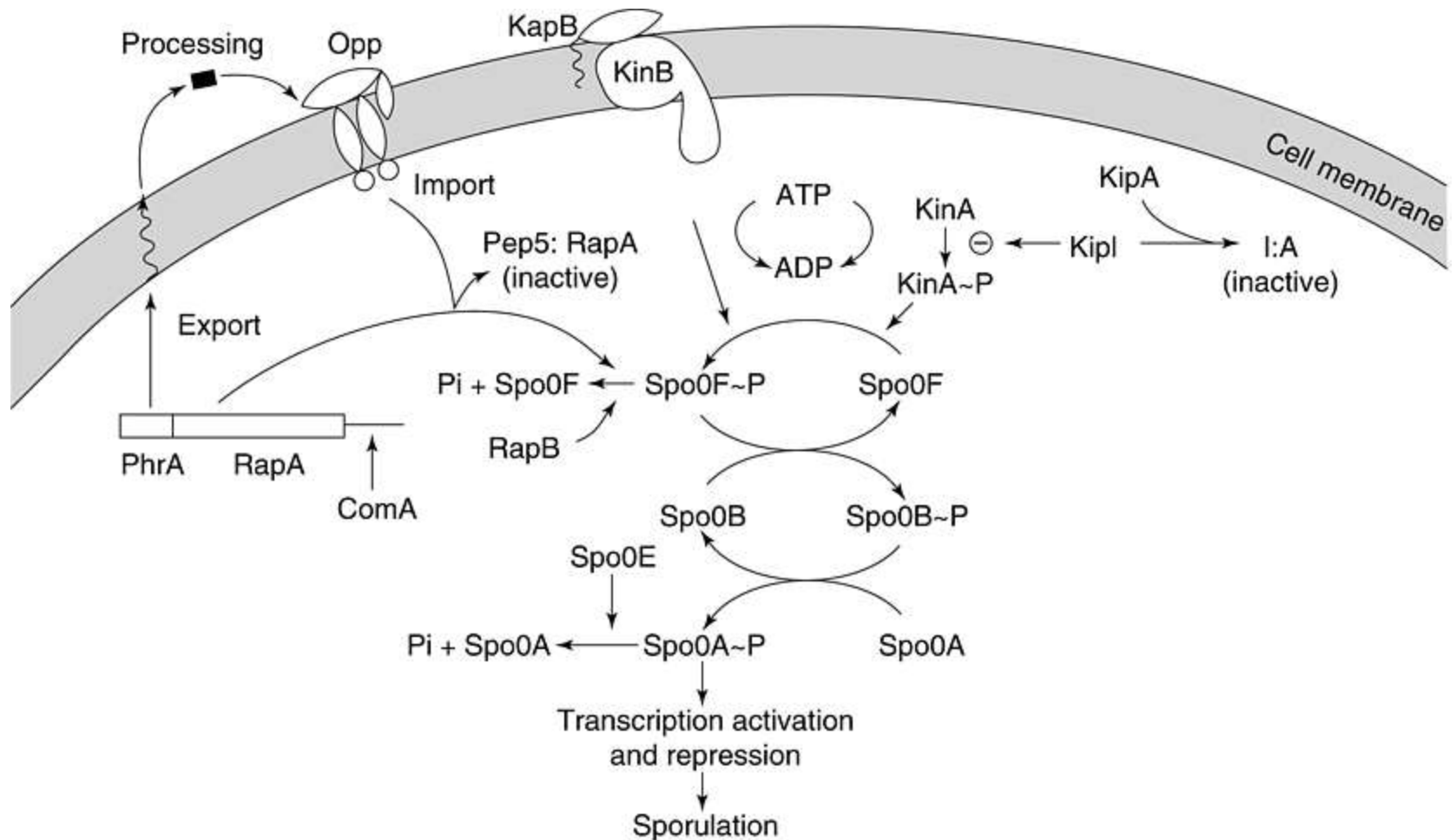


Phosphorylation cascades are commonly involved in intracellular signalling

- Two-component systems in *E. coli* respond to environmental cues and transmit information to DNA
- generic term: **Signal transduction**

B. subtilis makes a decision to sporulate based on many different factors

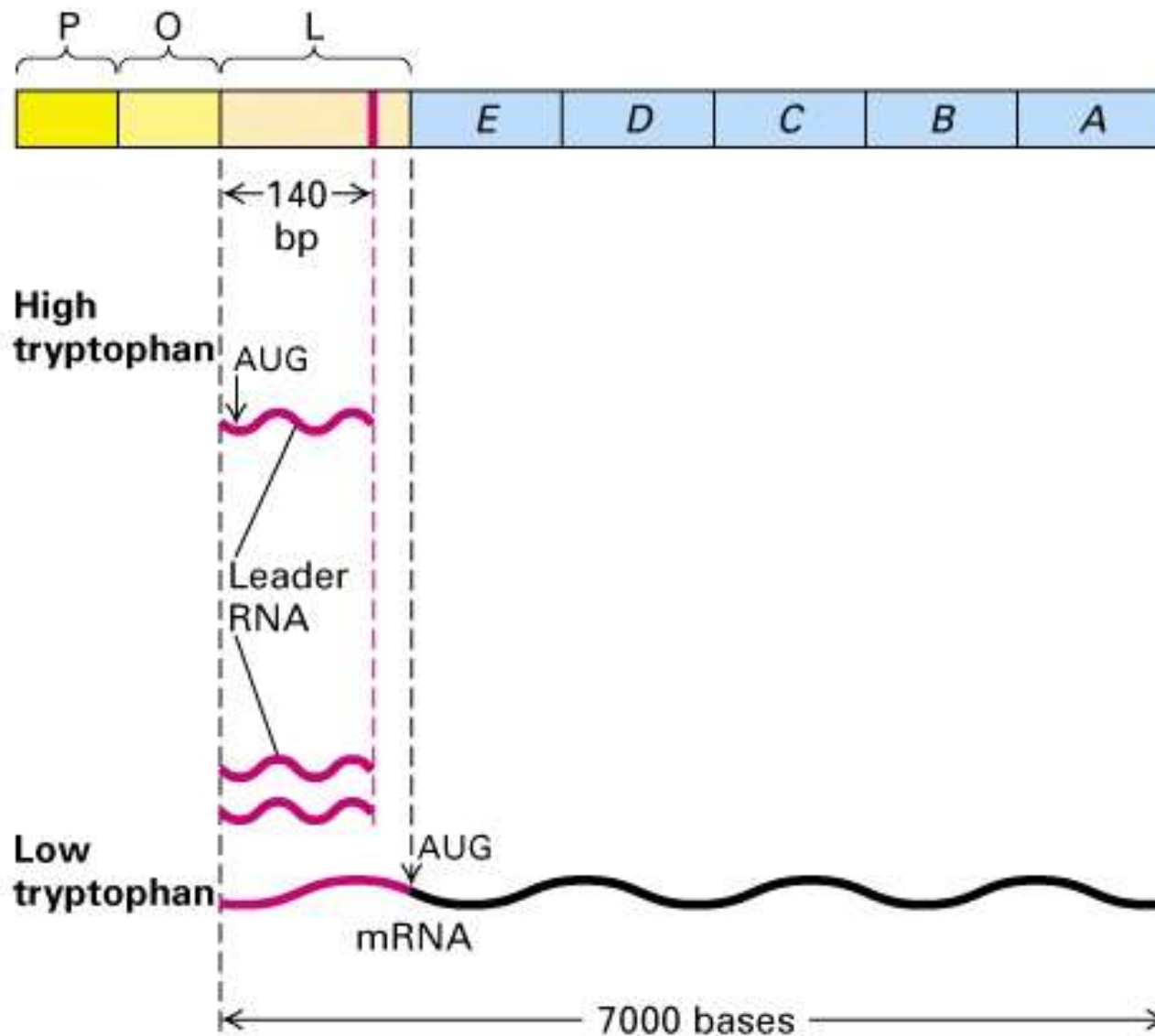
It all comes down to phosphorylation of SpoOA



