CHAPTER 13 LECTURE SLIDES

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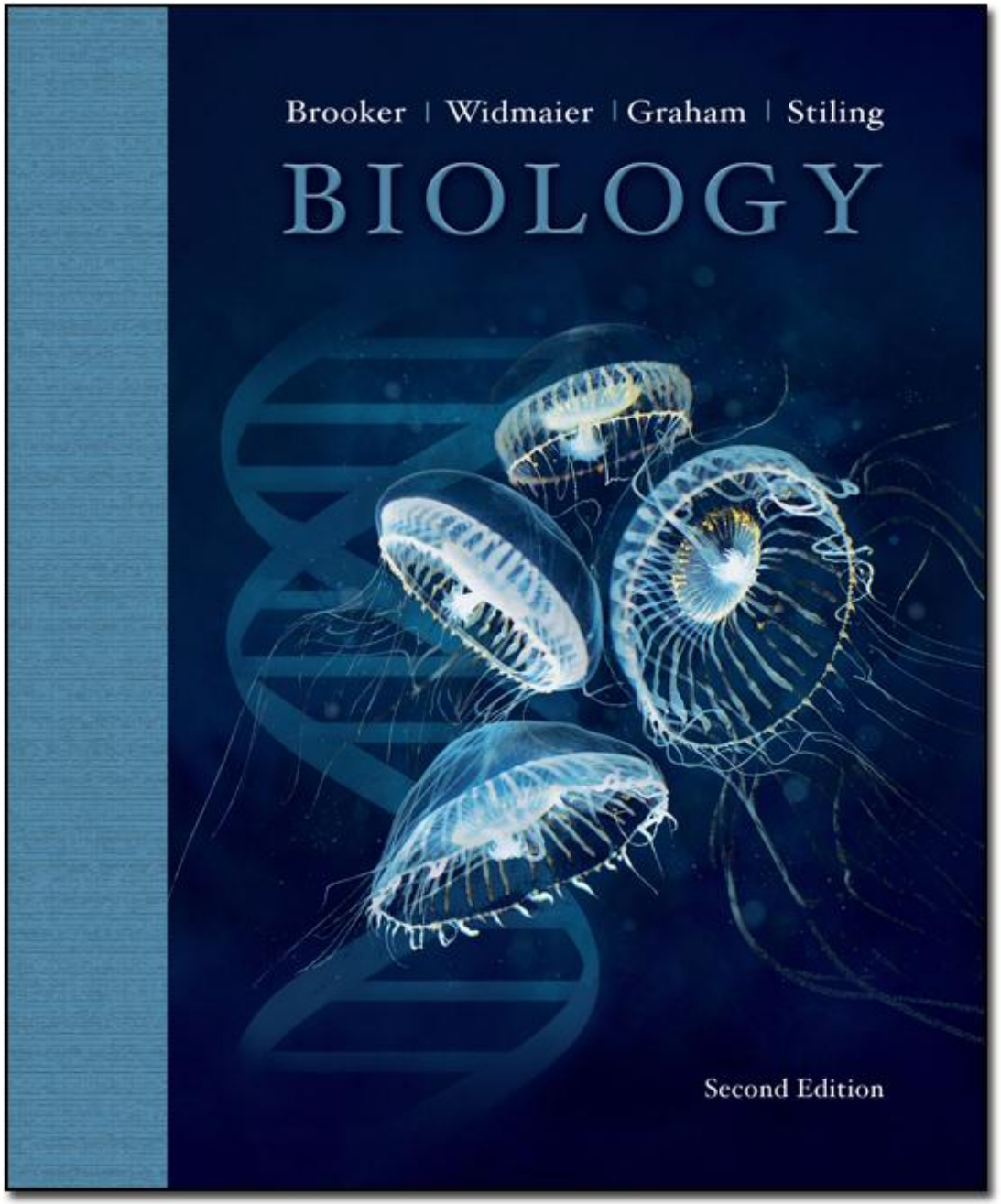


