HBC209

MEDICAL BIOCHEMISTRY
HBC200/UPC200/VBC200

LECTURE I: 22nd Feb 2022

Dr. Martin MULINGE
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Course Outline

WK	DATE	TOPIC
1	11.01	Structure and conformation of ribo-and deoxyribonucleotides, heteropolymeric nature of DNA and its role as informational molecule.
2	18.01	Central dogma. Base pairing, three dimensional structures and forms of DNA.
3	25.01	DNA replication, semi-conservative replication, processes involved in replication. Okazaki pieces, topoisomerases and gyrases.
4	01.02	Proofreading replication errors and DNA repair and causes of DNA damage.
5	08.02	Transcription and reverse transcription. Post-transcriptional modification.
6	15.02	Genetic code. Translation; reading frames. Ribosomal units. Initiation, elongation and termination. Inhibitors of transcription, replication and translation.
		CAT I

Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 1 04-08/10/2021	Tissue metabolism: Differential metabolism in Liver, Muscle, adipose brain and erythrocytes. Integration of metabolism	Prof. Mukuria
WEEK 2 11-15/10/2021	Biochemistry of muscle contraction	,,
WEEK 3 01-05/02/2021	Neurochemistry: Brain energy sources. The blood brain barrier. Neurotransmitters.	,,
WEEK 4 18-22/10/2021	Biotransformation of Xenobiotics: Drug metabolism: Biotransformation of drugs, phase I and phase II reactions	,,
WEEK 5 25-29/10/2021	Pharmacokinetics phase of drug transformations	,,
WEEK 6 01-05/11/2021	Nucleotide metabolism: Overview of structure and function of nucleotides. Purine and Pyrimidine metabolism: degradative and biosynthetic pathways, regulation of nucleotide metabolism.	,,
WEEK 7 08-12/11/2021	Disorders of nucleotide metabolism and drugs targeting nucleotide metabolism: (antifolates, glutamine antagonists, reverse transcriptase inhibitors) and their importance in cancer therapy.	,,
16/11/2021	MID SEMESTER CAT	Prof. Mukuria
WEEK 8 22-26/11/2021	Molecular biology: DNA structure, replication in eukaryotes and prokaryotes	Dr. Mobegi
WEEK 9 29-03/12/2021	Molecular biology: DNA structure, replication in eukaryotes and prokaryotes	,,
WEEK 10 06-10/12/2021	Invitro DNA replication- PCR: principle and applications.	,,
WEEK 11 13-17/12/2021	Transcription in prokaryotes and eukaryotes and post-transcriptional modifications	,,
	CHRISTMAS BREAK	

Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 12 03-07/12/2022	Translation/genetic code, Pre- & Post-translational modifications.	,,
WEEK 13 10-14/01/2022	Translation: Posttranslational protein modification. Inhibitors of protein synthesis and their role in chemotherapy.	,,
WEEK 14 17-21/01/2022	Parasite biochemistry: Special metabolism in parasitic protozoa and helminths	,,
WEEK 15 24-28/01/2022	Parasite biochemistry: Special metabolism in parasitic protozoa and helminths	,,
WEEK 16 31-04/02/2022	Protein folding and targeting in the cell. Diseases and syndromes associated with the two processes DNA mutations and repair mechanisms: types, effects Mutagens and their effect on DNA and suppressor mutations clinical correlations.	
WEEK 17 07-11/02/2022	Bioinformatics: Introduction to basic bioinformatics and its application in disease detection, drug design and Modern medicine	
WEEK 18 14-18/02/2022	END FIRST SEMESTER EXAMS	Prof. Mukuria Dr Mobegi

Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 20 **21-25/02/2022**	**Gene regulation: Structural and transcriptional regulation of gene expression in prokaryotes and eukaryotes repression and induction of transcription of prokaryotic gene, bacterial operon concept, negative vs positive control, other regulatory mechanisms.**	**Dr. Mulinge**
WEEK 21 28-04/03/2022	DNA repair and DNA recombination. Disease/syndromes associated with DNA repair	,,
WEEK 22 07 - 11/03/2022	Molecular virology: Classification and properties of viruses, replication and life cycle of viruses. Interferons, Oncogenes and oncogenic viruses. Viroids and prions. Application –HIV	,,
WEEK23 14 - 18/03/2022	Molecular virology: Classification and properties of viruses, replication and life cycle of viruses. Interferons, Oncogenes and oncogenic viruses. Viroids and prions. Application –HIV	,,
WEEK 24 21 - 25/03/2022	Bacterial Biochemistry: Bacterial cell structure: Cell envelope; Cell cytoplasm; Cellwall and its biosynthesis. Bacterial toxins, virulence and pathogenesis.	,,
WEEK 25 28 - 01/04/2022	Bacterial chemotherapy: Mechanisms of action of antibiotics Bacterial resistance to antimicrobial chemotherapy.	,,
WEEK 26 04 - 08/04/2022	Biochemical endocrinology: Endocrine, paracrine and autocrine mode of secretions. Classification of hormones. Mechanism of hormone action: Signal and signal transduction, Receptors: intracellular and membrane bound receptors. Second messenger role in signal transduction: cAMP, cGMP, lipids, Calcium ions.	,,
WEEK 27 11 - 15/04/2022	Biochemical endocrinology: Synthesis, storage, release, transport, mode of action and degradation of peptide, steroid and prostaglandins derived hormones	,,
12/04/2022	MID 2nd SEMESTER CAT	Dr Mulinge

Course Outline

WEEK	TOPIC	LECTURER (S)
	BREAK	
WEEK 28 25 - 29/04/2022	Molecular genetics: Organization of the human genome, chromosomes and karyotypes. Nuclear and mitochondrial chromosomes.	PROF. NGUU
WEEK 29 02 - 06/05/2022	Gene structure and organization and gene family. Satellite DNA and DNA families. C value of a genome Cot ½ values of DNA and its relation to repetition.	,,
WEEK 30 09 - 13/05/2022	Mendelian laws: Pedigree analysis, Mendelian laws of inheritance (single gene inheritance: autosomal and X-linked inheritance, dominant and recessive inheritance. Extension and exceptions of Mendelian inheritance, nonclassical patterns of single gene inheritance.	,,
WEEK 31 16 - 20/05/2022	Genetic diseases: Single gene (Mendelian) disorders, chromosomal, mitochondrial, multifactorial inheritance and examples of associated disorders.	,,
WEEK 32 23 - 27/05/2022	Population genetics: phenotypes, genotypes and gene and genotype frequencies, Hardy-Weinberg law, equilibrium, frequency of X-linked genes, and genotypes, genetic drift. Genetic polymorphism- factors, molecular basis of mutation, origin of mutation and frequency of new mutations.	,,
WEEK 33 30 - 03/06/2022	Clinical cytogenetics: Numerical chromosomal aberrations, (Euploidy and aneuploidy, sex chromosome aneuploidy and clinical correlations, structural chromosome aberrations-translocations, deletions, trans versions and associated disorders, polymorphic markers and linkage analysis. Genetic diagnosis and therapy: prenatal screening, genetic diagnosis and gene therapy.	,,
WEEK 34 - 36	END YEAR EXAMINATIONS	Prof. Nguu Prof. Mukuria Dr. Mulinge Dr Mobegi

Course Outline (2)

WK	DATE	TOPIC
7	22.02	Control of gene expression: repression and induction of transcription, bacterial operons, other regulatory mechanisms.
8	29.02	Eukaryotic promoters, enhancers and repressors.
9	07.03	DNA-protein interactions.
10	14.03	Manipulations of DNA; restriction enzymes, cutting and ligation.
11	21.03	Plasmids selection. Detection of recombinant plasmids and cloning genes.
12	28.03	Construction of genomic gene libraries.
13	04.04	**REVISION & CAT II**
14-15		**UNIVERSITY EXAMINATIONS**

Regulation of Gene Expression

Regulation of gene expression

Not all genes are turned on (expressed) all the time

In general, they are turned on only when needed.

Why regulate gene expression?

Regulation allows cells to respond to environmental conditions by synthesizing selected gene products only when they are needed.

Types of Gene expression

A Constitutive gene expression

Expression of genes at about the same level under all environmental conditions

e.g. "housekeeping genes" like primase or ssDNA binding proteins

B Regulated gene expression

Control of the rate of protein or RNA synthesis as an adaptive response to stimuli.

induction: increase in gene expression

repression: decrease in gene expression

cell does not waste energy making enzymes it does not need.

Transcriptional regulation

- regulation of RNA synthesis: the critical step in the regulation of most bacterial genes is the binding of RNA polymerase to DNA at the promoter.
  - The other potential points of control, while sometimes important in the expression of certain genes, serve more often to fine-tune the amount of protein produced.

The most common method of gene regulation in all cells

- ✓ Regulatory proteins
- ✓ Regulatory protein binding sites
- ✓ Effector molecules

RNA polymerase participates in all three phases of transcription

Initiation – core RNA polymerase plus sigma (σ) factor

- Core has four subunits: two alpha (α), one beta (β), one beta prime (β')
- DNA is unwound and polymerization begins

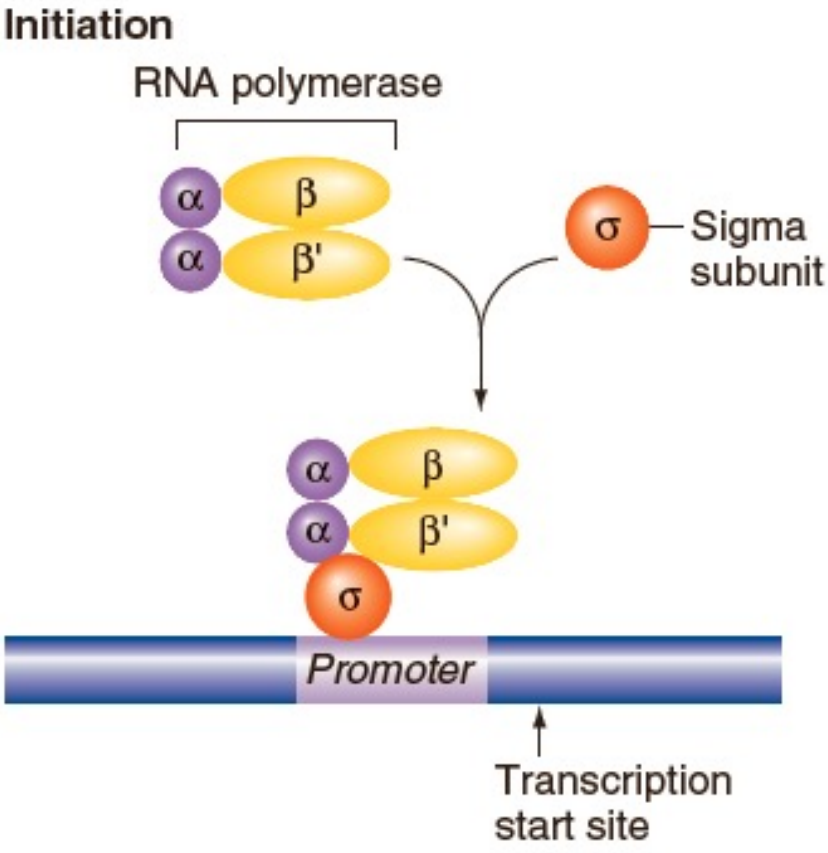
Elongation – core RNA polymerase without σ factor

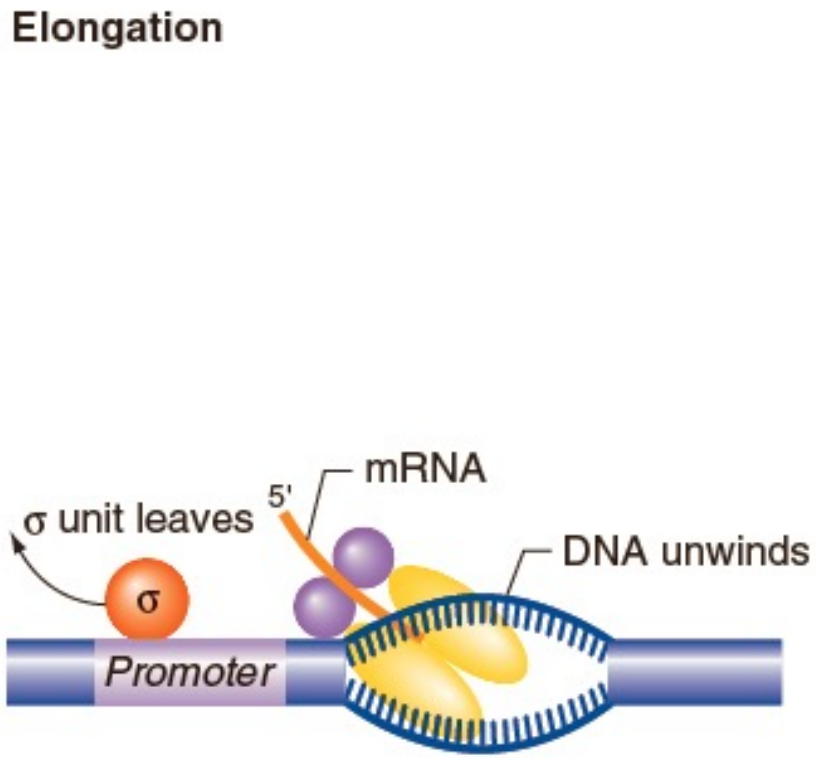
- Continues until RNA polymerase recognizes termination signal

Termination – two kinds in bacteria

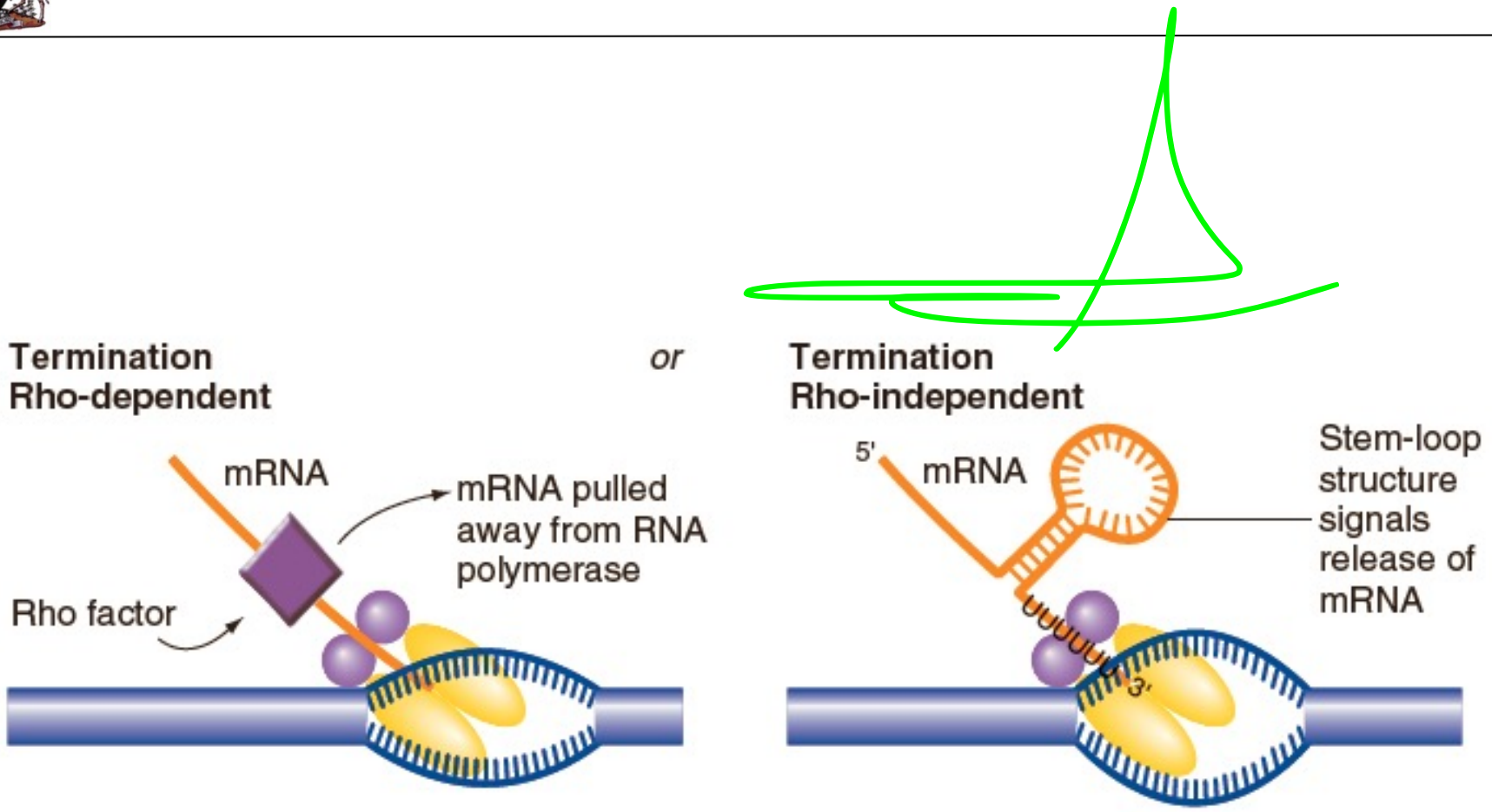
- Rho-dependent – Rho (ρ) protein binds to RNA polymerase and removes it from RNA
- Rho-independent – 20 nt sequence in RNA forms stem-loop