Attenuation

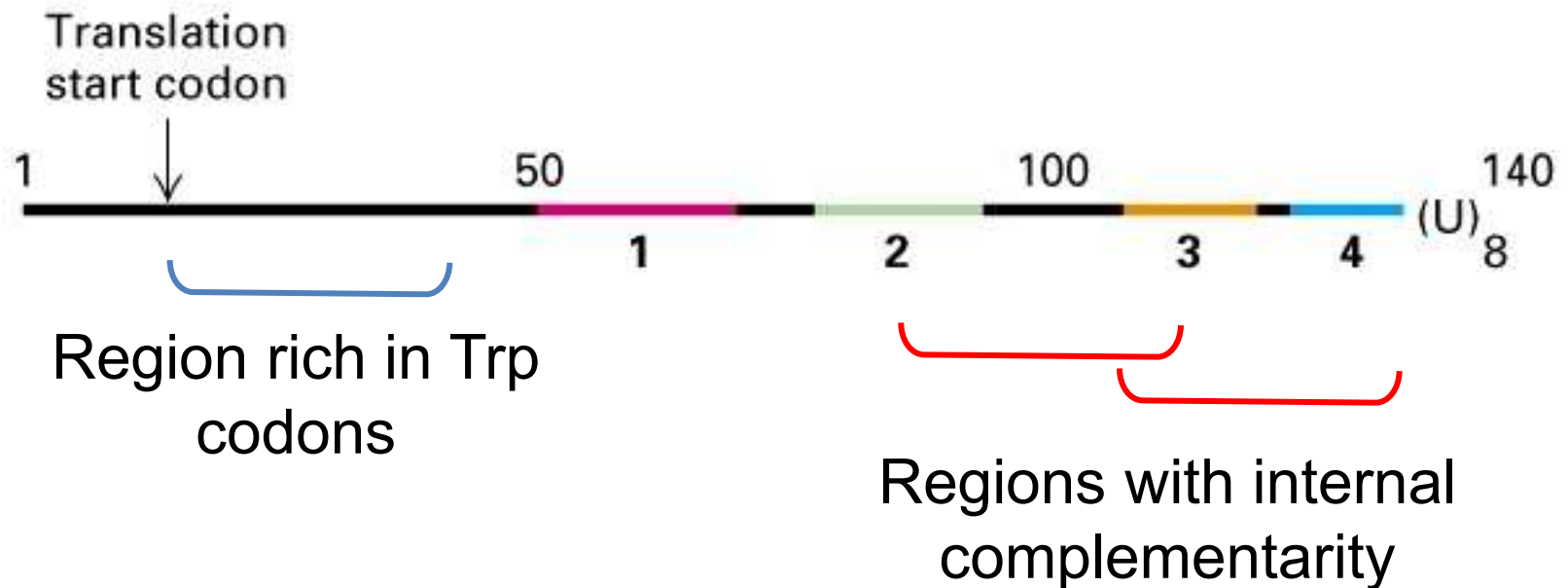
- Characteristic for RNAs regulated by attenuation are several **regions of internal complementarity** (inverted repeats) within the leader sequence
- The leader is further characterized by the presence of multiple codons for the amino acid whose metabolism is concerned (e.G. Trp)
- High Trp leads to premature transcription termination – low Trp permits transcription

Attenuation – trp operon

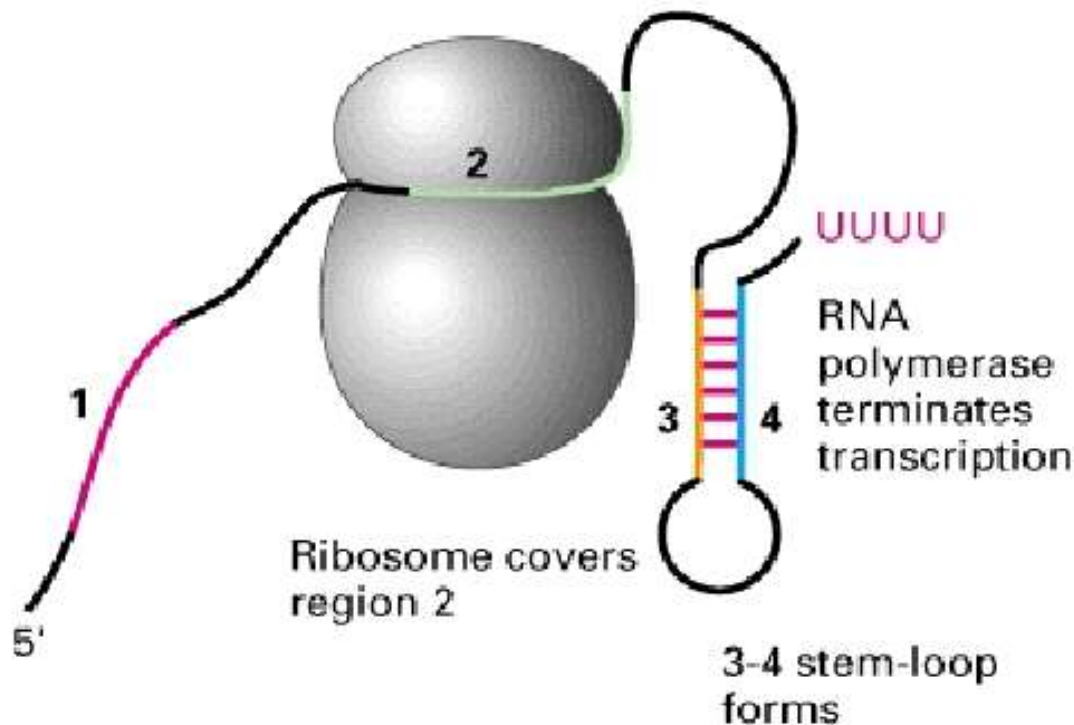


Attenuation: transcription termination induced by alternative RNA secondary structures