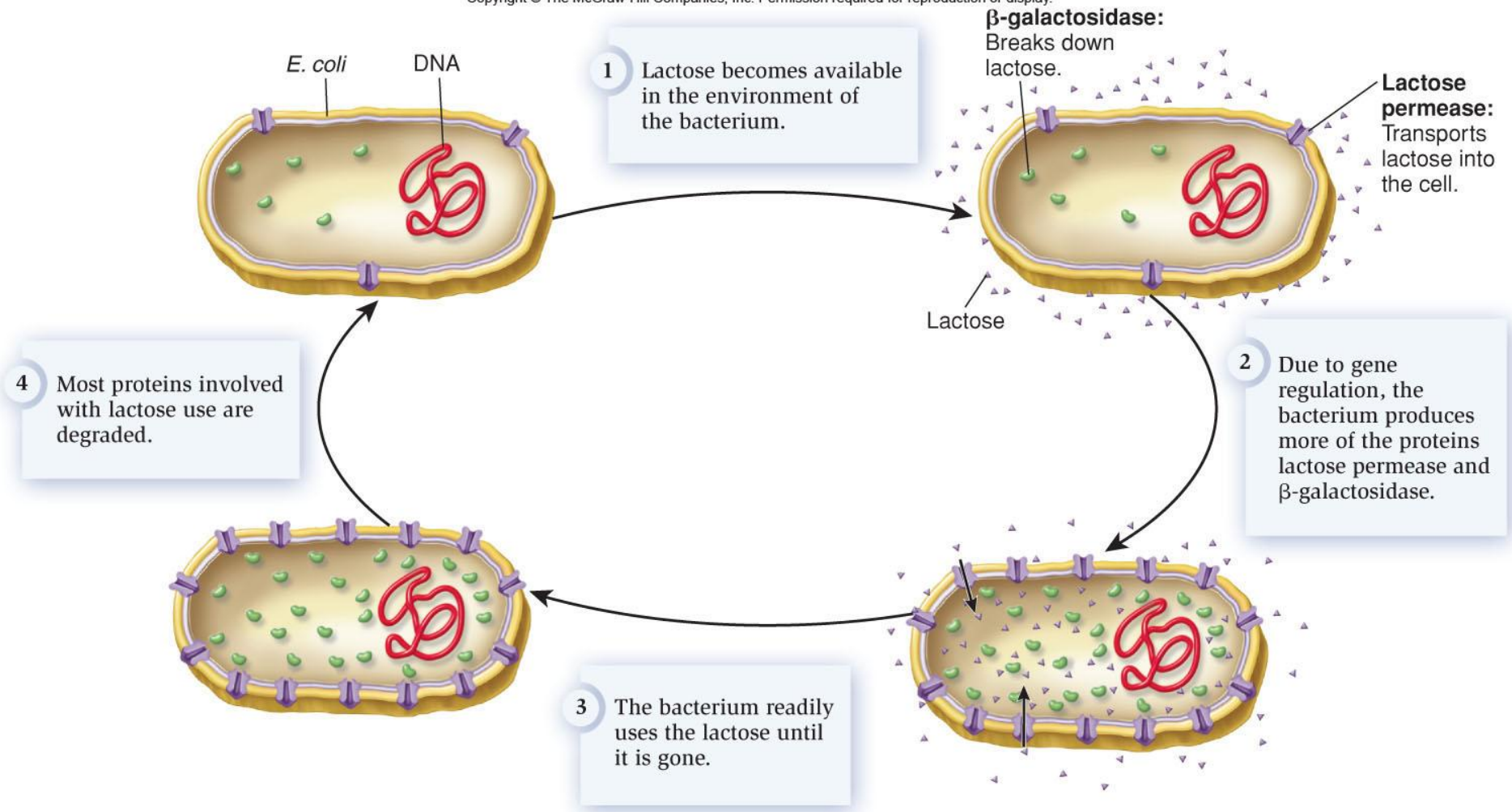
- Gene regulation refers to the ability of cells to control their level of gene expression
- Majority of genes regulated so proteins produce at certain times and in specific amounts
- Constitutive genes are unregulated and have essentially constant levels of expression

Overview

- Benefits of gene regulation
  - Conserves energy – proteins produced only when needed
  - Ensures genes expressed in appropriate cell type and at the correct stage in development