Role of RNA polymerase in initiation and elongation phases of transcription





Two kinds of transcription termination in bacteria



A. Regulatory proteins

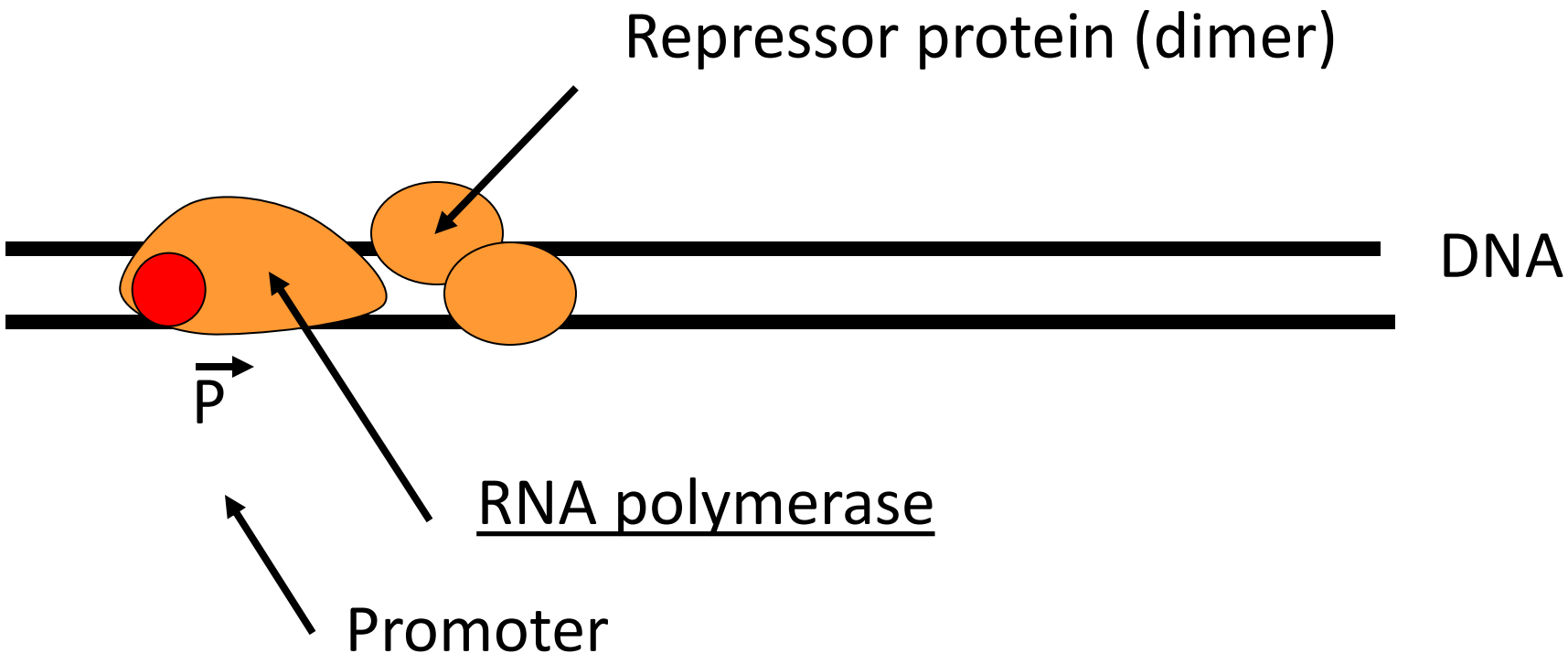
- Transcriptional regulation is mediated by regulatory proteins.

- Cells have many different regulatory proteins.

- Specific regulatory proteins control the transcription of specific groups of genes.

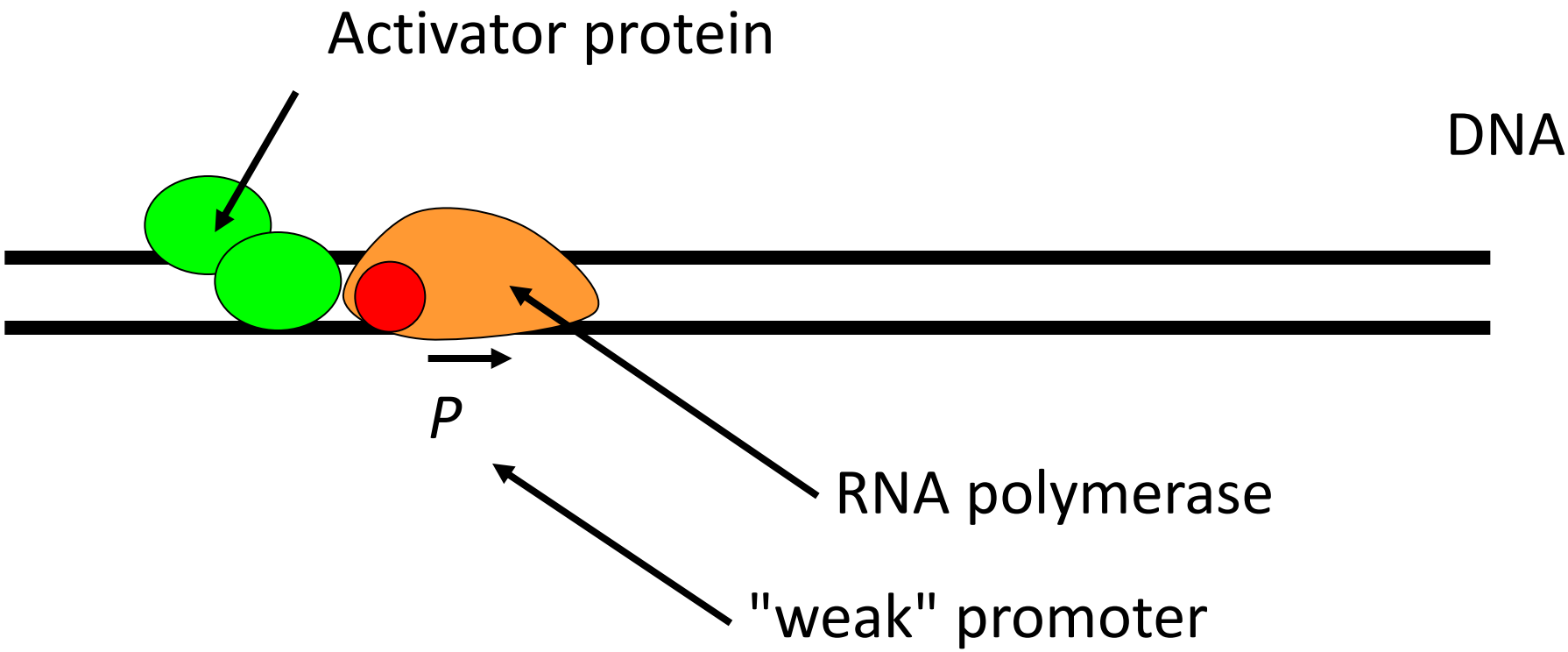
- Examples of regulatory proteins are "repressor proteins" and "activator proteins."

Repressor proteins



Repressor proteins decrease transcription when bound to DNA by interfering with the activity or binding of RNA polymerase.

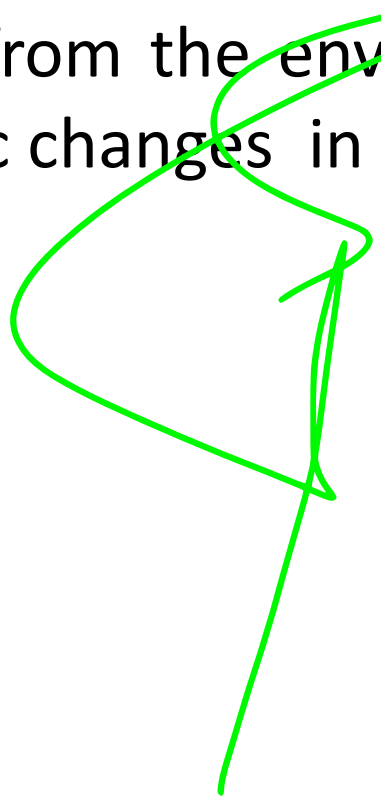
2. Activator proteins



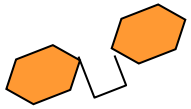
Activator proteins <u>increase</u> transcription when bound to DNA by helping RNA polymerase bind to weak promoters.

C. Effector molecules

Small molecules from the environment (or made inside cells) that signal specific changes in gene expression.



Classes of effectors

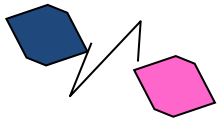


maltose

a. inducers

small molecules that mediate gene induction

e.g. catabolic substrates:
sugars, amino acids, fatty acids



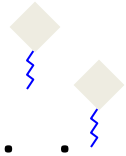
lactose

Classes of effectors

b. corepressors

small molecules that mediate gene repression

e.g. biosynthetic products:
amino acids, purines, pyrimidines, fatty acids etc.



arginine

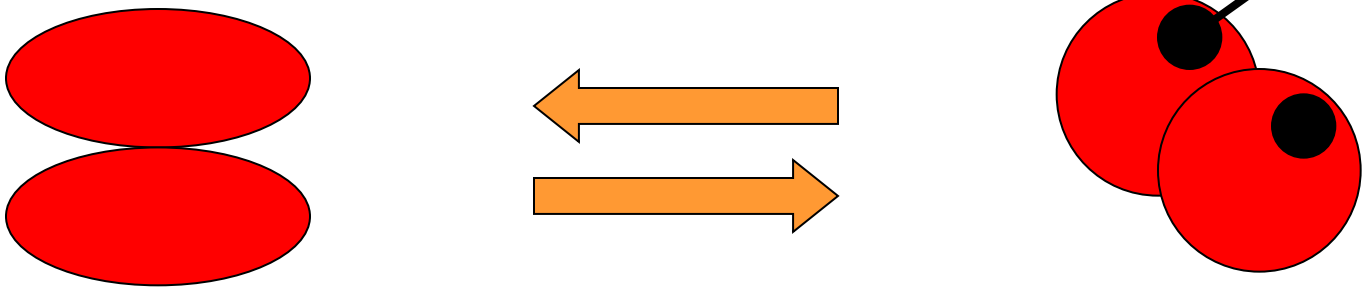
tryptophan

How effectors work

Effectors change the DNA binding affinity of regulatory proteins for their binding sites.

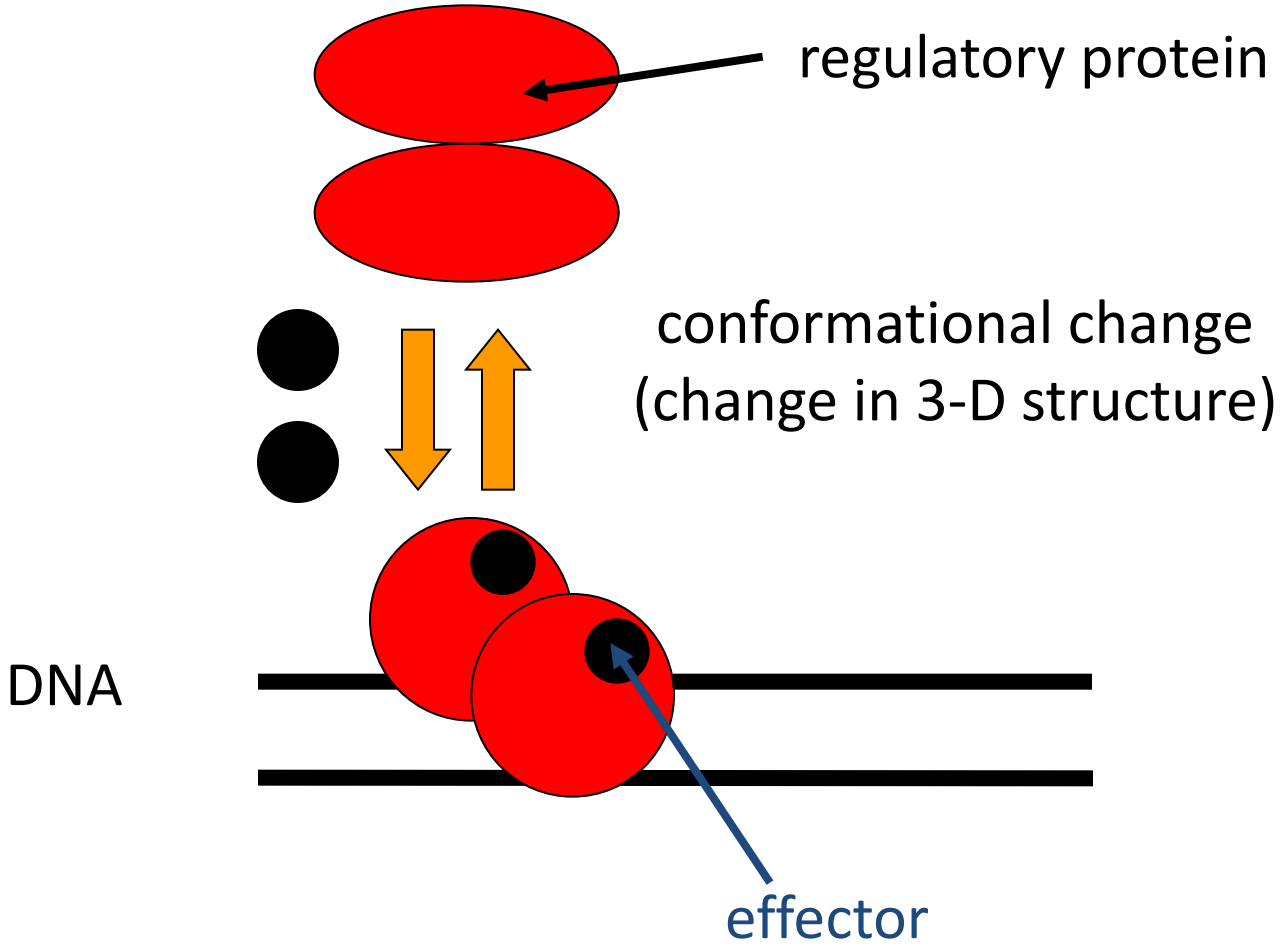
regulatory protein

effector

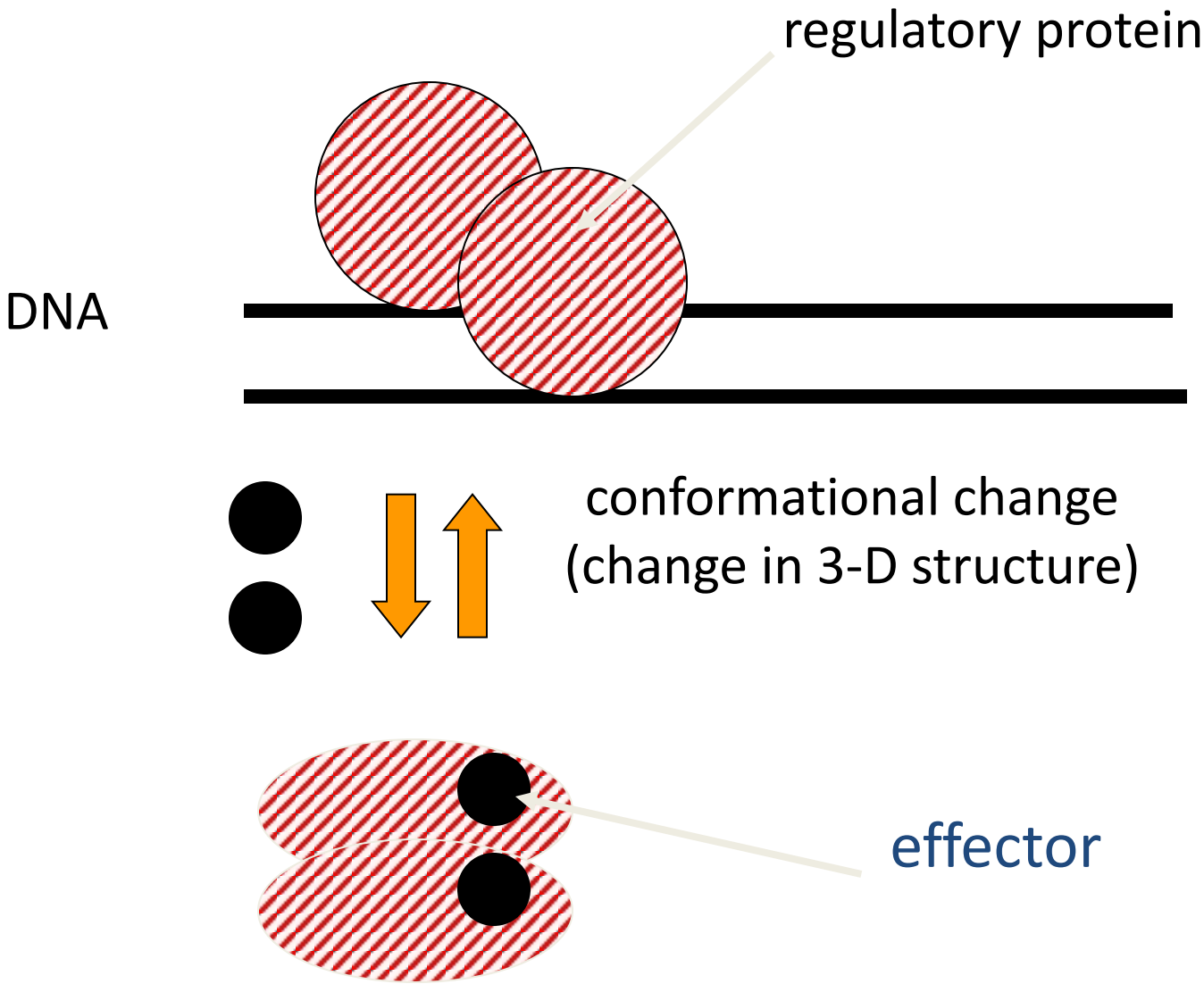


conformational change
(change in 3-D structure)

A. Some effectors increase DNA binding affinity

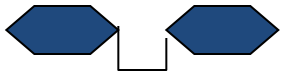


B. Some effectors decrease DNA binding affinity



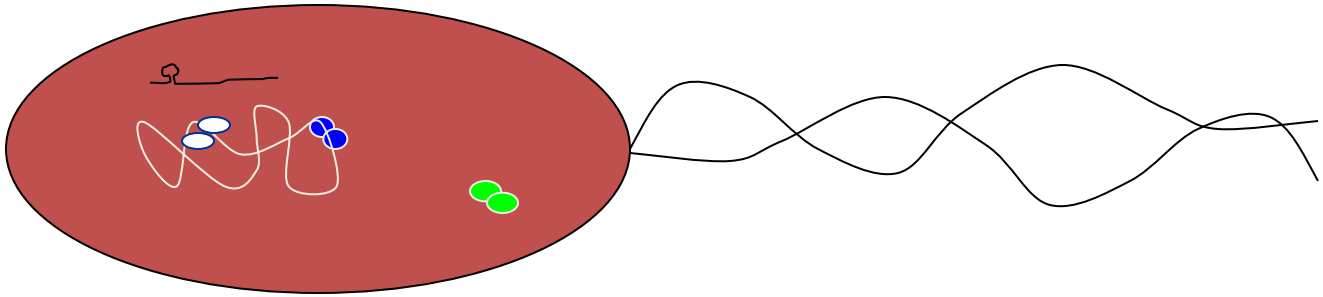
REGULATION OF GENE EXPRESSION IN PROKARYOTES

Cells can respond to environmental changes by regulating gene expression.