(a) *trp* leader RNA

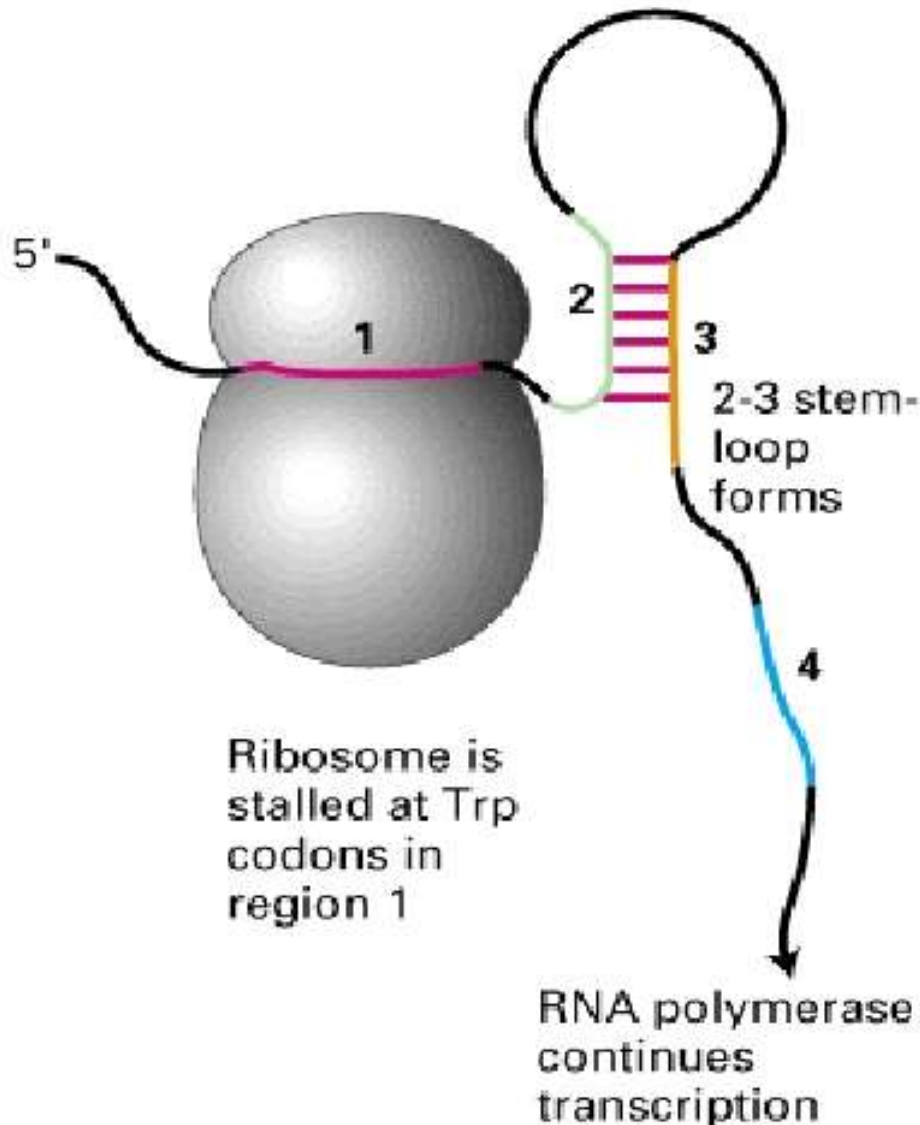


Trp attenuation: high tryptophan conditions



1. Availability of Tryptophan-tRNA causes ribosomes to rapidly translate **Trp-codon rich region**
2. Regions 3 and 4 form a hairpin loop 5' of the poly-U region, leading to transcription termination

Trp attenuation: low tryptophan conditions



1. Unavailability of Tryptophan-tRNA causes ribosomes to stall within **Trp-codon rich region**, allowing the formation of a hairpin loop between regions 2 and 3
2. region 4 is unavailable to form the structure essential for transcription termination

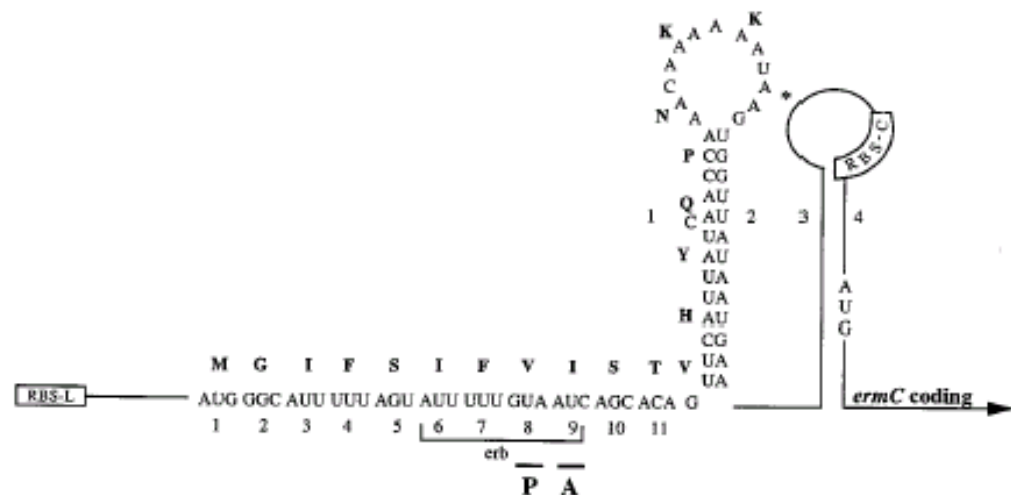
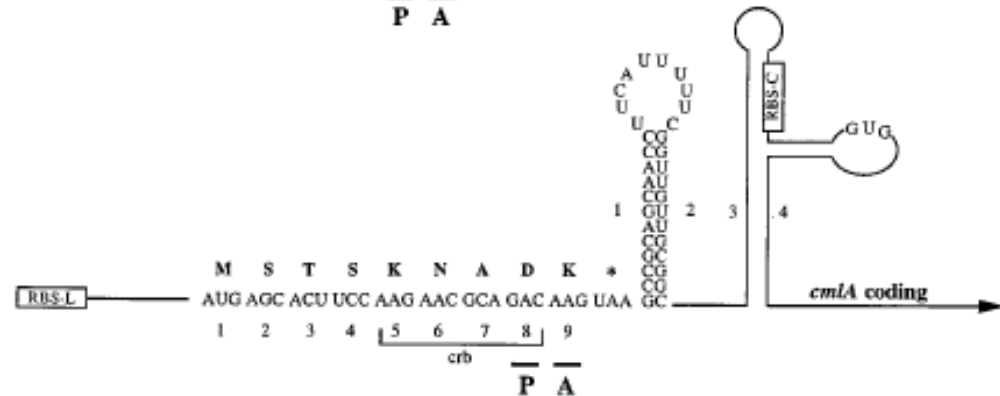
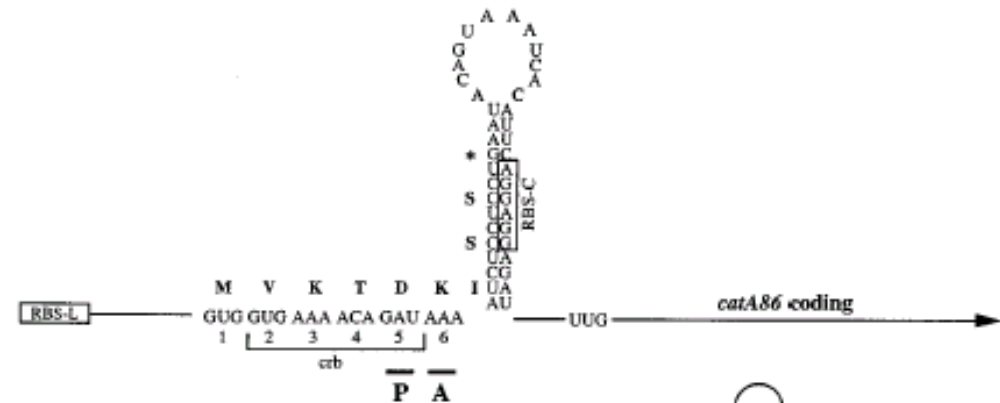
Leader Sequences

- (A) Met - Lys - Arg - Ile - Ser - Thr - Thr - Ile - Thr - Thr - Thr - Ile - Thr - Ile - Thr - Thr -
5' AUG AAA CGC AUU AGC ACC ACC AUU ACC ACC ACC AUC ACC AUU ACC ACA 3'
- (B) Met - Lys - His - Ile - Pro - Phe - Phe - Phe - Ala - Phe - Phe - Phe - Thr - Phe - Pro - Stop
5' AUG AAA CAC AUA CCG UUU UUC UUC GCA UUC UUU UUU ACC UUC CCC UGA 3'
- (C) Met - Thr - Arg - Val - Gln - Phe - Lys - His - His - His - His - His - His - His - Pro - Asp -
5' AUG ACA CGC GUU CAA UUU AAA CAC CAC CAU CAU CAC CAU CAU CCU GAC 3'

Translation attenuation

Often found in
antibiotic resistance
gene regulation –
some antibiotics
target the ribosome.