Gene regulation in prokaryotes

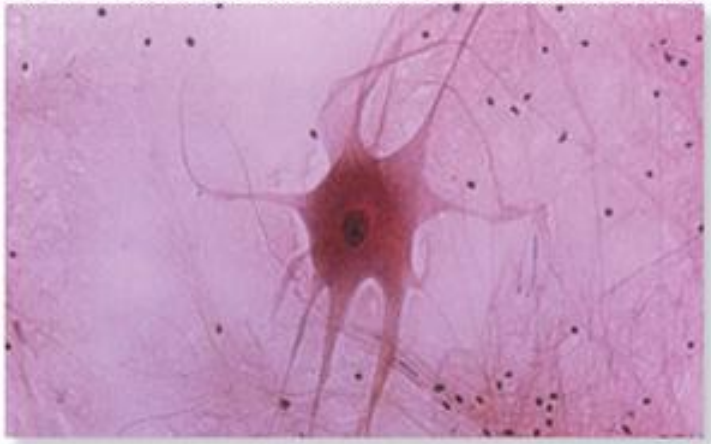
- Often used to respond to changes in the environment
- *Escherichia coli* and lactose example
- When lactose is not present, *E. coli* does not make a lactose permease (lactoset transporter) and β- galactosidase
- When lactose is available, the proteins are made
- When lactose levels drop, the proteins are no longer made



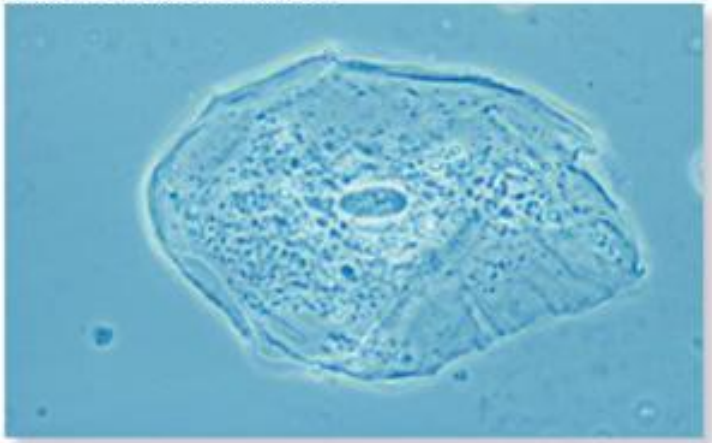
Gene regulation in eukaryotes

- Produces different cell types in an organism or cell differentiation
- All of the organism's cells contain the same genome but express different proteomes
  - Different proteins
  - Different amounts of the same protein