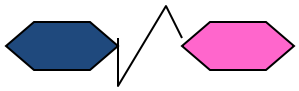


maltose

arginine

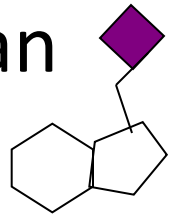


lactose



glucose

tryptophan



Regulation of expression can occur at many steps

Transcriptional control

- Binding of RNA polymerase to promoter
  - Most critical step in regulation of most prokaryotic genes
- Shift from initiation to elongation
- Release of mRNA at termination

Posttranscriptional control

- Stability of mRNA
- Efficiency of translation initiation
- Stability of polypeptide

Utilization of lactose by *E. coli* provides a model system of gene regulation

Lactose utilization requires two enzymes

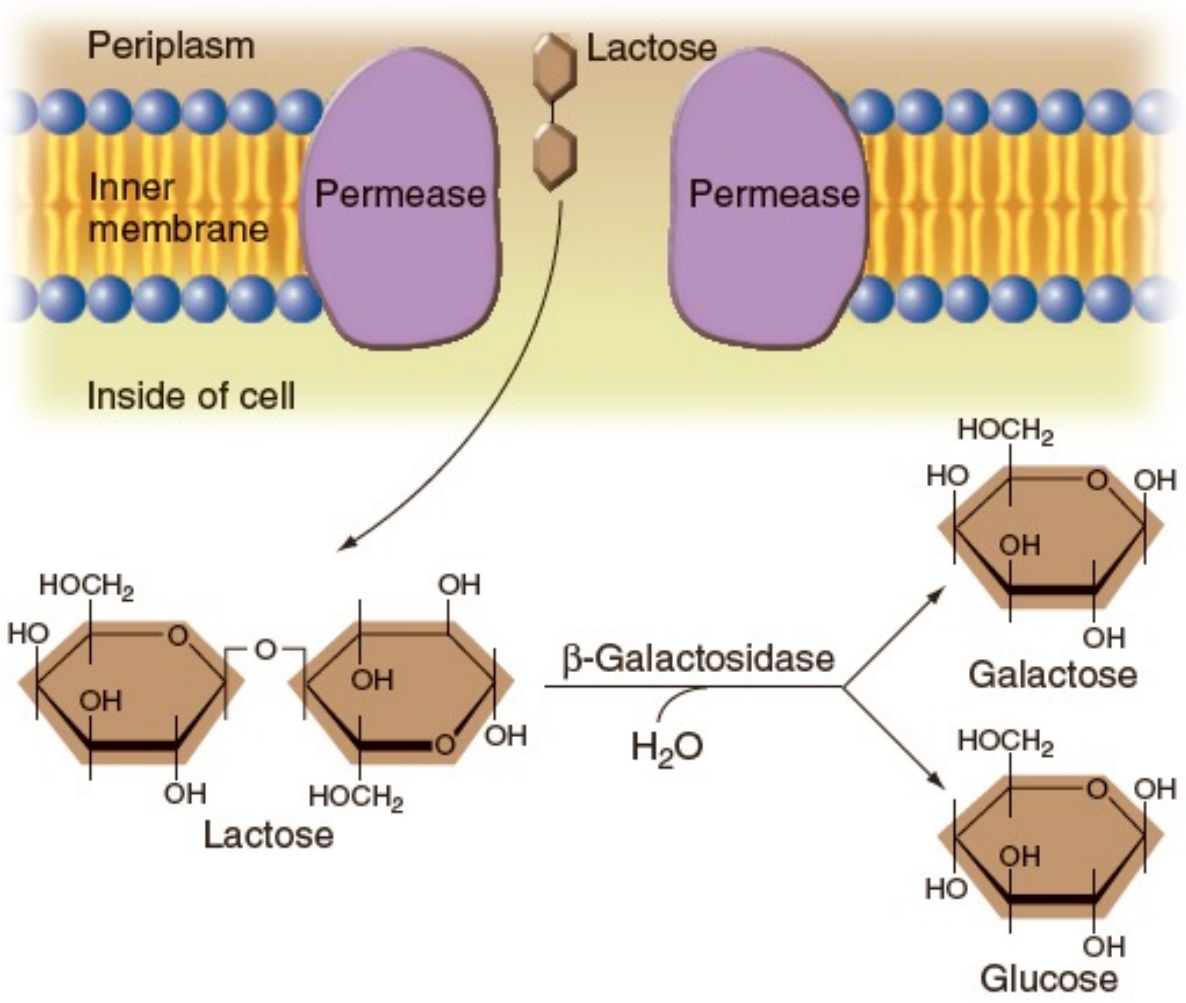
- Permease transports lactose into cell
- β-Galactosidase (β-Gal) splits lactose into glucose and galactose

In the absence of lactose, both enzymes are present at very low levels

Lactose is the inducer of the genes encoding permease and β-Gal

- Induction – stimulation of synthesis of a specific protein
- Inducer – molecule responsible for induction

Lactose utilization in an *E. coli* cell



Advantages of using lactose utilization by *E. coli* as a model for understanding gene regulation

***Lac*⁻ mutants** can be maintained on media with glucose and so *lac* genes are not essential for survival

- If both glucose and lactose are present, *E. coli* cells will use glucose first

Simple assays for *lac* expression - use of *ortho*-Nitrophenyl-β-galactoside (ONPG) or X-gal as substrates for β-gal (color change)

Lactose induces a 1000-fold increase in β-gal activity

Detection and characterization of hundreds of *lac*⁻ mutants defective in lactose utilization

Studies of *lac*⁻ mutants revealed the operon theory of gene regulation

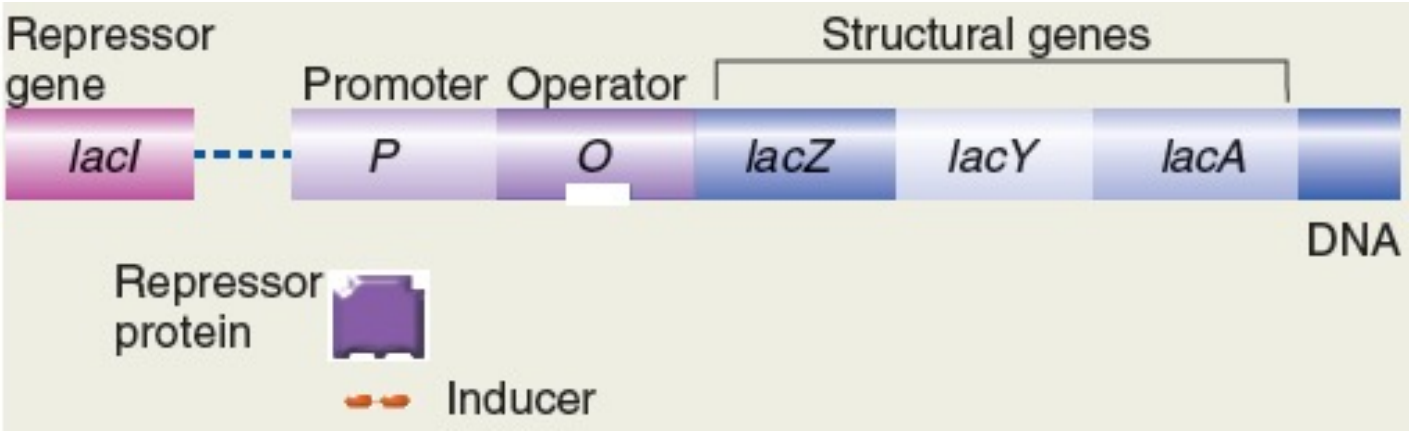
Jacques Monod and Francois Jacob – Pasteur Institute

- Compared the effects of many different types of *lac* mutants on induction and repression of enzyme activity for lactose utilization
- Operon theory - one signal can simultaneously regulate expression of several clustered genes
- Hypothesized that *lac* genes are transcribed together as a single mRNA (polycistronic) from a single promoter

The lactose operon in *E. coli*

The players

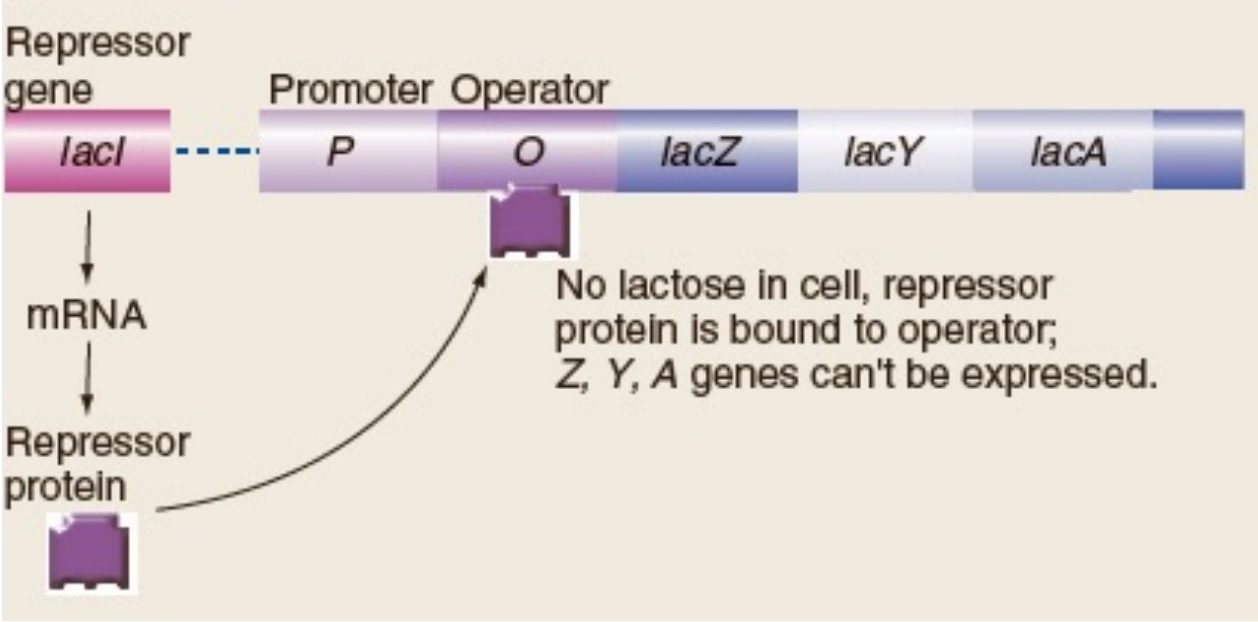
- Three structural genes - *lacZ*, *lacY*, and *lacA*
- Promoter - site to which RNA polymerase binds
- *Cis*-acting operator site – controls transcription initiation
- *Trans*-acting repressor - binds to the operator (encoded by *lacI* gene)
- Inducer - prevents repressor from binding to operator



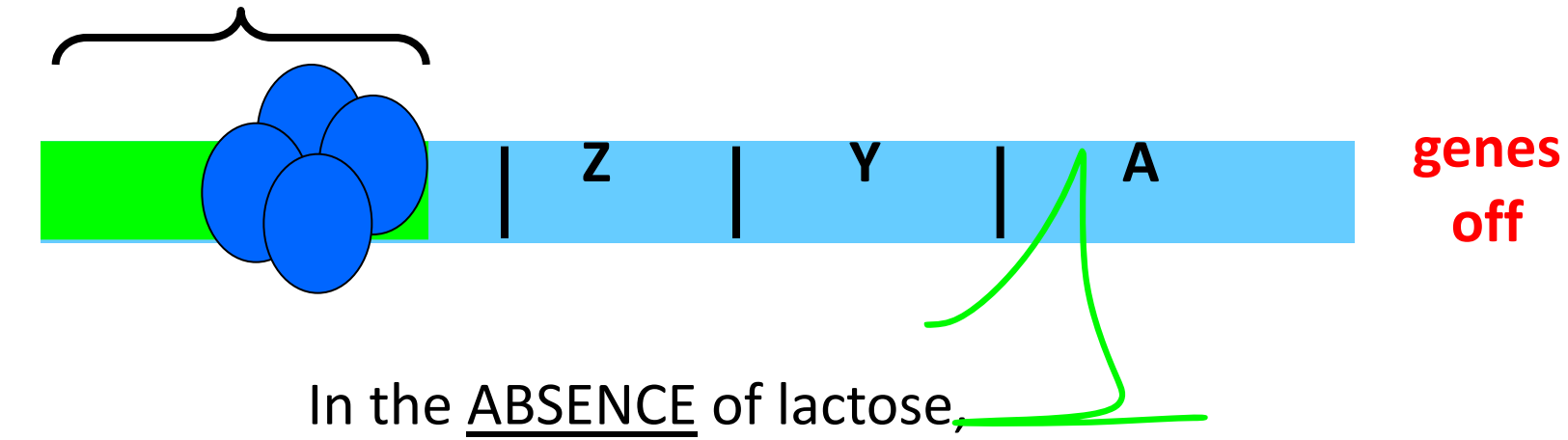
Repression of *lac* gene expression

In the absence of lactose, repressor protein binds to the operator and prevents transcription

lac repressor is a negative regulatory element



Lac operon when Lactose is NOT available - animation

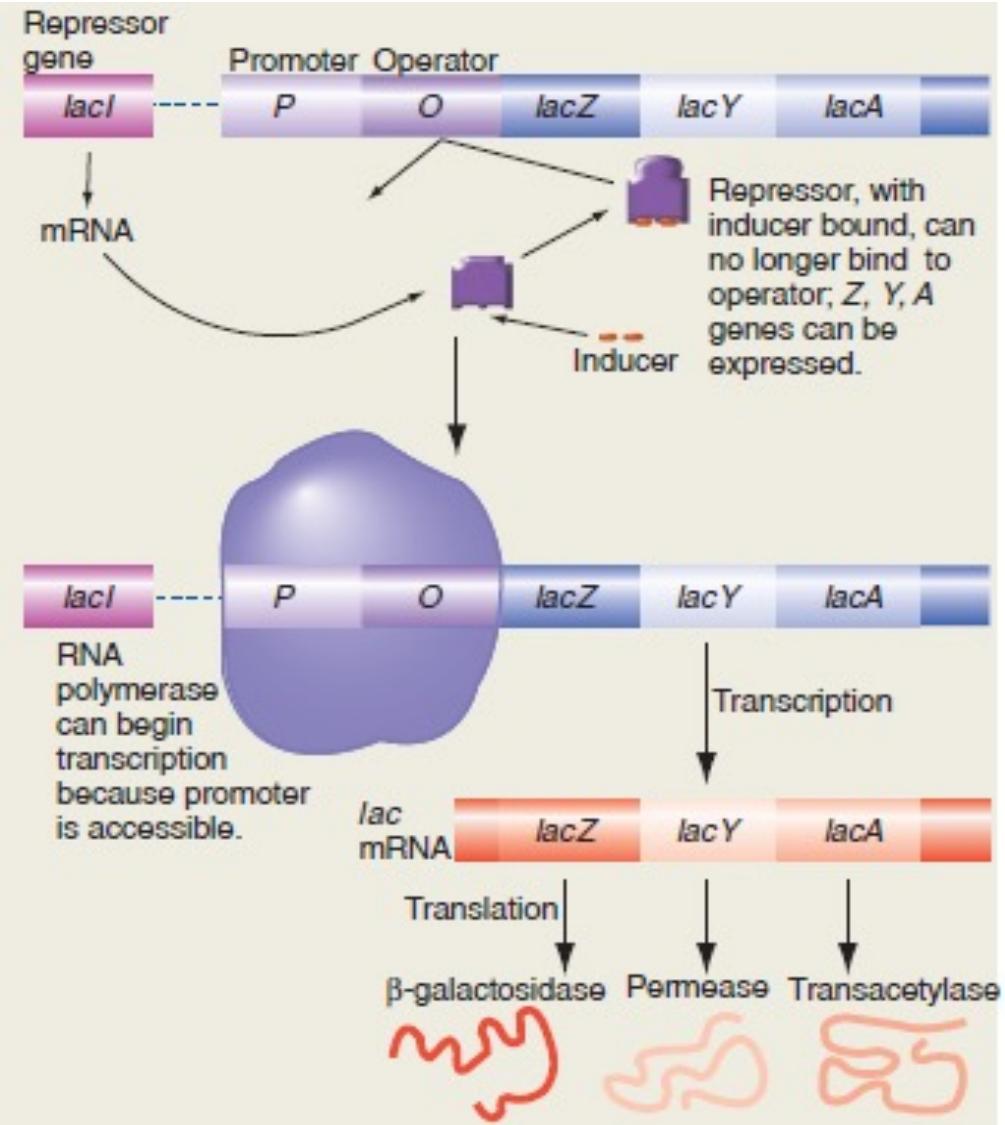


In the ABSENCE of lactose,
the *lac* repressor protein binds DNA.

When lactose is unavailable, the catabolic enzymes are NOT needed.