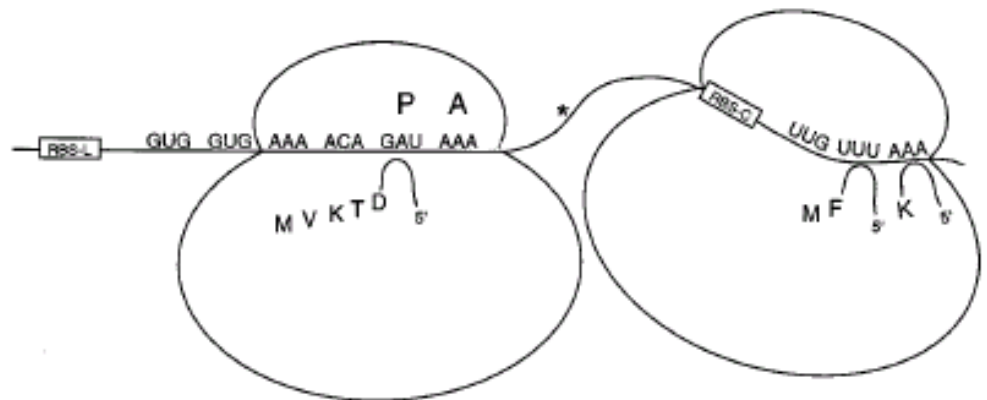
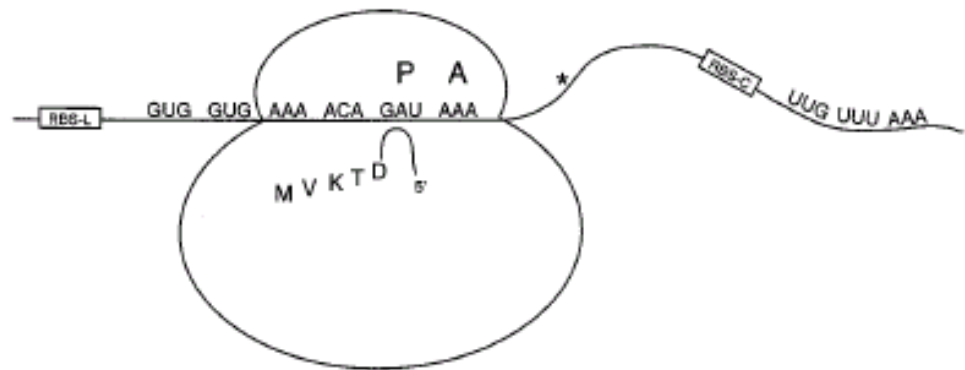
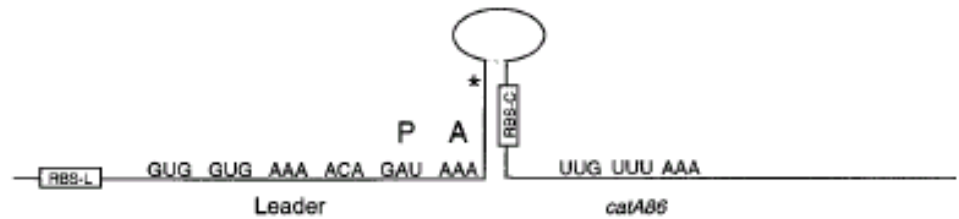
Relies on a unique mRNA structure and a leader peptide to function



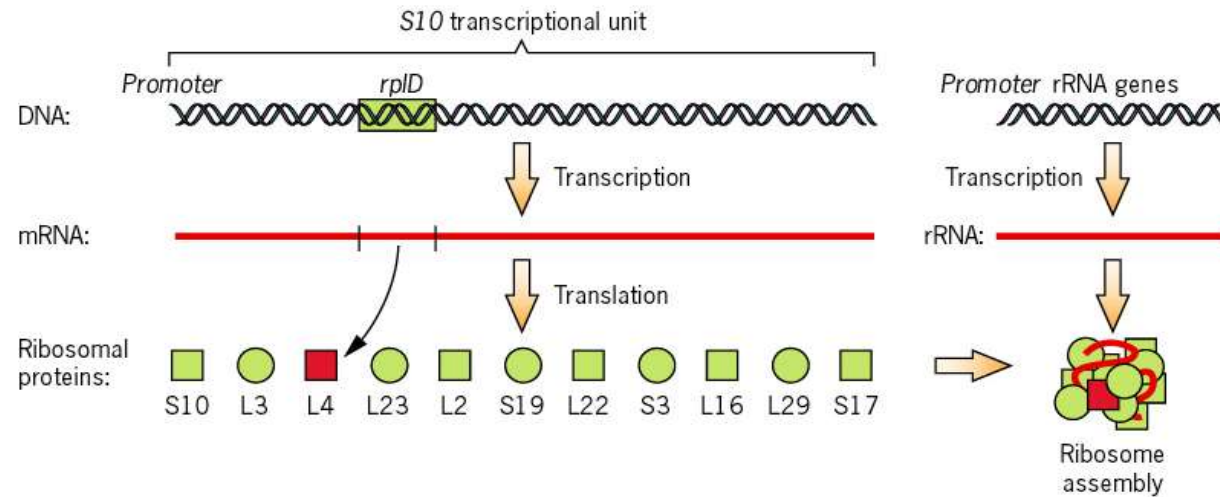
Translation attenuation

mRNA secondary structure occludes the ribosome binding site

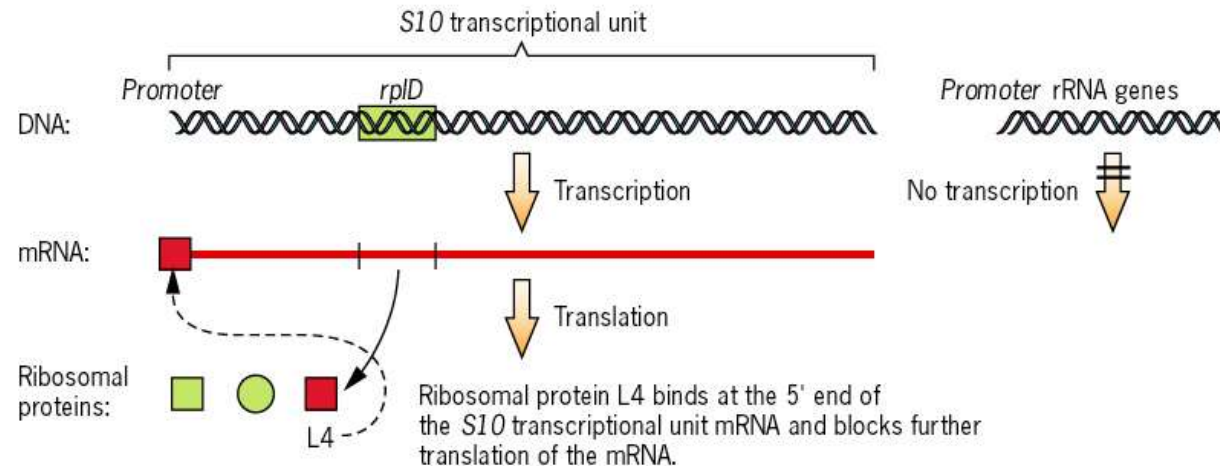
Stalling of translation at the leader peptide disrupts 2ndary structure and allow expression



Translational control of *E.coli* ribosomal protein genes



(b) The *S10* mRNA is translated when free ribosomal RNA is present.



(c) Protein L4 blocks translation of the *S10* mRNA when no free ribosomal RNA is present.