(a) Skeletal muscle cell



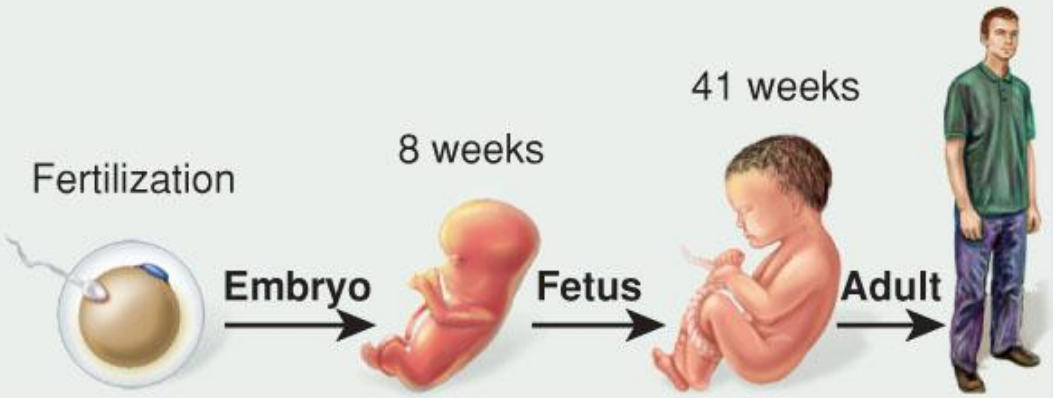
(b) Nerve cell



(c) Skin cell

Developmental gene regulation in mammals

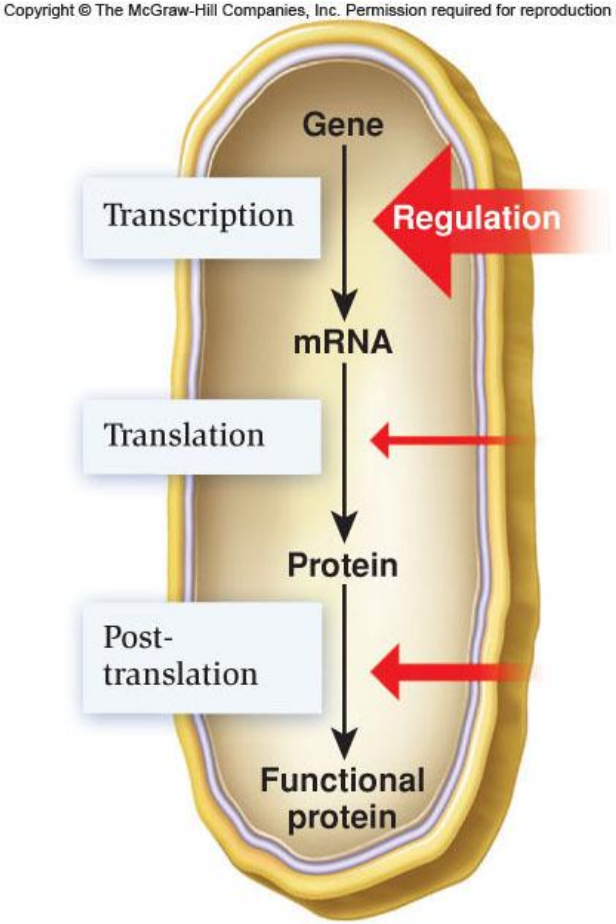
- Fetal human stage characterized by continued refinement of body parts and a large increase in size
- Gene regulation determines which globin polypetides are made to become functional hemoglobin
- Fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin
  - Allows fetus to harvest oxygen from maternal blood



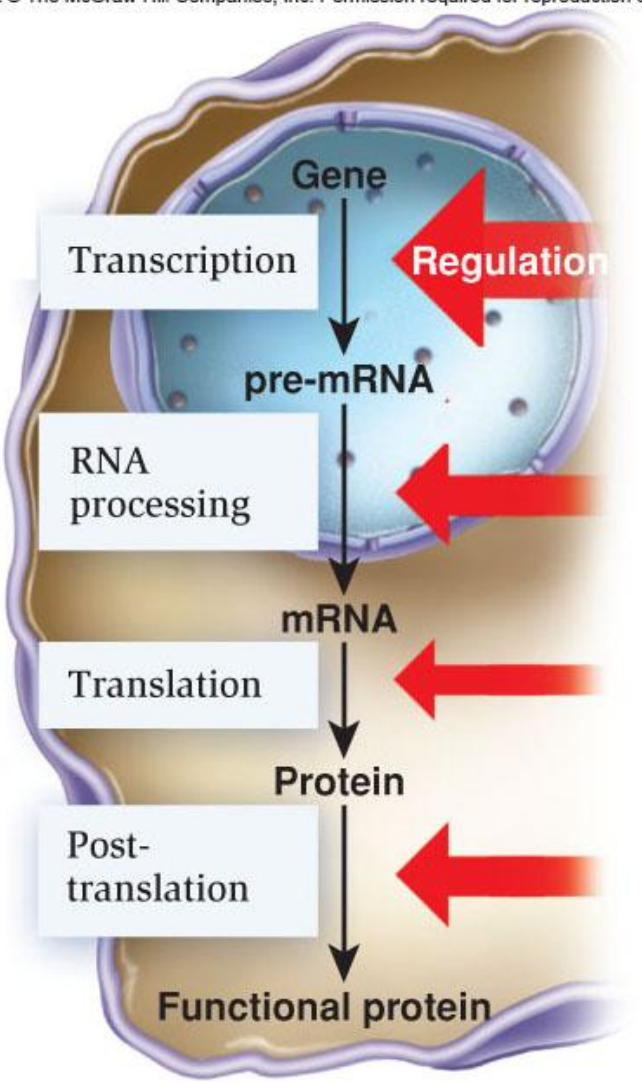
	Embryo	Fetus	Adult
Hemoglobin protein	2 ζ-globins 2 ϵ-globins	2 α-globins 2 γ-globins	2 α-globins 2 β-globins
Oxygen affinity	highest	high	moderate
Gene expression			
α-globin gene	off	on	on
β-globin gene	off	off	on
γ-globin gene	off	on	off
ζ-globin gene	on	off	off
ϵ-globin gene	on	off	off

Gene regulation can occur at different points

- Bacterial gene regulation
  - Most commonly occurs at the level of transcription
  - Or control rate mRNA translated
  - Or regulated at protein or post-translation level