Induction of *lac* gene expression

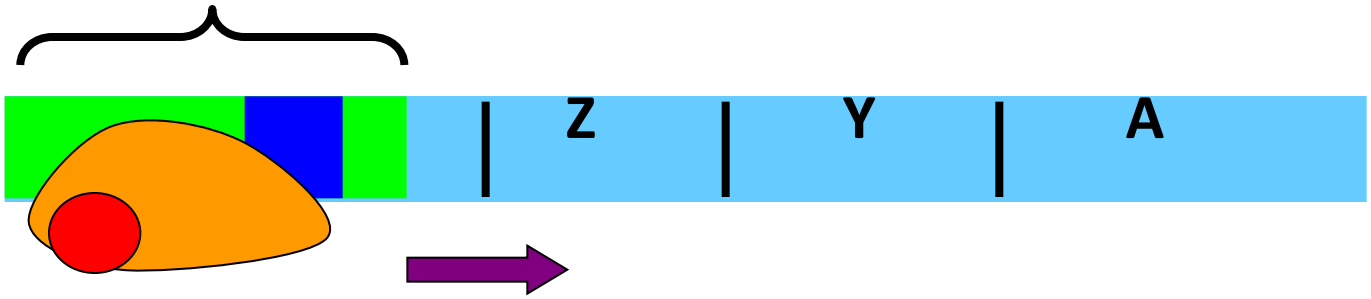
1. When lactose is present, allolactose, an inducer derived from the sugar, binds to the repressor. This binding changes the shape of the repressor, making it unable to bind to the operator.
2. With the release of the repressor from the operator, RNA polymerase gains access to the *lac operon promoter and* initiates transcription of the three lactose-utilization genes into a single polycistronic mRNA.



inducer, we now know that the inducer is actually allolactose, a molecule derived from and thus related to lactose.

Lac operon when Lactose is available - animation

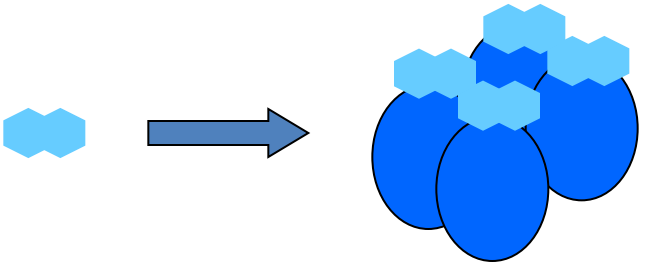
***lac* promoter**



genes on

RNA polymerase

lactose → allolactose



repressor does not bind DNA

Jacob and Monod defined the roles of the *lac* genes by genetic analysis of many *lacI*$^-$ mutants

Complementation analysis identified three genes in a tightly linked cluster

- *lacZ* encodes β-galactosidase
- *lacY* encodes permease
- *lacA* encodes transacetylase
- Most studies focused on *lacZ* and *lacY*

Constitutive expression of β-galactosidase and permease was caused by mutations in the *lacI* gene

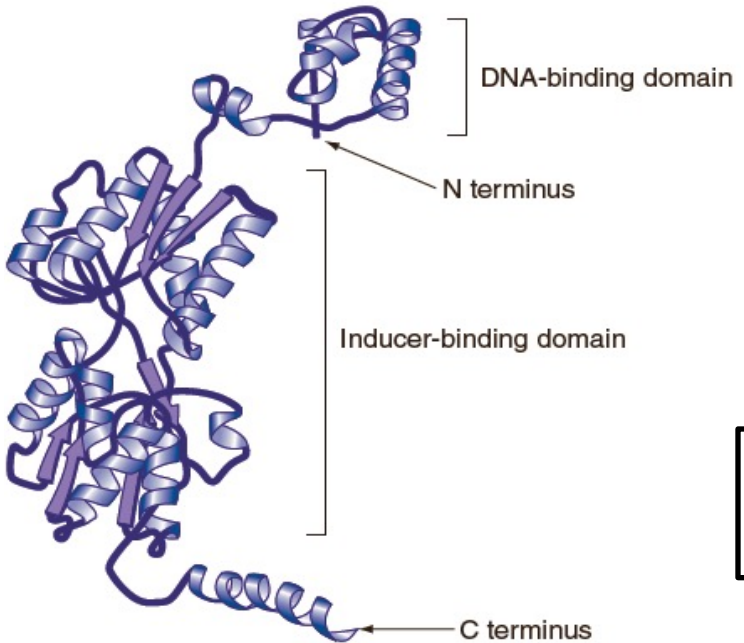
- Constitutive mutants (*lacI*$^-$) express the enzymes in the absence and presence of inducer

How the inducer acts to trigger synthesis of lac enzymes

Binding of inducer to repressor changes the shape of the repressor so that it can longer bind to DNA

- When no inducer is present, repressor is able to bind to DNA

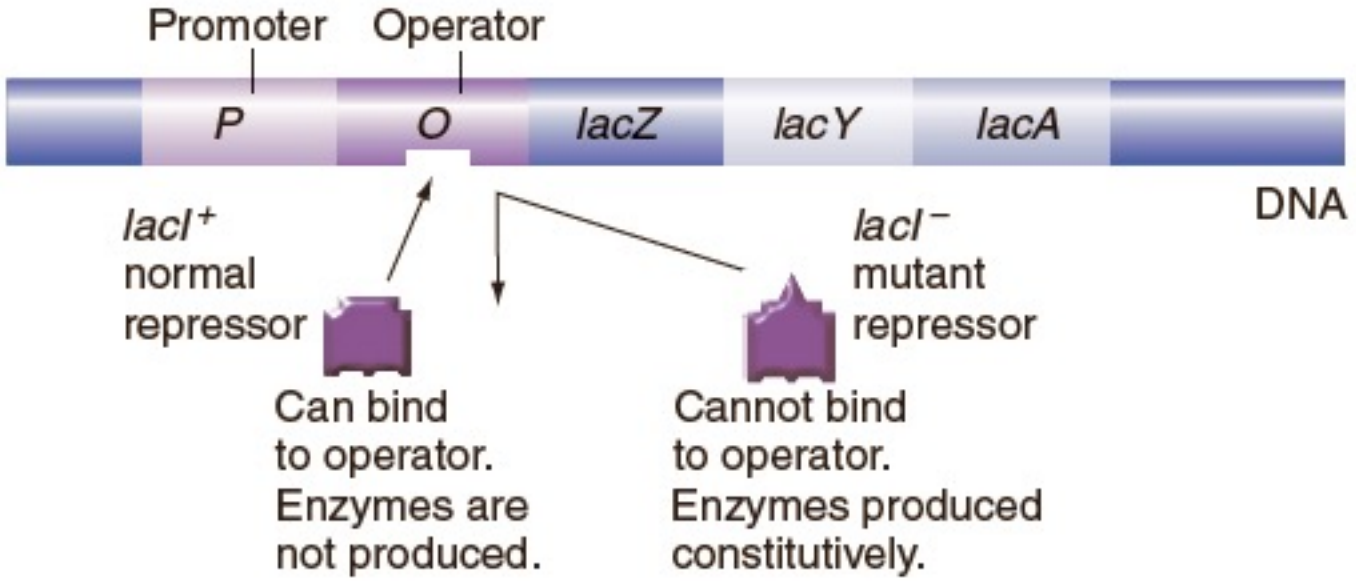
Repressor is an allosteric protein – undergoes reversible changes in conformation when bound to another molecule



lac repressor has two separate domains

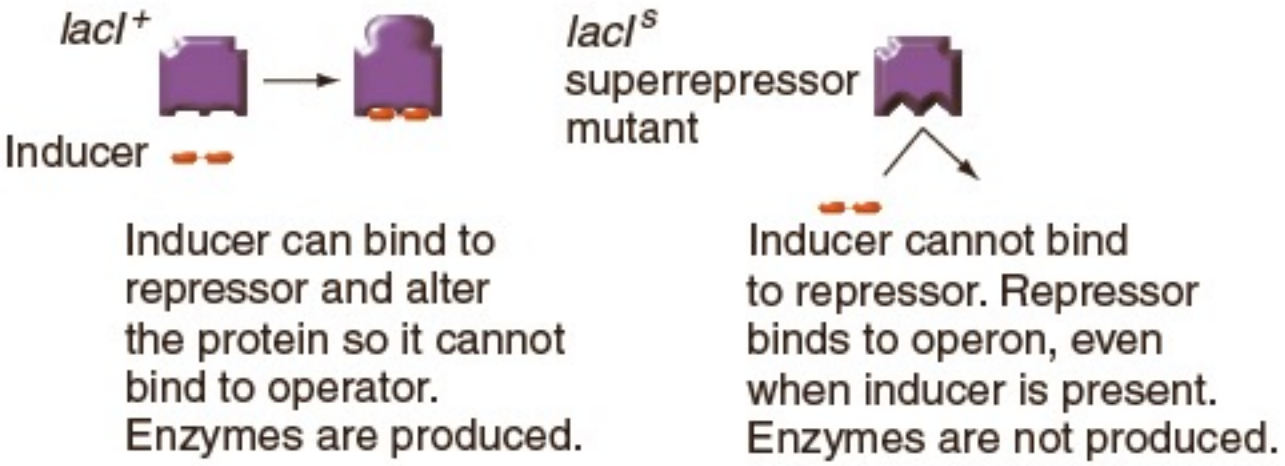
lacI⁻ mutants have a mutant repressor that cannot bind to operator

In *lacI*⁻ mutants, *lac* genes are <u>expressed</u> in the absence and the presence of inducer (constitutive expression)



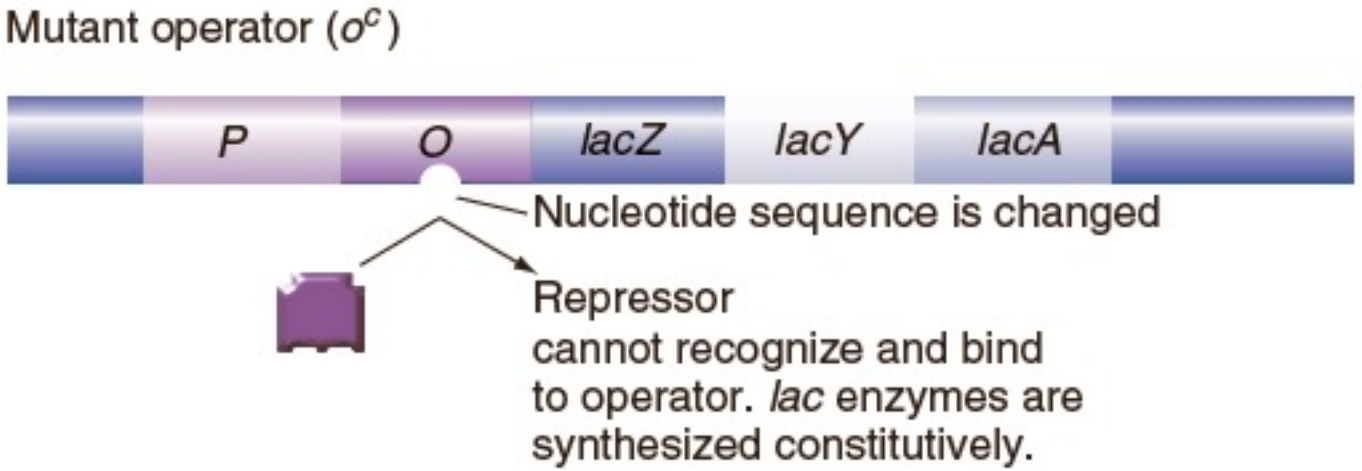
lacI^{s} mutants have a superrepressor that binds to operator but cannot bind to the inducer

In *lacI*^{S} mutants, *lac* genes are **repressed** in the absence and the presence of inducer



lacO^c mutants have a mutant operator that cannot bind the repressor

In *lacO*^{c} mutants, lac genes are <u>expressed</u> in the absence and the presence of inducer (constitutive expression)



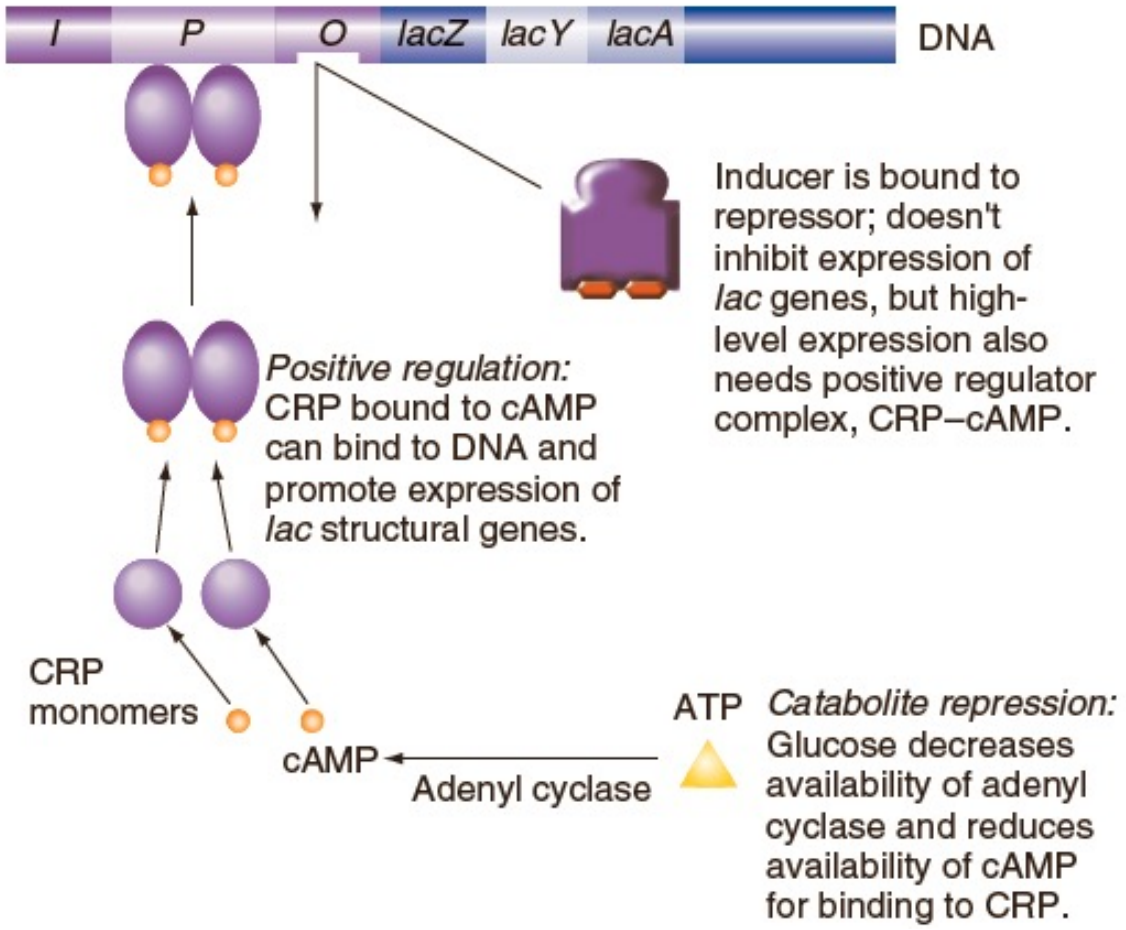
lac operon of *E. coli* is regulated by both lactose and glucose

- When both glucose and lactose are present, only glucose is utilized

- ✓ Lactose induces *lac* mRNA expression, but only in the absence of glucose
  - Lactose prevents repressor from binding to *lacO*
  - *lac* repressor is a negative regulator of *lac* transcription
- ✓ *lac* mRNA expression cannot be induced if glucose is present
  - Glucose controls the levels of cAMP
  - cAMP binds to cAMP receptor protein (CRP)
  - CRP-cAMP is a positive regulator of *lac* transcription

Positive regulation by CRP–cAMP

Catabolite repression – overall effect of glucose is to prevent *lac* gene expression

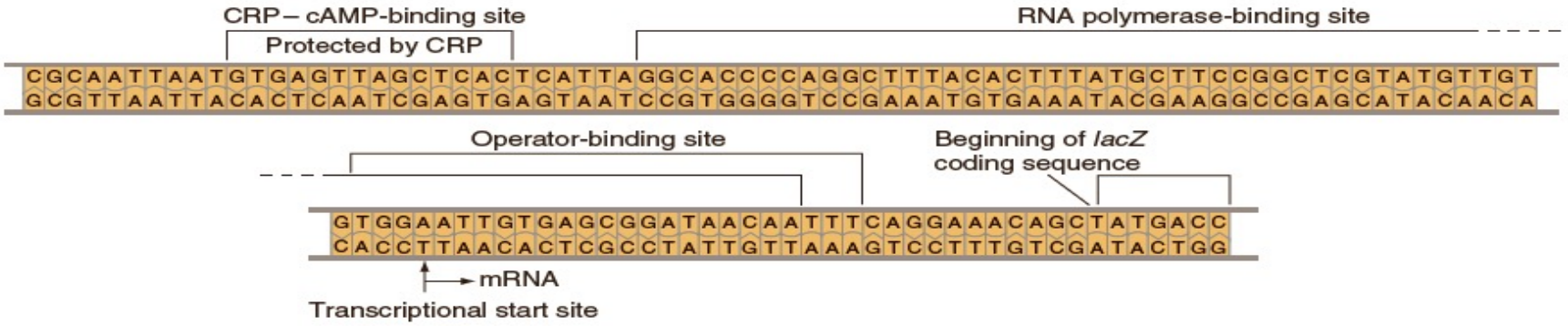


Positive regulation by CRP–cAMP

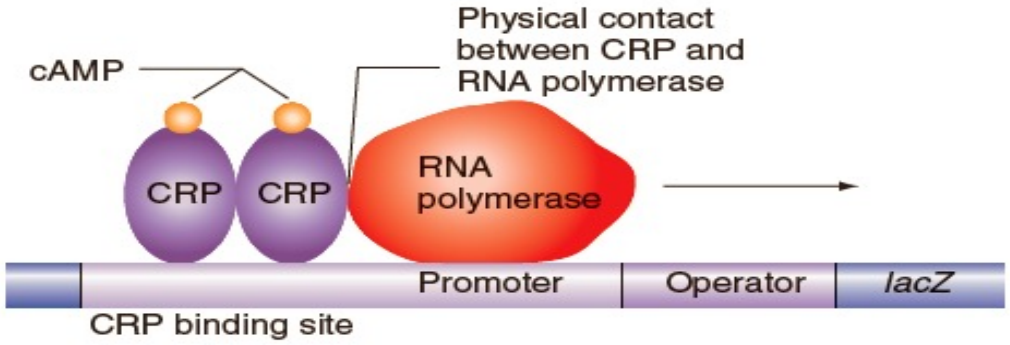
- Inside bacterial cells, the small nucleotide known as cAMP (cyclic adenosine monophosphate) binds to a protein called cAMP receptor protein, or CRP. The binding of cAMP to CRP enables CRP to bind to DNA in the regulatory region of the *lac operon, and this DNA binding of CRP increases* the ability of RNA polymerase to transcribe the *lac genes*. **Thus, CRP functions as a positive regulator** that enhances the transcriptional activity of RNA polymerase at the *lac promoter, while cAMP is an effector* whose binding to CRP enables CRP to bind to DNA near the promoter and carry out its regulatory function.
- Glucose indirectly controls the amount of cAMP in the cell by decreasing the activity of adenyl cyclase, the enzyme that converts ATP into cAMP. Thus, when glucose is present, the level of cAMP remains low; when glucose is absent, cAMP synthesis increases. As a result, when glucose is present in the culture medium, there is little cAMP available to bind to CRP and therefore little induction of the *lac operon,* even if lactose is present in the culture medium.
- The overall effect of glucose in preventing *lac gene transcription is known* as **catabolite repression, because the presence of a preferred** catabolite (glucose) represses transcription of the operon. In addition to functioning as a positive regulator of the *lac operon, the CRP–cAMP complex increases transcription* in several other catabolic gene systems, including the *gal* operon (whose protein products help break down the sugar galactose).
- As you would expect, these other catabolic operons are also sensitive to the presence of glucose, exhibiting a low level of expression when glucose is present and cAMP is in short supply. Mutations in the gene encoding CRP that alter the DNA-binding domain of the protein reduce transcription of the *lac and* other catabolic operons.
- The binding of the CRP–cAMP complex is an example of a global regulatory strategy in response to limited glucose in the environment.