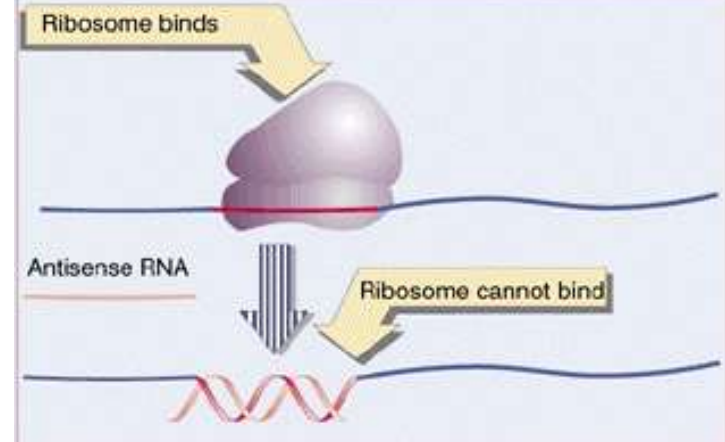
Regulation by small RNA molecules

Transacting regulators

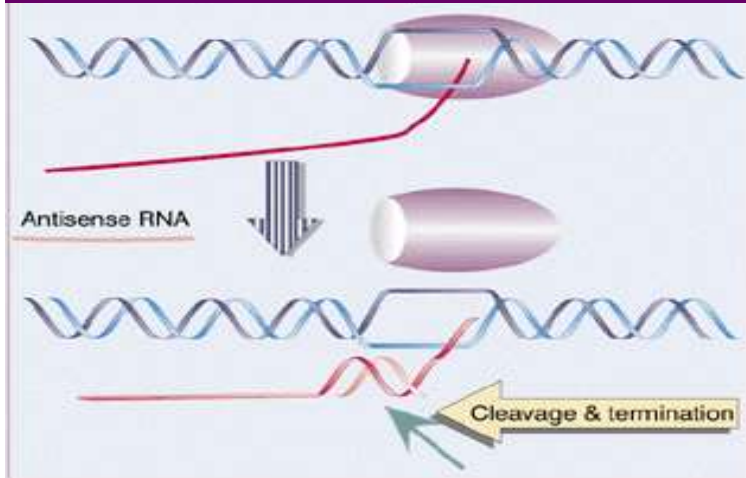
Regulator RNAs

Antisense RNA

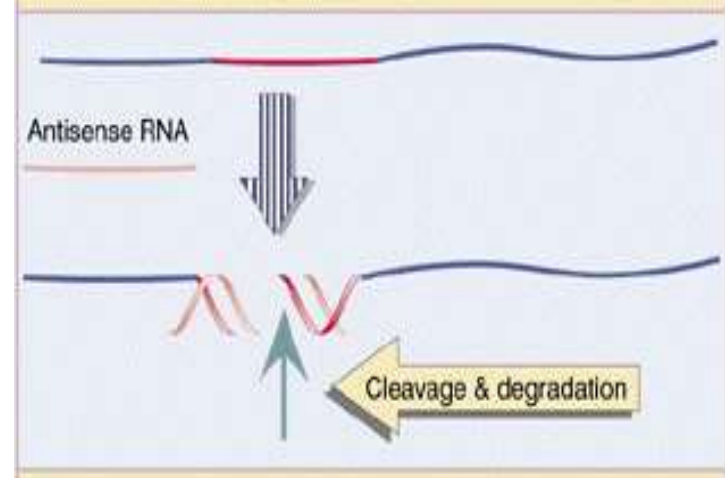
Occlude RBS



Mimic terminator

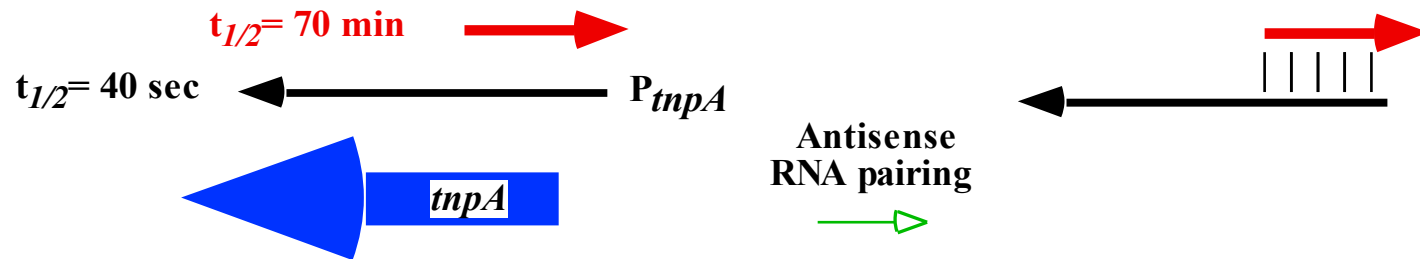


Stability -endonuclease target



mRNA stability and the amount of mRNA

Transposase mRNA



The antisense transcript has a much **longer half life** than the transposase transcript, such that under steady state conditions there are about 5 copies of the antisense transcript in the cell and about 0.2 copies of the sense transcript.

Translation of the *Tn10* transposase gene is repressed by an **antisense RNA**.

Blocks the RBS. Ensures that the amount of transposase made by the cell remains quite **low**.

Represses expression of transposase from any *Tn10* brought into a cell that already contains a copy of *Tn10*.

Regulation at the level of protein

Processing

Expressed early in an inactive form

Processed to provide the active form

Examples: sigma factors of *Bacillus subtilis* and λ lysin

Processing of periplasmic or outer membrane proteins to release the active form by leader peptidases