Interaction of regulatory proteins with RNA polymerase

Many negative regulators (e.g. lac repressor) prevent transcription initiation by blocking the functional binding of RNA polymerase



Many positive regulators (e.g. CRP-cAMP) establish contact with RNA polymerase that enhances transcription initiation. Without interaction with CRP-cAMP, RNA polymerase can bind to the promoter but is less likely to unwind DNA and initiate transcription



Positive regulation of the *araBAD* operon by AraC

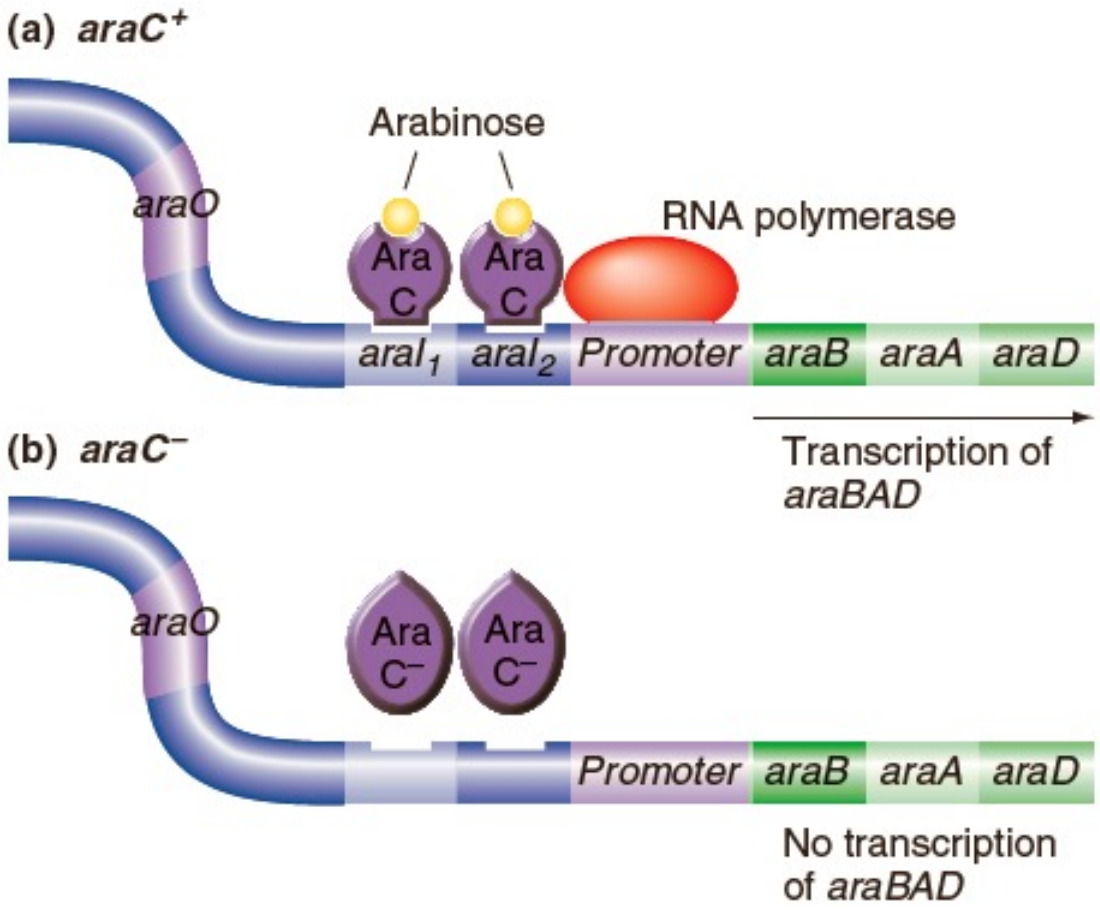
Three structural genes required in the breakdown of the sugar arabinose
- *araB*, *araA*, and *araD*

- Arabinose genes are in an operon and are induced when arabinose is present

AraC is a positive regulator of the *araBAD* operon

- Loss of function of AraC results in no expression of the *araBAD* operon when arabinose was present

AraC is a positive regulator

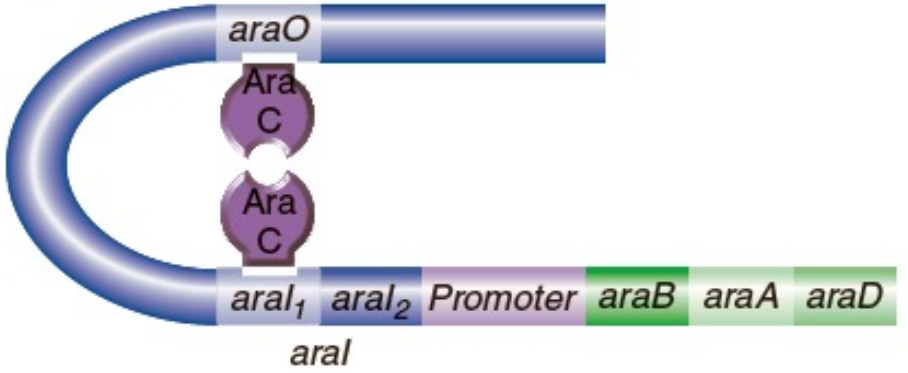


AraC acts as both a repressor and an activator

AraC can bind to three sites (araO, $araI_1$, and $araI_2$) with different affinities

(a) No arabinose present:

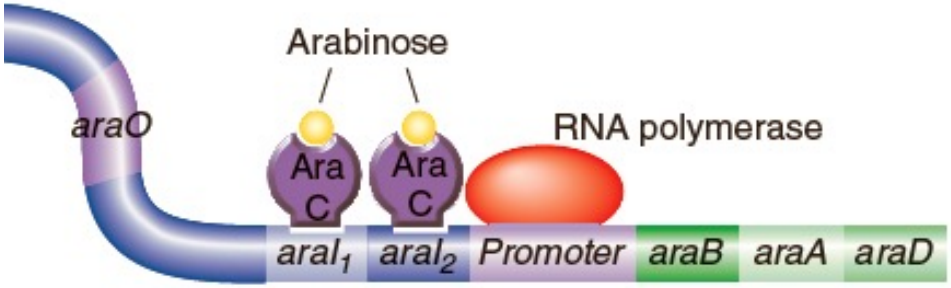
When AraC is bound to araO and to $araI_1$, looping of DNA occurs and prevents transcription



(b) Arabinose present:

Arabinose causes allosteric change in AraC so that it cannot bind to araO

AraC interacts with RNA polymerase only when both $araI_1$ and $araI_2$ are occupied



Regulation of the tryptophan (*trp*) operon in *E. coli*

Structural genes for tryptophan (Trp) biosynthesis are expressed only in the absence of Trp

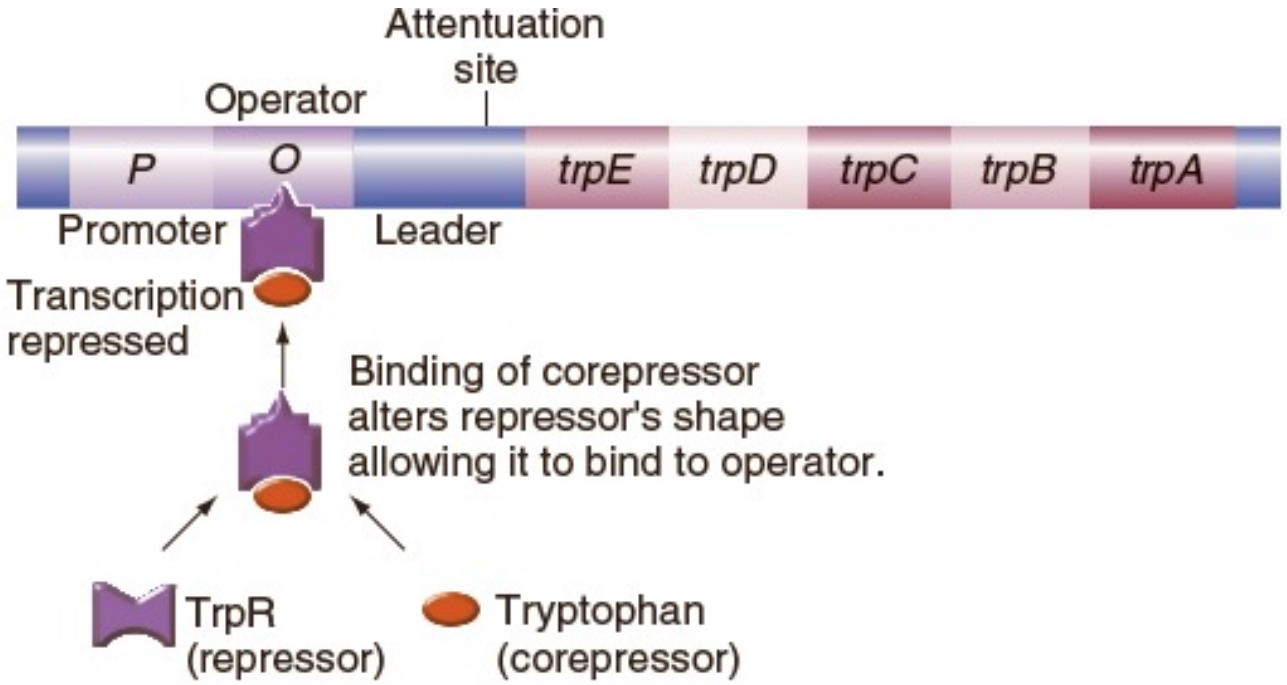
Two mechanisms for trp operon regulation

- *TrpR* gene encodes the trp repressor that can bind to the Trp operator (*TrpO*)
  - When Trp is present, TrpR repressor binds to *TrpO*
  - When Trp is absent, TrpR repressor cannot bind to *TrpO*
- Attenuation controls termination of transcription in the trp leader (*TrpL*)
  - When Trp is present, transcription terminates in *TrpL*
  - When Trp is absent, transcription doesn't terminate in *TrpL*

Tryptophan acts as a corepressor

Binding of tryptophan to TrpR repressor allows TrpR to bind to *TrpO* and inhibit transcription of the five structural genes

In the absence of tryptophan, TrpR repressor cannot bind to *TrpO*



Evidence that TrpR repressor is not the only regulator of the *trp* operon

Constitutive expression of Trp biosynthesis doesn't occur in *TrpR*$^-$ mutants

If TrpR were the sole regulator, maximal expression of *trp* genes would occur in the absence or presence of tryptophan

Second regulatory mechanism is attenuation – control of gene expression by premature termination of transcription

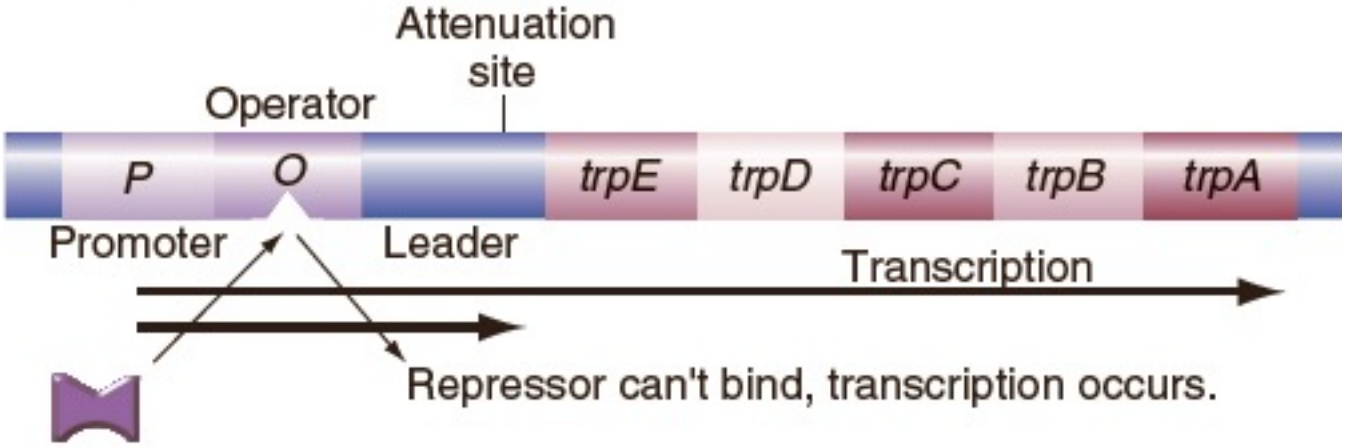
	With Tryptophan* (%)	Without Tryptophan (%)
TrpR$^+$	8	100
TrpR$^-$	33	100

*In the growth medium

Transcription from the *trp* promoter produces two alternative mRNAs

Attenuation controls termination of transcription in the *trp* leader (*TrpL*)

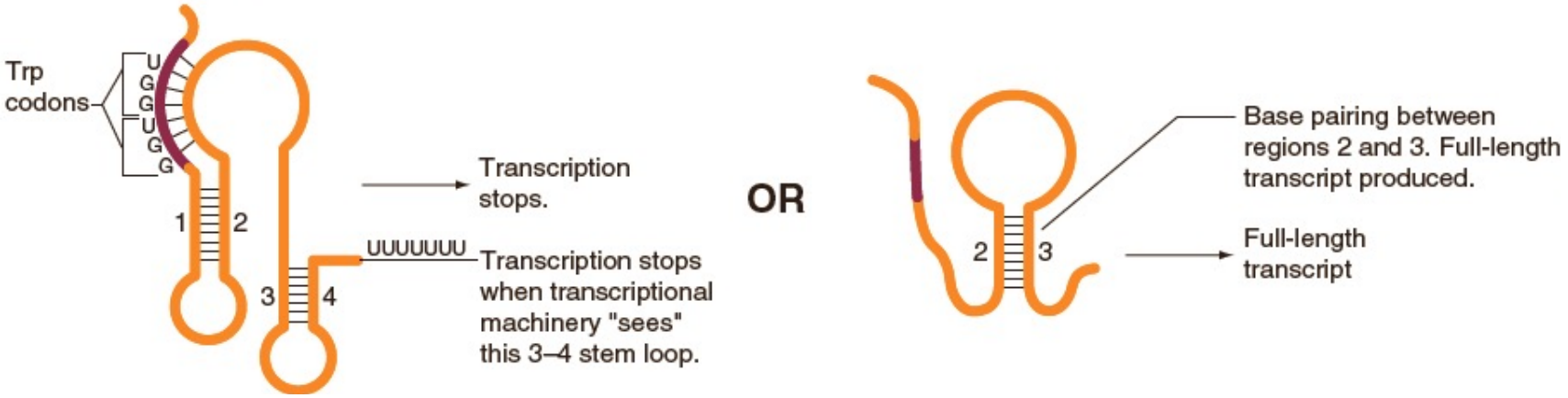
- Truncated mRNA - terminates in *TrpL*, only 140 bases
- Full-length mRNA - continues through *TrpL* and encodes all five structural genes



Alternate stem-loop structures in *trpL* RNA

Different regions of *trpL* have complementary base-pairing

- Formation of the 1-2 stem-loop allows formation of the 3-4 stem-loop
- Formation of the 2-3 stem-loop prevents formation of the 3-4 stem-loop
- The 3-4 stem loop is a transcription terminator