Easy to determine due to the presence of signal sequences

Examples: alkaline phosphatase, *P. aeruginosa* elastase

Regulation at the level of protein

Stability

Degradation by proteases

Examples: Sigma32 by FtsH, SigmaS by Clp protease

Short half-life

Status

Active or inactive requires modification ---> conformational change

Examples: Phosphorylation of NtrC, ADP ribosylation of Toxin

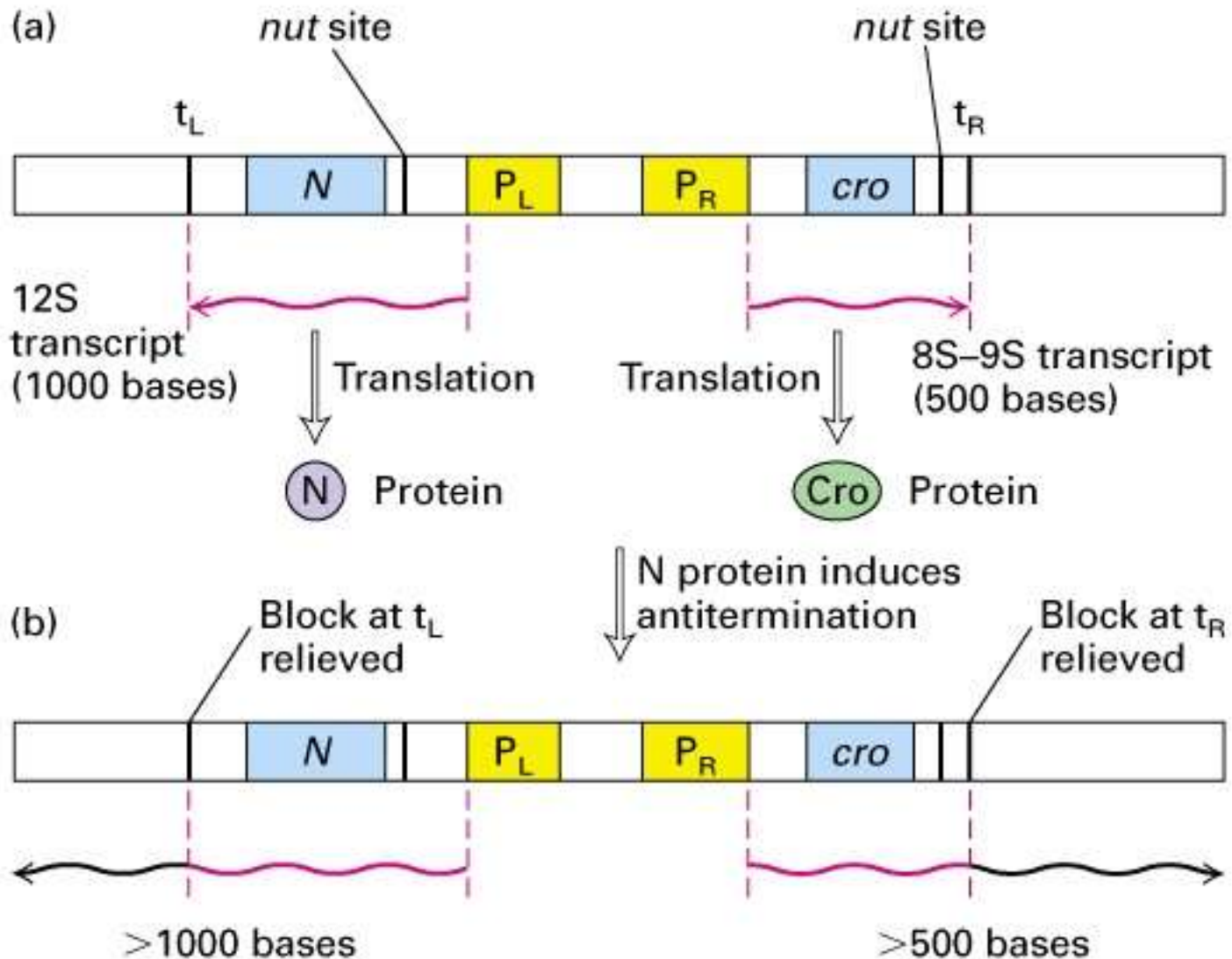
Release from an inactive complex

Examples: Anti-sigma factors

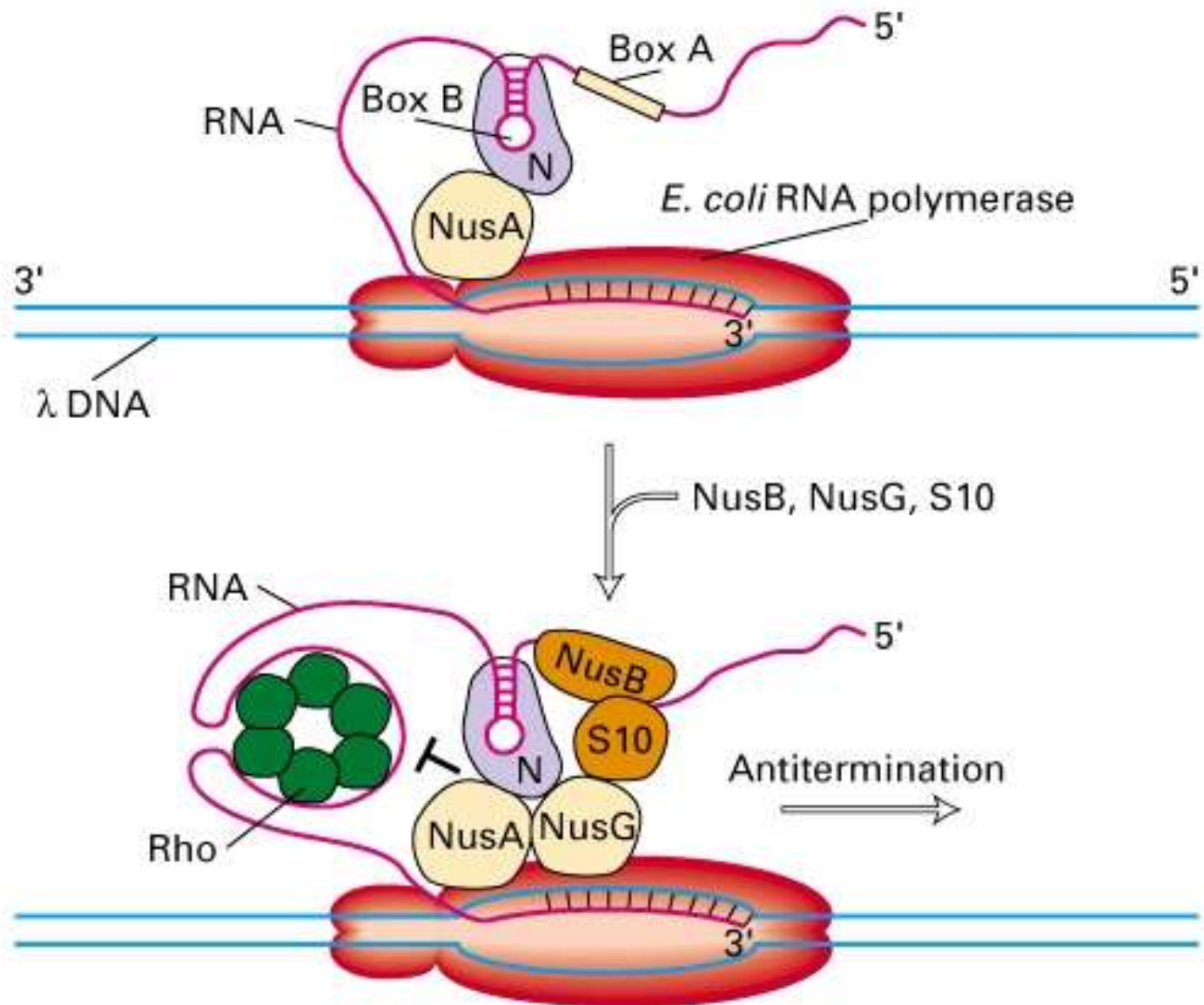
Anti-termination

- Phage Lambda (l) produces an anti-termination protein to allow transcription of late-phase phage proteins
 - Early phase transcription of phage Lambda (l) DNA from two promoters produces two proteins critical for its function
 - Cro is a DNA binding protein and a regulator of the phage gene activity
 - Protein 'N' is an anti-termination protein which allows transcription to continue beyond the first rho-dependent termination sites