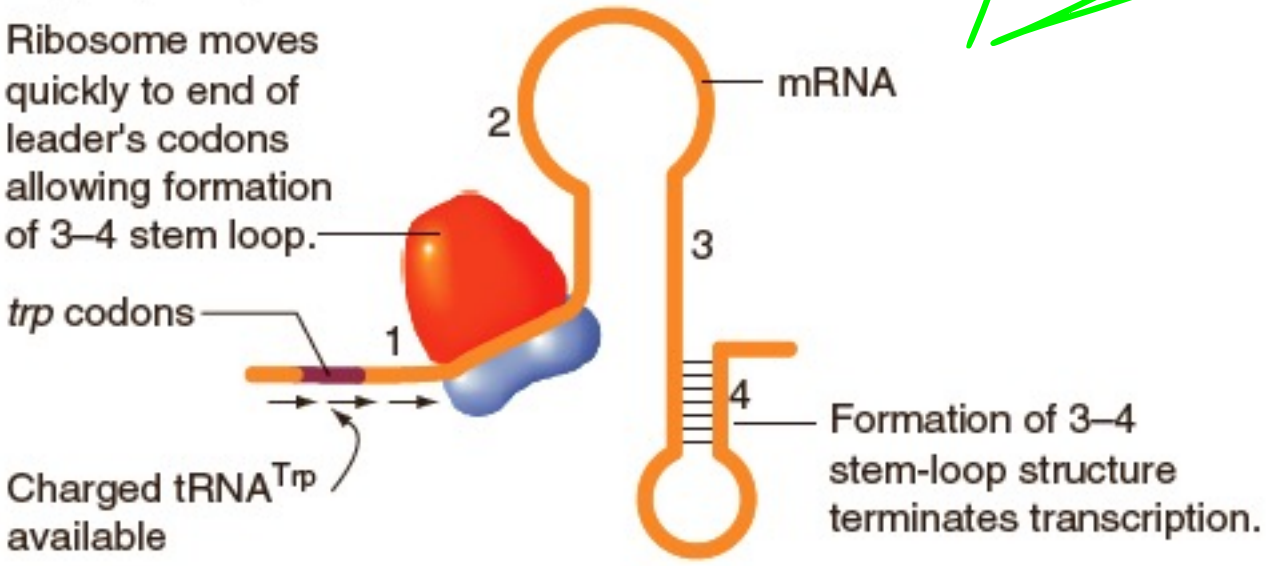


When tryptophan is present, transcription terminates in *trpL* because of translation

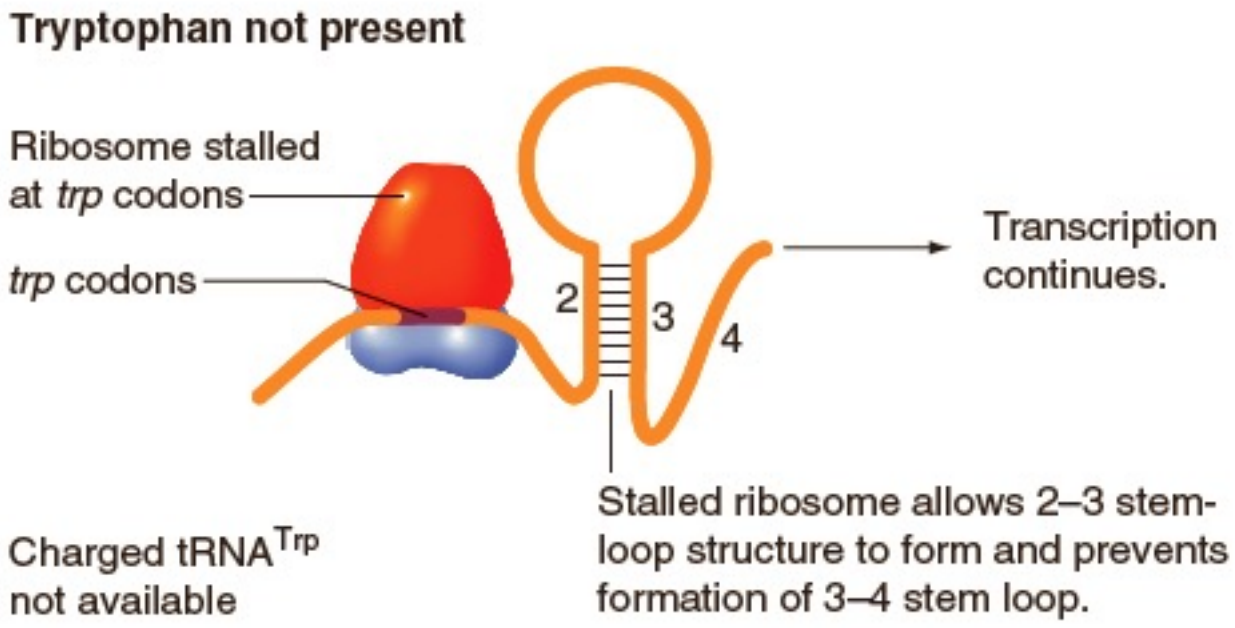
The *trpL* mRNA is translated and includes two *trp* codons

Movement of ribosomes through *trpL* mRNA depends on the availability of $tRNA^{Trp}$

- When Trp is present, $tRNA^{Trp}$ is available and rapid ribosome movement allows the formation of 3-4 stem-loop

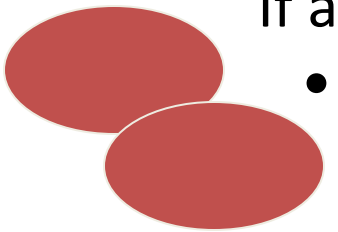


When tryptophan isn't present, transcription doesn't terminate in *trpL*



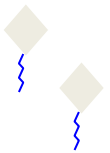
arg operon

Regulation of the *arg* operon for arginine biosynthesis

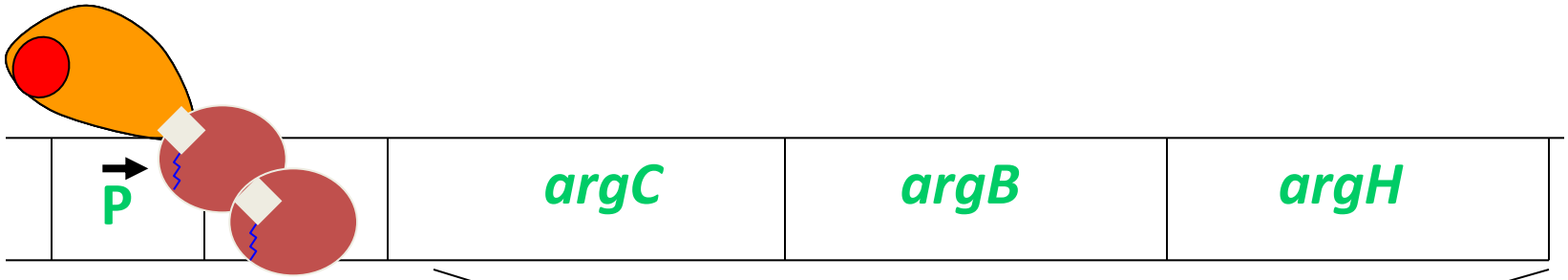


If arginine is present in large amounts

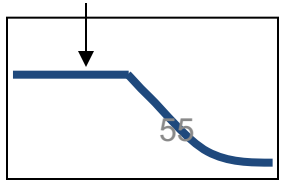
- arg biosynthetic enzymes NOT needed



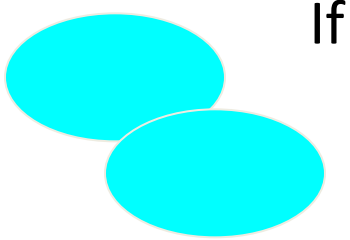
- arg binds repressor
- arg-repressor binds DNA
- RNA polymerase can't bind to promoter



∴ transcription rate decreases

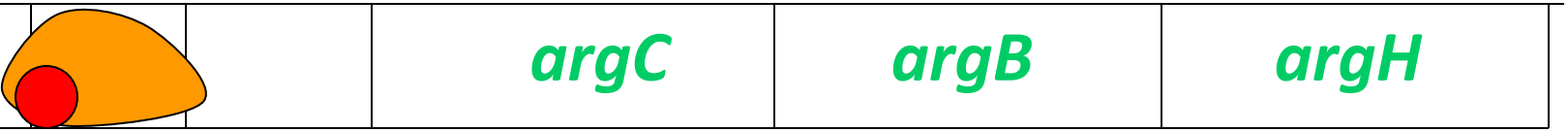


arg operon

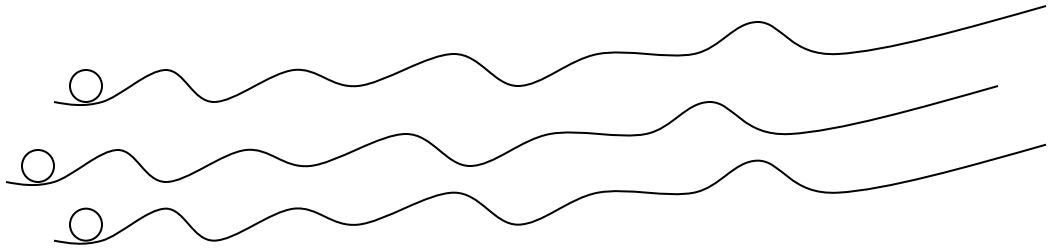


If arg is absent, the cell needs to make arg

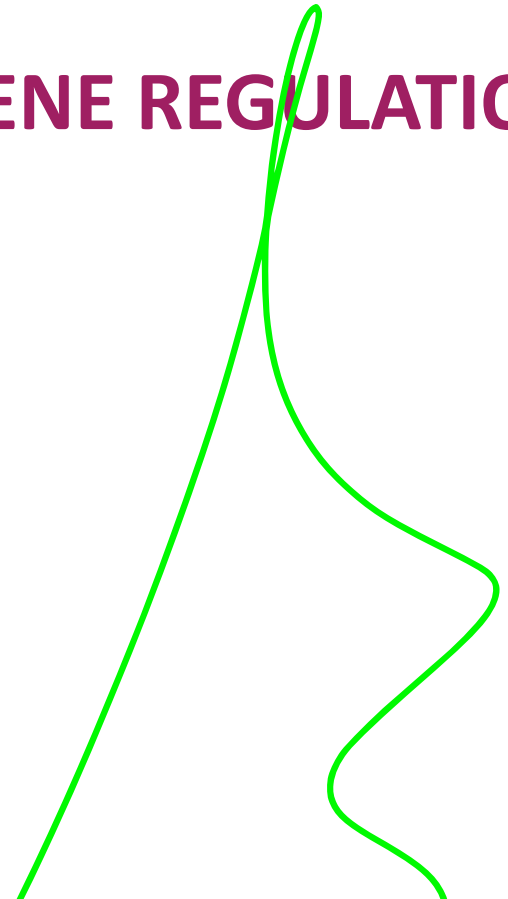
- repressor doesn't bind DNA
- RNA polymerase can bind
- transcription of *arg* genes occurs



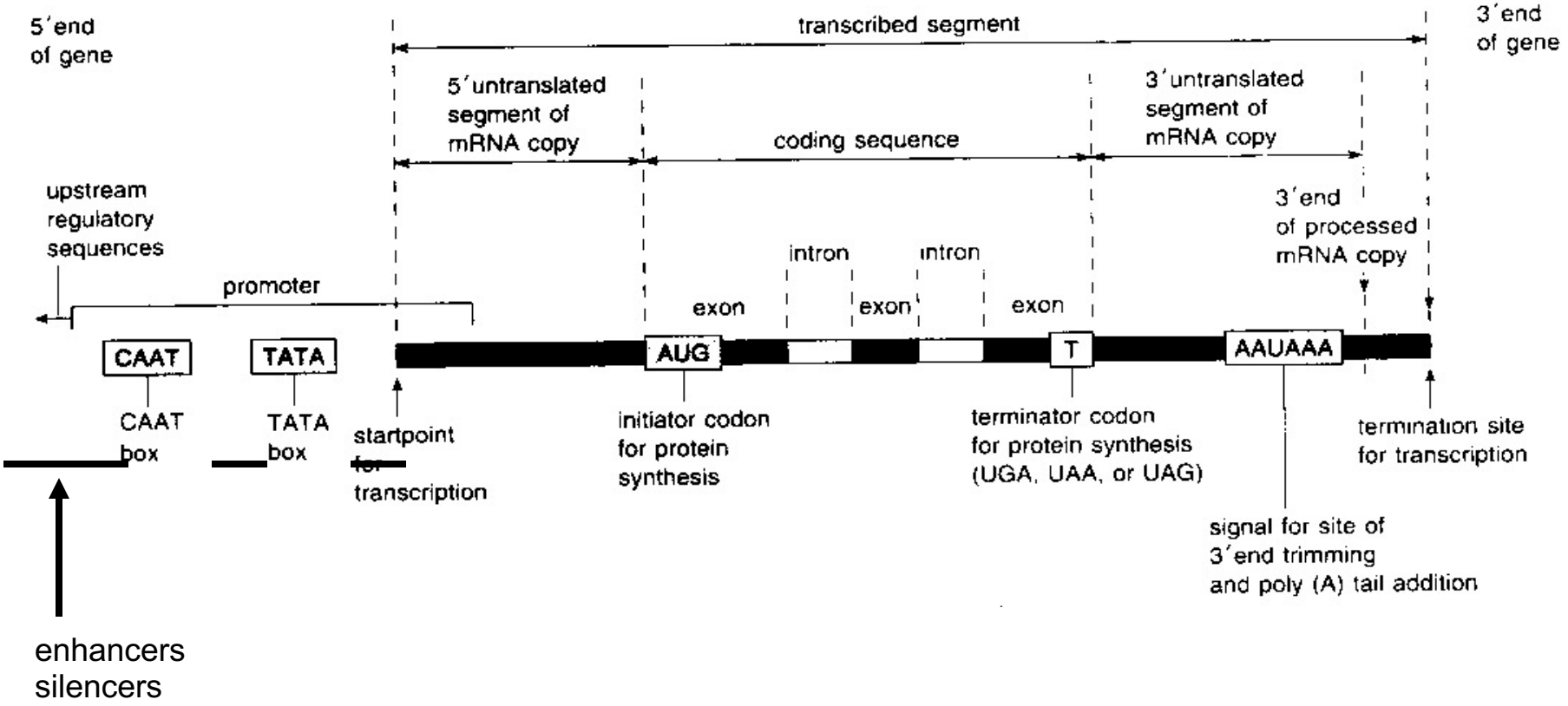
operator *arg* biosynthetic genes



EUKARYOTIC GENE REGULATION



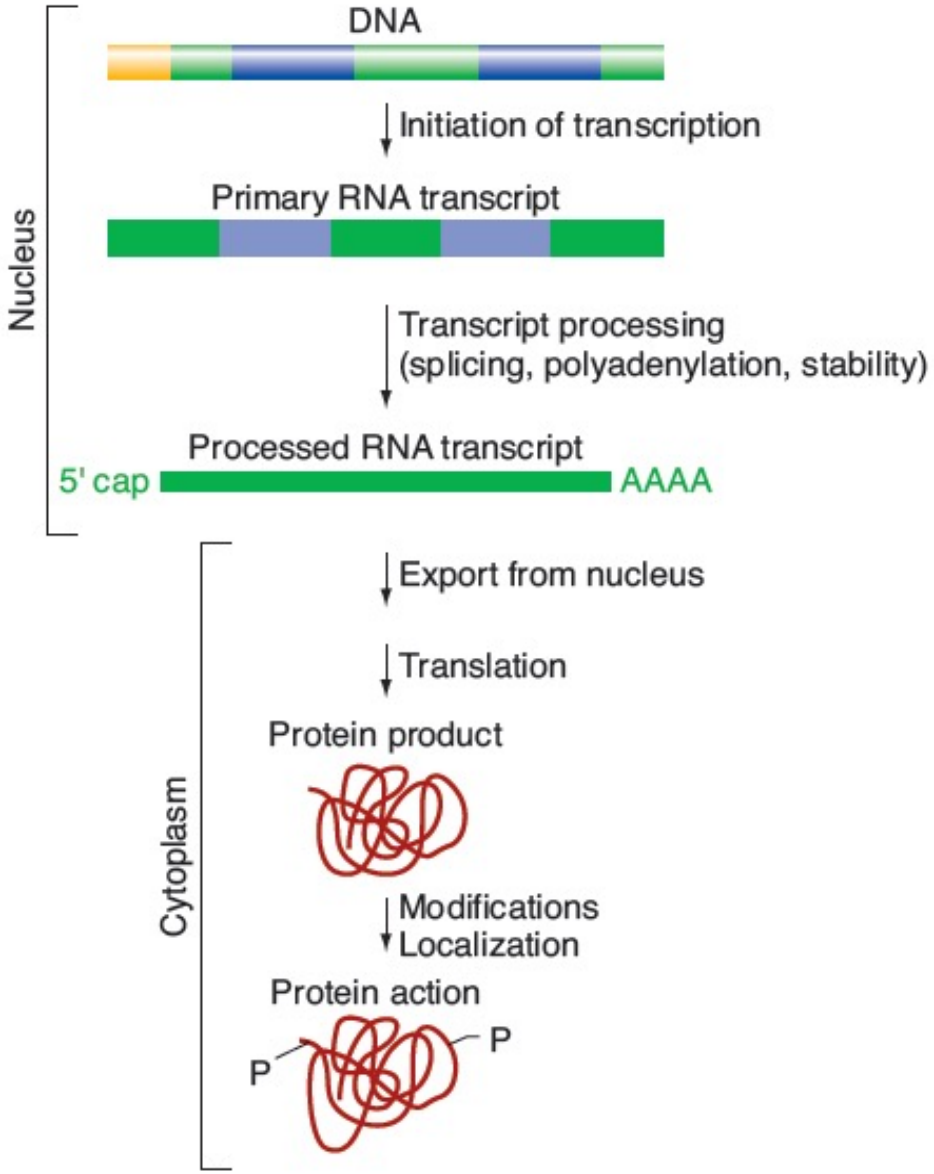
Eukaryotic gene organization



Key regulatory differences between eukaryotes and prokaryotes

Characteristic	Prokaryote	Eukaryote
Control of transcription through specific DNA-binding proteins	Yes	Yes
Re-utilization of same DNA-binding motifs by different DNA-binding proteins	Yes	Yes
Activator proteins	Yes	Yes
Repressor proteins	Yes	Yes
Specificity of binding to DNA by regulatory protein	Specific	Highly specific
Affinity of binding	Strong	Very strong
Role played by chromatin structure	No	Yes
Coordinate control achieved with operons	Yes	Rare
Differential splicing	No	Yes
Attenuation	Yes	No
mRNA processing	No	Yes
Differential polyadenylation	No	Yes
Differential transport of RNA from nucleus to cytoplasm	No	Yes

Multiple steps where production of the final gene product can be regulated in eukaryotes



Roles of the Three RNA Polymerases

Three types of RNA polymerases in eukaryotes

- RNA pol I – transcribes rRNA genes (found in nucleolus)
- RNA pol II – transcribes all protein-coding genes (mRNAs) and micro-RNAs (found in nucleoplasm)
- RNA pol III – transcribes tRNA genes and some small regulatory RNAs (found in nucleoplasm)

Promoters

- Three eukaryotic RNA polymerases have:
  - Different structures
  - Transcribe different classes of genes
- The three different 3 polymerases recognize different promoters

Class II Promoters

- Promoters recognized by RNA polymerase II (class II promoters) are similar to prokaryotic promoters
- Considered to have two parts:
  - Core promoter having 4 elements
  - Upstream promoter element

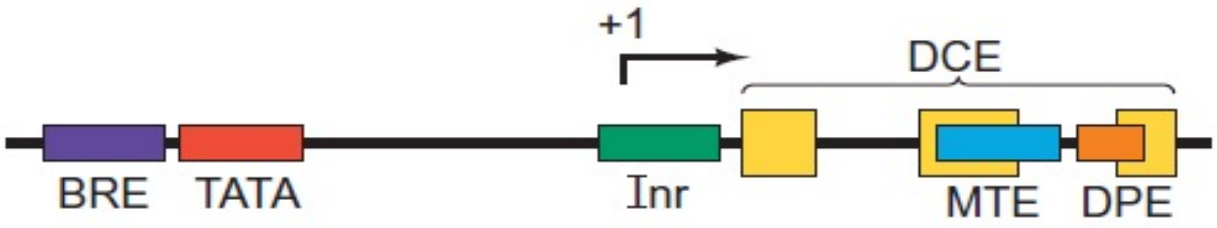


Figure 10.18 A generic class II core promoter. This core promoter contains up to six elements. These are, 5′ to 3′: the TFIIB-recognition element (BRE, purple); the TATA box (red); the initiator (green); the downstream core element, in three parts (DCE, yellow); the motif ten element (MTE, blue); and the downstream promoter element (DPE, orange). The exact locations of these promoter elements are given in the text.

Core Promoter Elements – TATA Box

- TATA box
  - Found on the nontemplate strand
  - Very similar to the prokaryotic -10 box
  - There are frequently TATA-less promoters
    - Housekeeping genes that are constitutively active in nearly all cells as they control common biochemical pathways
    - Developmentally regulated genes

Core Promoter Elements

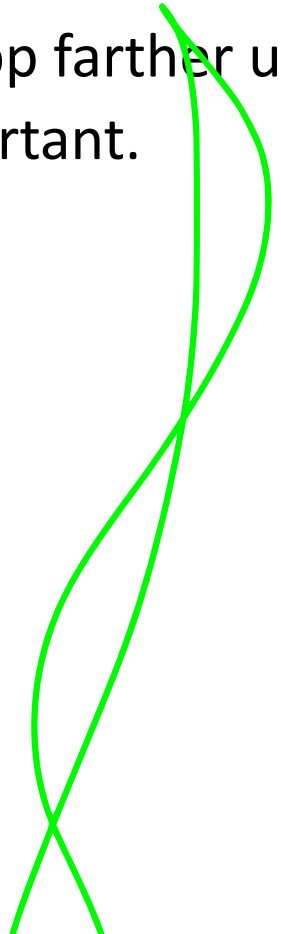
- In addition to TATA box, core promoters are:
  - TFIIB recognition element (BRE)
  - Initiator (Inr)
  - Downstream promoter element (DPE)
- At least one of the four core elements is missing in most promoters
- TATA-less promoters tend to have DPEs
- Promoters for highly specialized genes tend to have TATA boxes
- Promoters for housekeeping genes tend to lack them

Upstream Elements

- Upstream promoter elements are usually found upstream of class II core promoters
- Differ from core promoters in binding to relatively gene-specific transcription factors
  - GC boxes bind transcription factor Sp1
  - CCAAT boxes bind CTF (CCAAT-binding transcription factor)
- Upstream promoter elements can be orientation-independent, yet are relatively position-dependent

Class I Promoters

- Class I promoters are not well conserved in sequence across species
- General architecture of the promoter is well conserved – two elements:
  - Core element surrounding transcription start site
  - Upstream promoter element (UPE) 100 bp farther upstream
  - Spacing between these elements is important.



Class III Promoters

- RNA polymerase III transcribes a set of short genes
- These have promoters that lie wholly within the genes
- There are 3 types of these promoters

- Type I (5S rRNA) has 3 regions:
  - Box A
  - Short intermediate element
  - Box C
- Type II (tRNA) has 2 regions:
  - Box A
  - Box B
- Type III (nonclassical) resemble those of type II

Class III Promoters

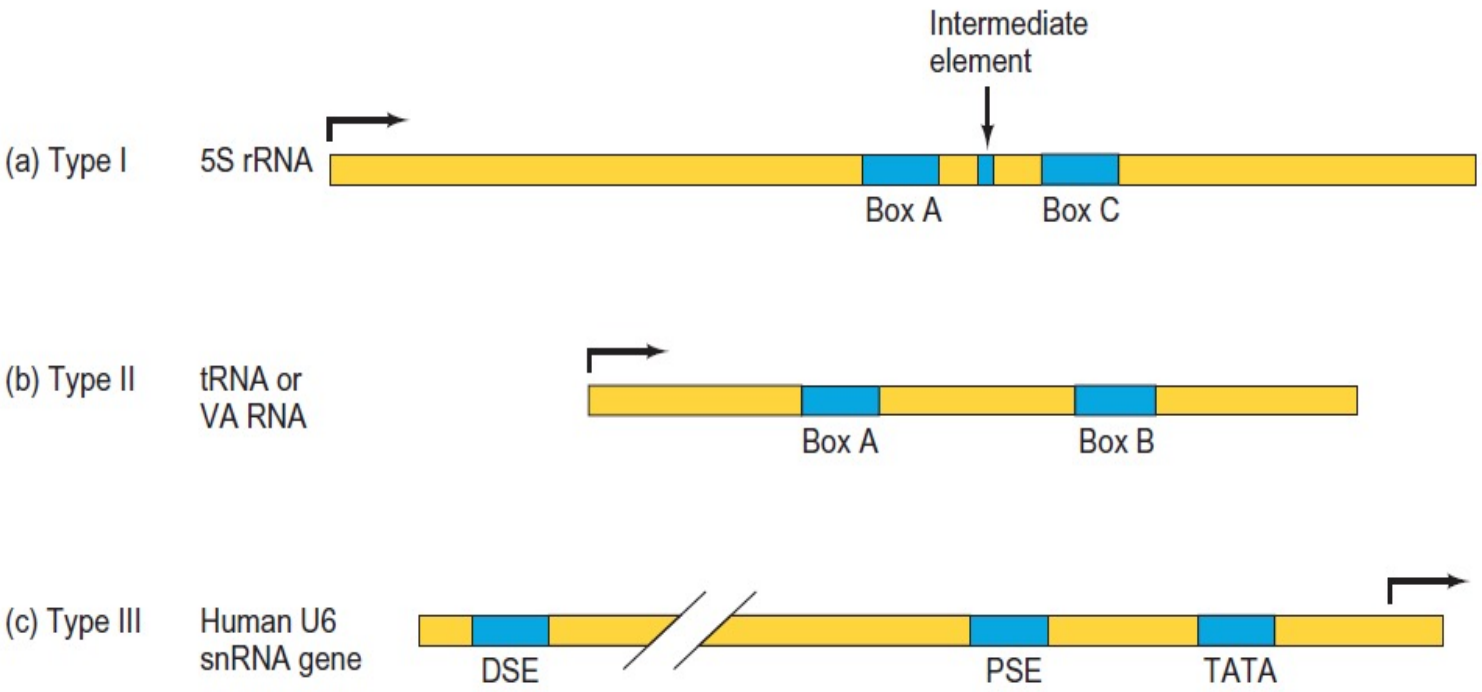


Figure 10.23 Promoters of some class III genes. The promoters of the 5S, tRNA and U6 RNA genes are depicted as groups of blue boxes within the genes they control. DSE and PSE are distal and proximal sequence elements, respectively.

cis-acting elements: promoters and enhancers

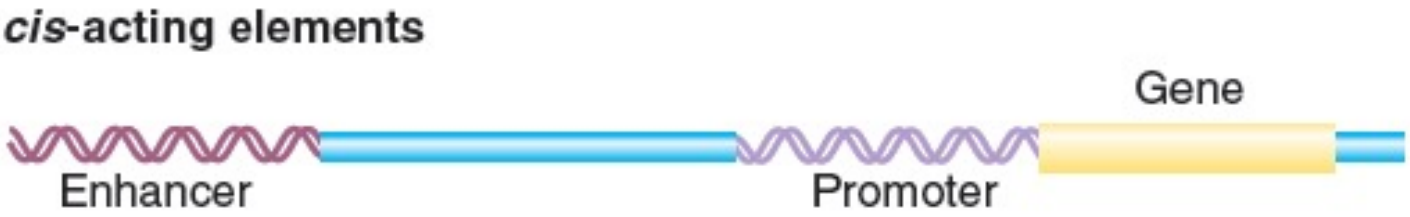
Promoters – usually directly adjacent to the gene

- Include transcription initiation site
- Often have TATA box: **TATA** $\frac{\mathbf{A}}{\mathbf{T}}$ **A** $\frac{\mathbf{A}}{\mathbf{T}}$

- Allow basal level of transcription

Enhancers – can be far away from gene

- Augment or repress the basal level of transcription



cis-acting regulatory elements are regions of DNA sequence that lie nearby on the same DNA molecule as the gene they control and act to increase or repress the basal level of transcription.

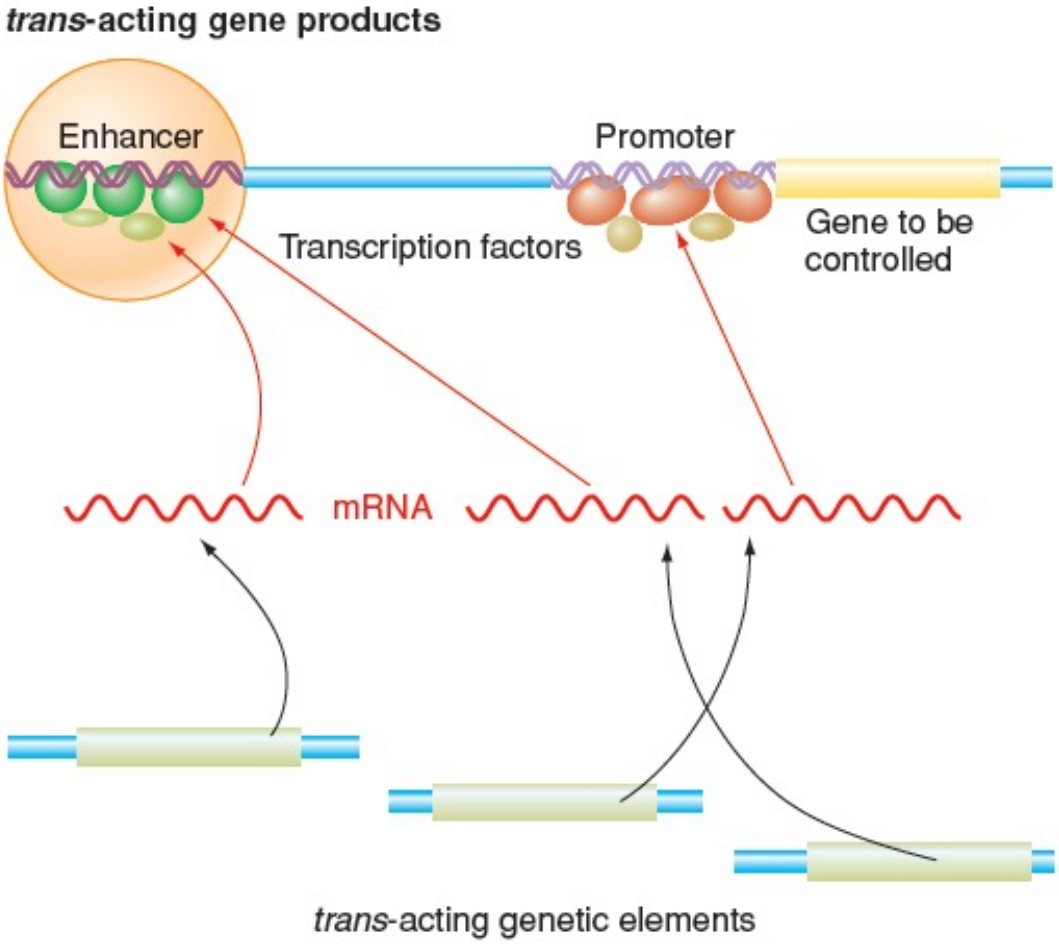
trans-acting factors interact with *cis*-acting elements to control transcription initiation

Direct effects of TFs :

- Through binding to DNA

Indirect effect of TFs:

- Through protein-protein interactions



trans-acting genetic elements encode products called transcription factors (TFs) that interact with *cis-acting elements, either directly through DNA binding or indirectly* through protein-protein interactions.

Basal transcription factors

Basal transcription factors assist the binding of RNA pol II to promoters (see Fig 16.6)

Key components of basal factor complex:

- TATA box-binding protein (TBP)
  - Bind to TATA box
  - First of several proteins to assemble at promoter
- TBP-associated factors (TAFs)
  - Bind to TBP assembled at TATA box

RNA pol II associates with basal complex and initiates basal level of transcription

Basal factors bind to promoters of all protein-encoding genes

Ordered pathway of assembly at promoter:

1. TBP binds to TATA box
2. TAFs bind to TBP
3. RNA pol II binds to TAFs

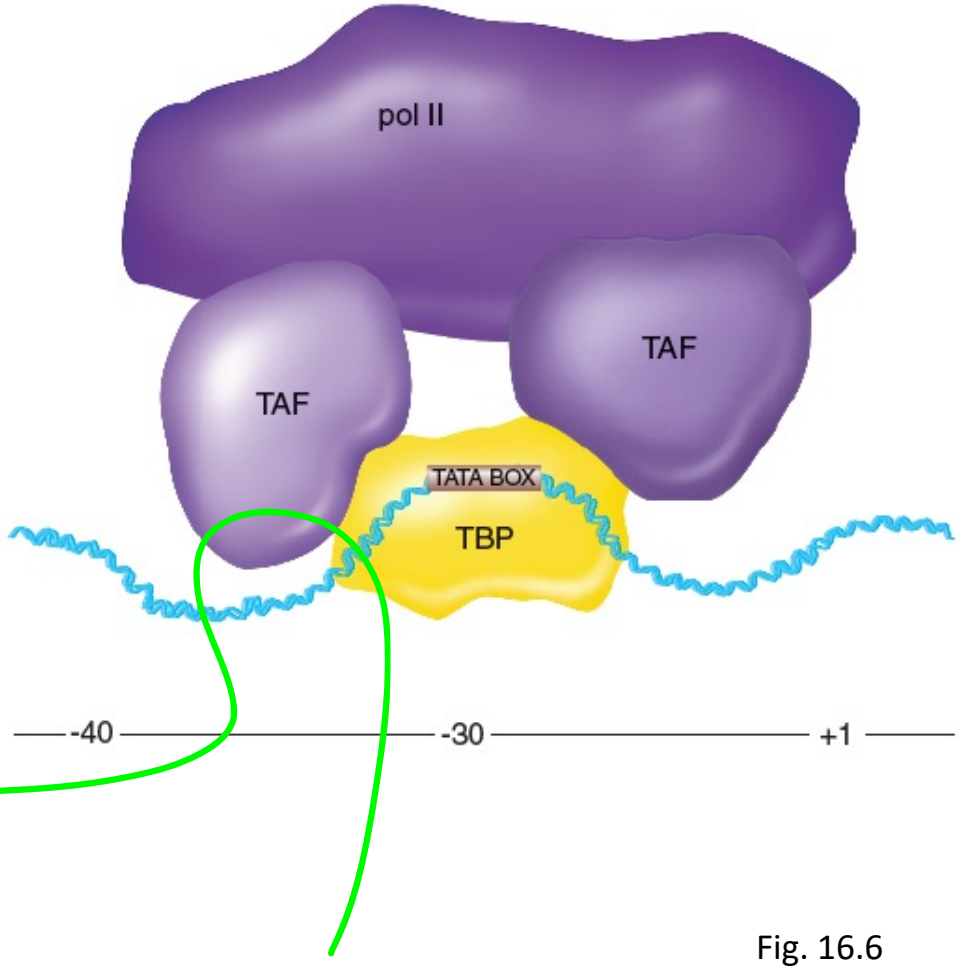


Fig. 16.6

Activators are transcription factors that bind to enhancers

Activators are responsible for much of the variation in levels of transcription of different genes.