Anti-termination

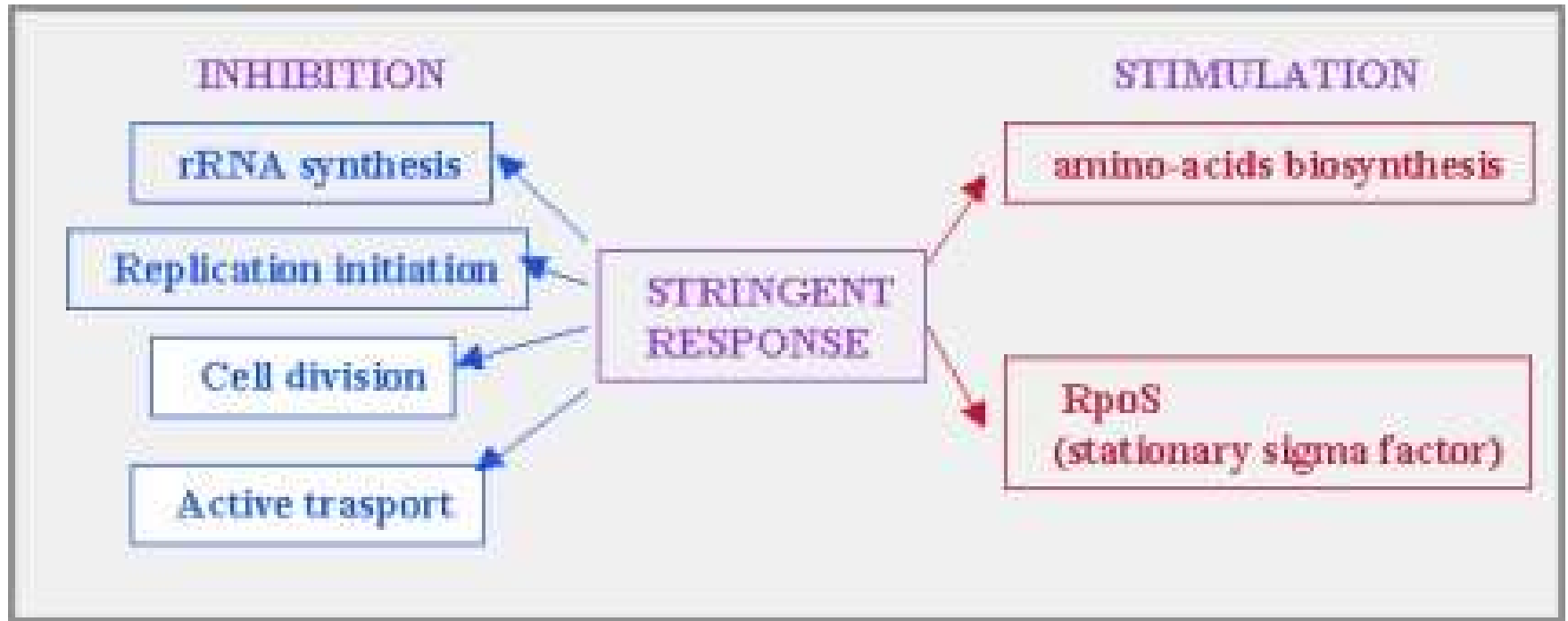


Anti-termination



Stringent response

First described in *E. coli* in 1962



Following amino-acid starvation, the cells reduce themselves to a minimal unit, sufficient for surviving the starvation period. Thus, they are able to recover quickly when nutrients become available again. (p)ppGpp

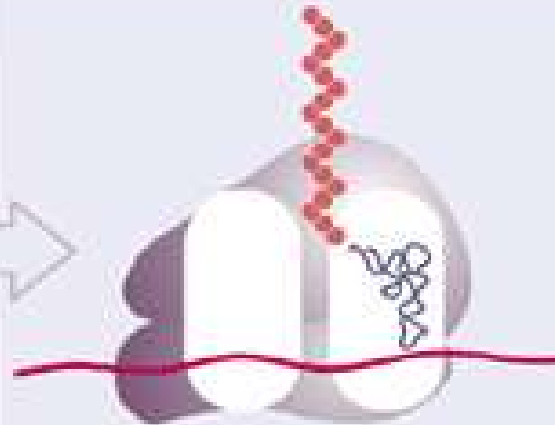
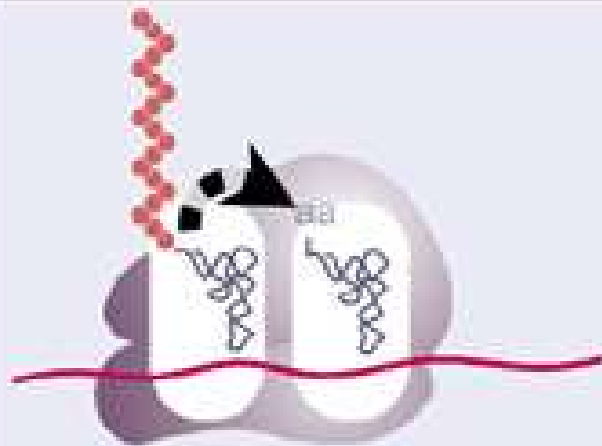
The stringent response is mediated by two unusual phosphorylated derivatives of GTP and GDP, called (p)ppGpp collectively, which accumulate to high levels within a few seconds after amino-acid starvation.

Stringent response

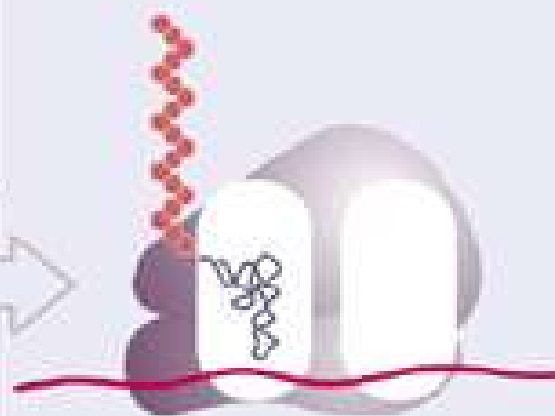
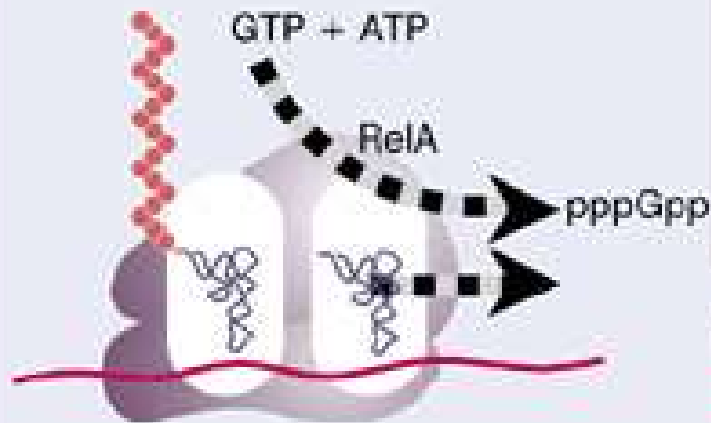
Amino acid starvation

1. The concentration of charged tRNAs drops.
2. A large transient accumulation of two novel small molecules is induced; the two molecules, originally called "magic spots", are now known to be the highly-phosphorylated nucleotides guanosine tetraphosphate (5'-ppGpp-3') and guanosine pentaphosphate (5'-pppGpp-3').
3. Total protein synthesis slows down, and the pattern of protein synthesis shifts dramatically. Ribosomal protein synthesis drops to near zero; synthesis of amino acid biosynthetic enzymes is induced.
4. Synthesis of rRNA and tRNA almost stops.
5. Initiation of new rounds of DNA replication stops.
6. Synthesis of phospholipid, carbohydrate, and murein slows down.

Stringent response



Aminoacyl-tRNA is substrate for peptide bond synthesis (followed by ribosome movement)



Uncharged tRNA triggers idling reaction (followed by discharge of tRNA)

Alternate sigma factors bind to sequences upstream from the promoter

- σ^{54} is specific for genes involved in nitrogen metabolism
- σ^{54} dependent transcription units respond to activating sites -80 to -160
- recognition sites for these activators are called *enhancers*
- enhancers are *cis-acting factors* that work at considerable distances from the target gene

Alternate sigma factors bind to sequences upstream from the promoter

Example:

- NtrC recognizes an enhancer upstream from the *glnA* gene (glutamine synthetase)
- NtrC is phosphorylated by NtrB (phosphokinase)
- activated NtrC* interacts with the RNAPol/ σ^{54} complex and promotes transcription