They Increase levels of transcription by interacting directly or indirectly with basal factors at the promoter

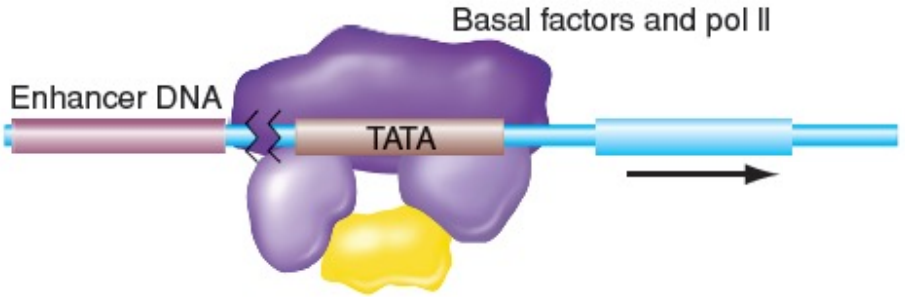
- 3-dimensional complex of proteins and DNA (Fig. 16.7)

Mechanisms of activator effects on transcription

- Stimulate recruitment of basal factors and RNA pol II to promoters
- Stimulate activity of basal factors already assembled on promoters
- Facilitate changes in chromatin structure

Binding of activators to enhancers increases transcriptional levels

Low level transcription occurs when only basal factors are bound to promoter



When basal factors and activators are bound to DNA, rate of transcription increases

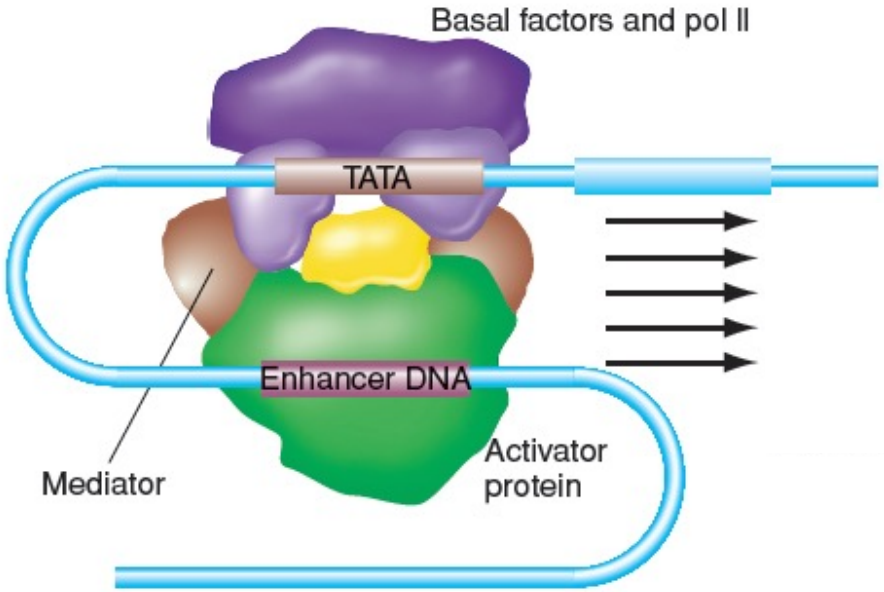


Fig. 16.7

Domains within activators

Activator proteins have **two** functional domains

- Sequence-specific DNA binding domain (Fig 16.8)
  - Binds to enhancer
- Transcription-activator domain
  - Interacts with other transcriptional regulatory proteins

Some activators have a third domain (Fig 16.9)

- Responds to environmental signals
  - Example - steroid hormone receptors

DNA-binding domains of activator proteins

Interacts with major groove of DNA

Specific amino acids have high-affinity binding to specific nucleotide sequence

The three best-characterized motifs:

- Helix-loop-helix (HLH)
- Helix-turn-helix (HTH)
- Zinc finger

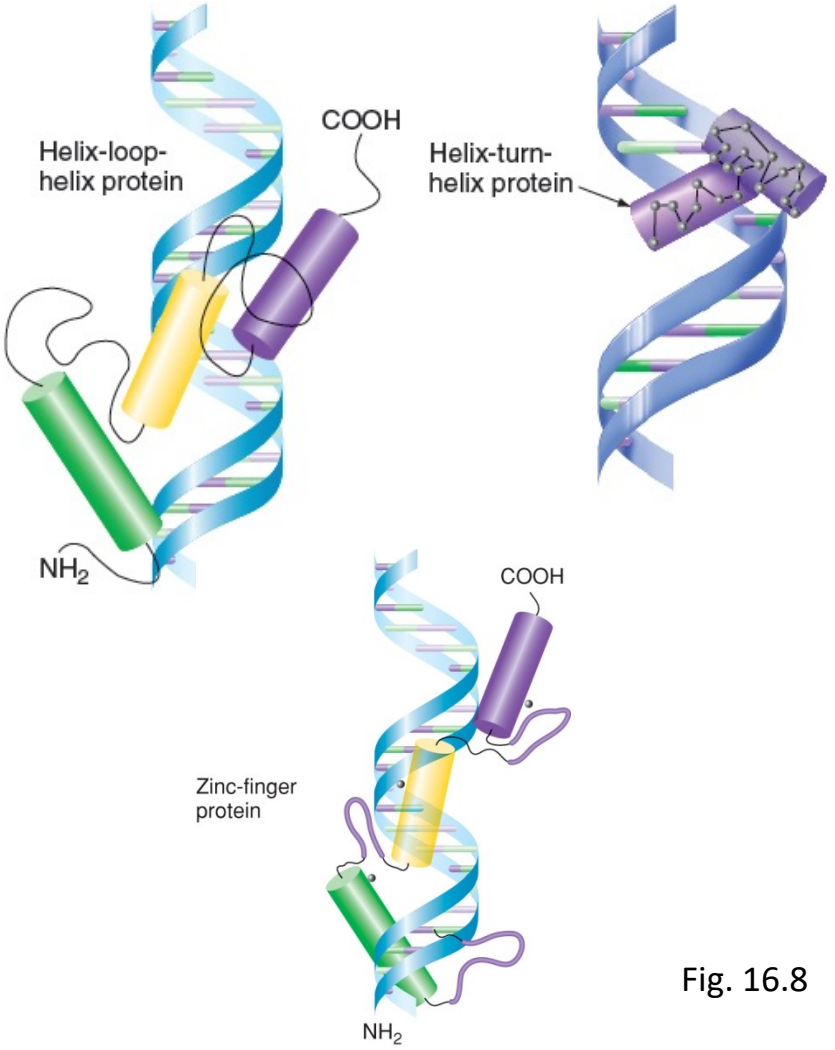
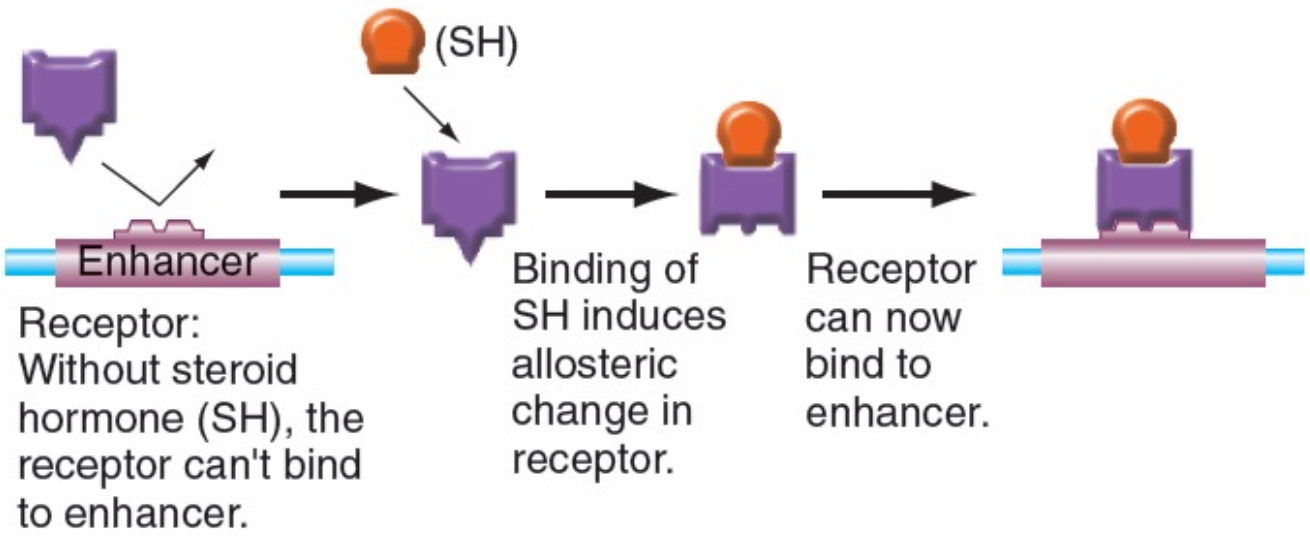


Fig. 16.8

Steroid hormone receptors are activators only in the presence of specific hormones

Steroid hormones don't bind to DNA but are coactivators of steroid hormone receptors

- In the absence of hormone, these receptors cannot bind to DNA and so cannot activate transcription
- In the presence of hormone, these receptors bind to enhancers for specific genes and activate their expression



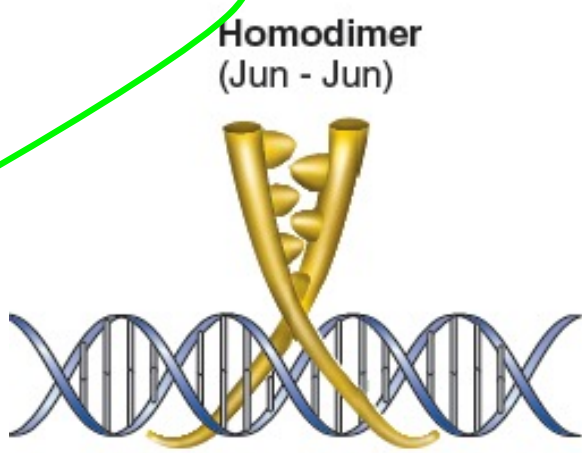
Many activators must form dimers to function

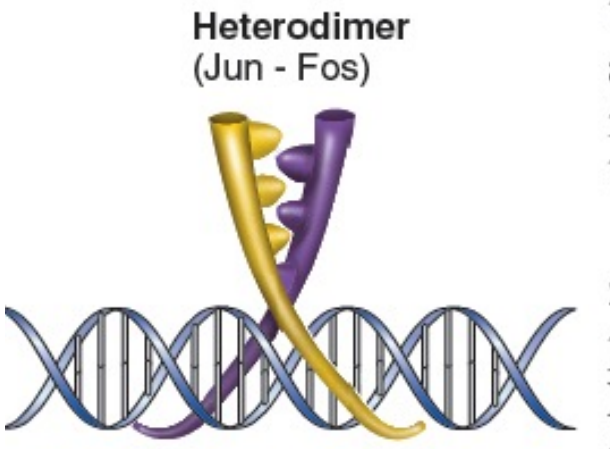
Homodimers: multimeric proteins made of identical subunits

Heterodimers: multimeric proteins made of nonidentical subunits

Examples - Fos and Jun, both have leucine zippers

- Fos forms heterodimers with Jun, but cannot form homodimers
- Jun can form homodimers
- Jun-Jun and Jun-Fos dimers bind to the same enhancer sequence, but have different affinities



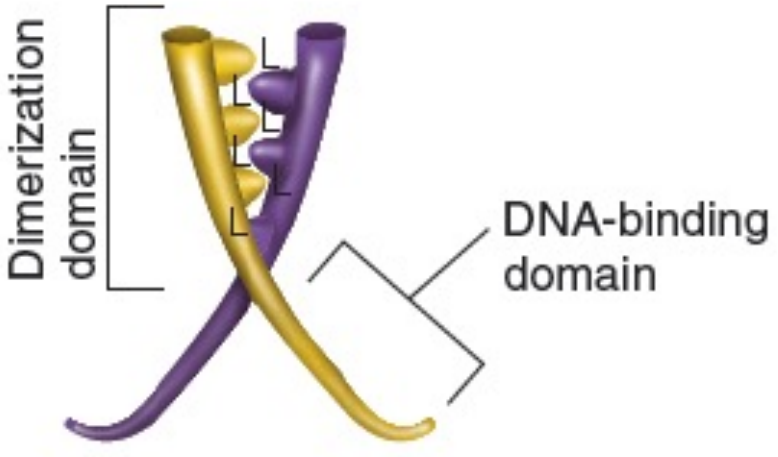


Dimerization domains make up another class of transcription factor domains

Dimerization domains are specialized for polypeptide-polypeptide interactions

Leucine zippers are a common dimerization motif in eukaryotes

Amino acid sequence twirls into an a helix with leucines protruding at regular intervals



Repressor proteins suppress transcription initiation through different mechanisms

Some repressors have no effect on basal transcription but suppress the action of activators

- Compete with activator for the same enhancer (Fig 16.12a)

  OR

- Block access of activator to an enhancer (Fig 16.12b)

Some repressors eliminate virtually all basal transcription from a promoter

- Block RNA pol II access to promoter

  OR

- Bind to sequences close to promoter or distant from promoter

Repressor proteins that act through competition with an activator protein

Repressor binds to the same enhancer sequence as the activator

- Has no effect on the basal transcription level

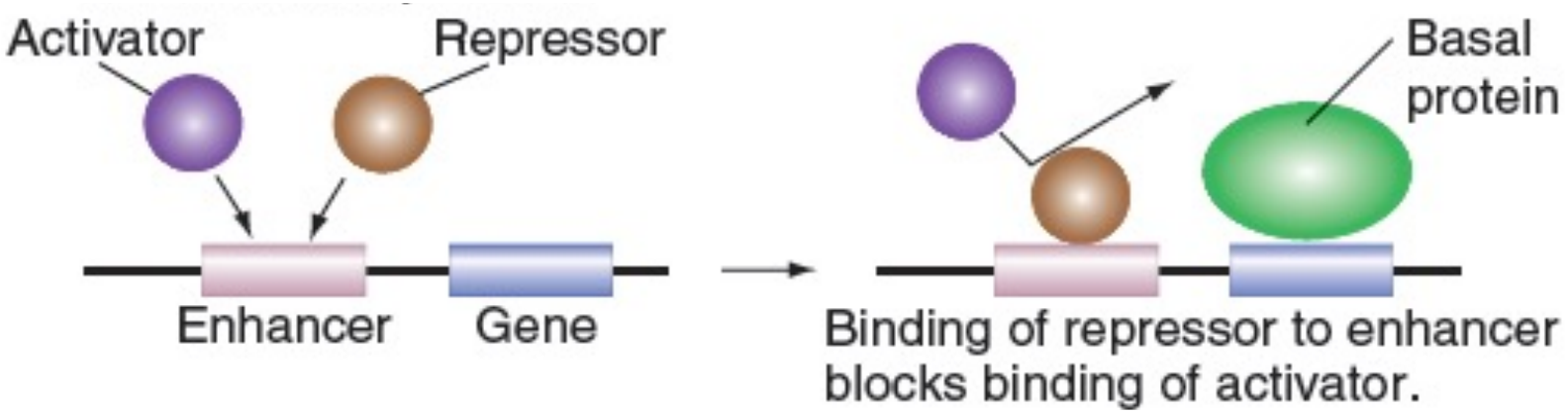
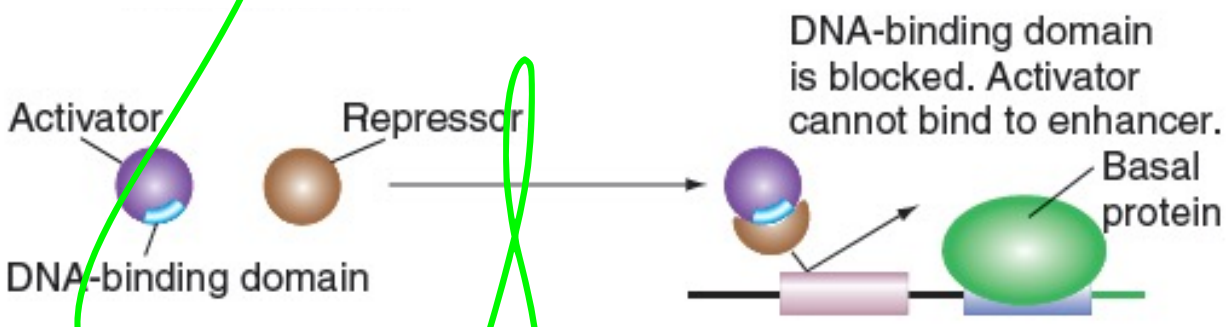


Fig. 16.12a

Repressor proteins that act through quenching an activator protein

Quenchers bind to the activator but do not bind to DNA

- Type I: Repressor blocks the DNA-binding domain



- Type II: Repressor blocks the activation domain

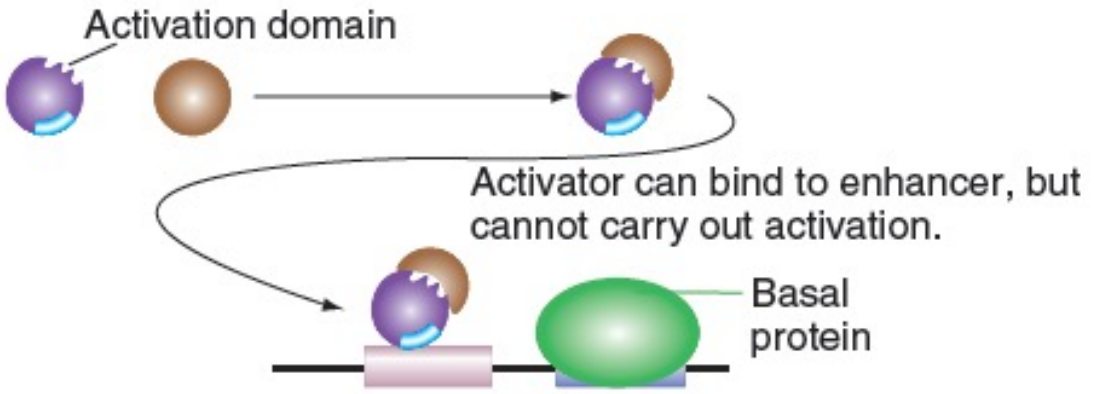


Fig. 16.12b

Complex regulatory regions enable fine-tuning of gene expression

Each gene can have many regulatory proteins

- In humans, ~2000 genes encode transcriptional regulatory proteins
- Each regulatory protein can act on many genes
- Each regulatory region can have dozens of enhancers

Enhanceosome – multimeric complex of proteins and other small molecules that associate with an enhancer

- Enhancers can be bound by activators and repressors with varying affinities
- Different sets of cofactors and corepressors compete for binding to activators and repressors

Q & A