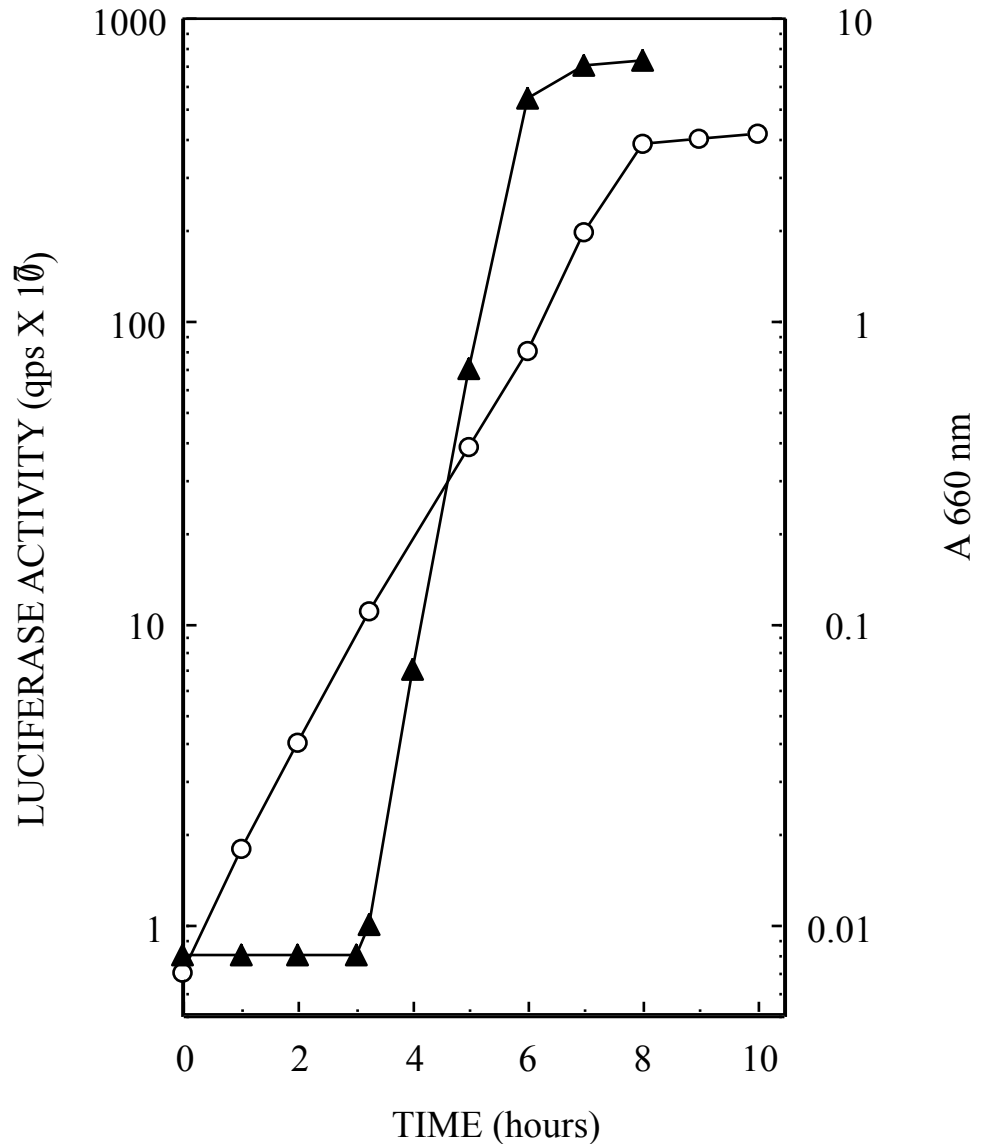
Quorum sensing – *lux* operon

Vibrio fischeri

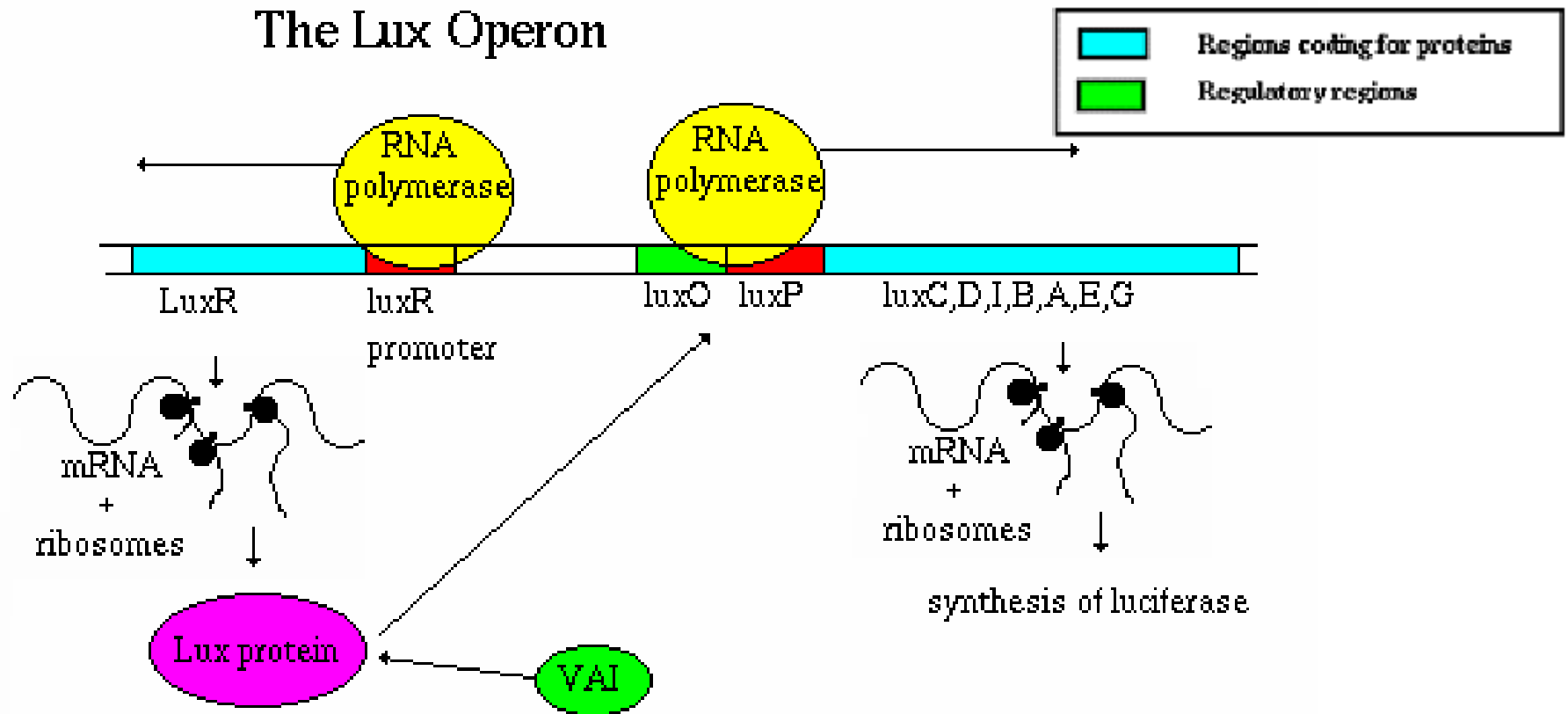
Population density-dependent

OR

Growth-phase-dependent



Quorum sensing – *lux* operon



VAI - (*Vibrio fischeri* autoinducer), **LuxR** - transcription activator of lux operon

N-acetyl homoserin lactone - AHL

AHL molecules and their functions

Bioluminescence

Vibrio anguillarum, *V. fischeri*, *V. harveyi*

Plant-microbe interactions

Rhizobium leguminosarum

R. meliloti - rhizosphere-specific genes

Virulence determinants

Pseudomonas aeruginosa - elastase, pyocyanin etc

Ralstonia solanacearum - a phytopathogen

Aeromonas hydrophila - serine proteases

Vibrio cholerae - HA/protease

Bordetella pertussis - pertussis toxin

Erwinia carotovora - a phytopathogen

Staphylococcus aureus

Polysaccharide production

Rhodobacter sphaeroides

Erwinia stewartii - Stewart's wilt of sweet corn pathogen

Klebsiella pneumoniae

Escherichia coli - colanic acid

Conjugal transfer

Agrobacterium tumefaciens Ti plasmid

Swarming motility

Serratia liquefaciens

Cell division

Escherichia coli

Extracellular lipase synthesis

Streptomyces lividans 66

Pigment production

Chromobacterium violaceum - violacein

Antibiotic production

Erwinia carotovora - carbapenem, b-lactam antibiotic

Pseudomonas aureofaciens- 3 phenazine antibiotics

Gram +ve lactic acid bacteria - antimicrobial peptide

Genetic competence

Streptococcus pneumoniae

Bacillus subtilis