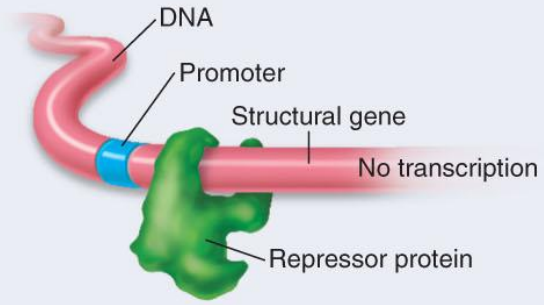


- Eukaryotic gene regulation
  - Transcriptional regulation common
  - RNA processing
  - Translation
  - Post-translation

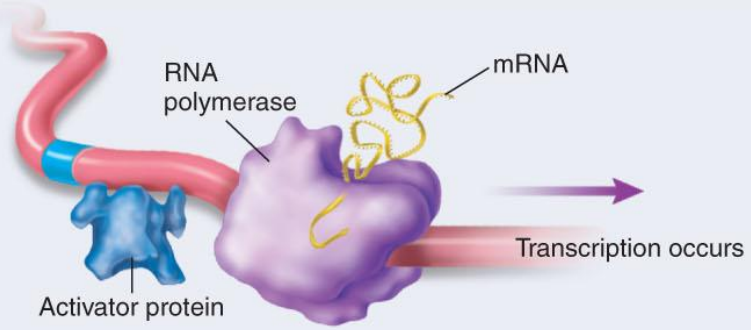


Transcriptional regulation in bacteria

- Involves regulatory transcription factors
- Bind to DNA in the vicinity of a promoter and affect transcription of one or more nearby genes
- Repressors inhibit transcription
  - Negative control
- Activators increase the rate of transcription
  - Positive control

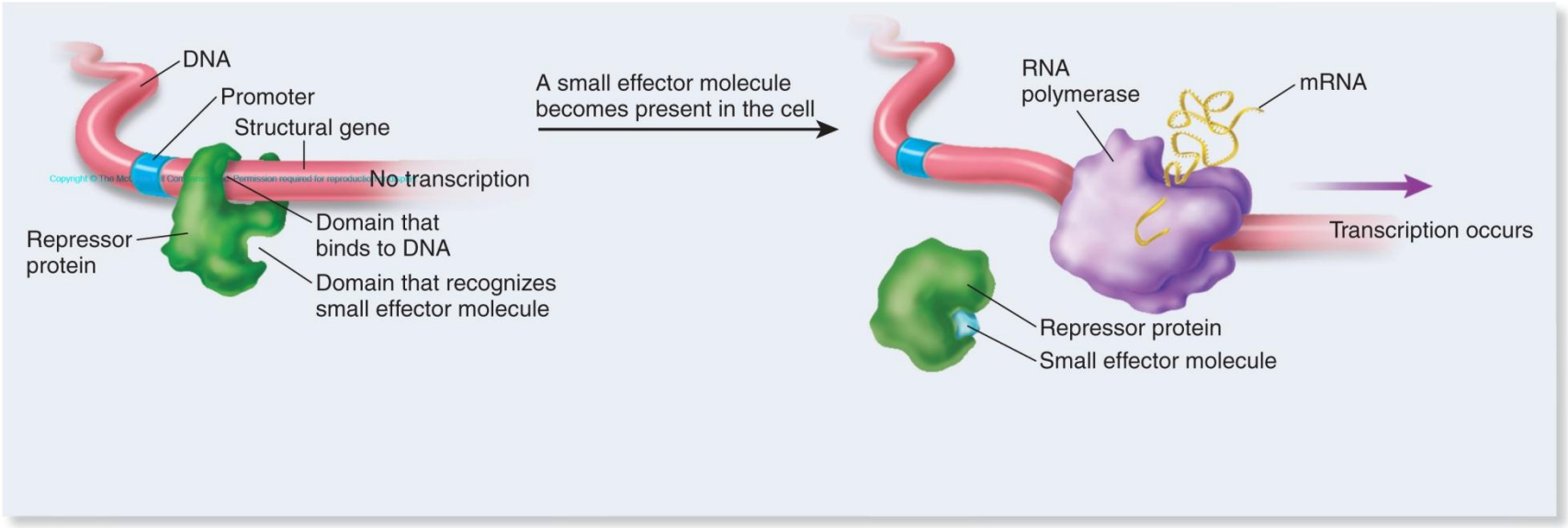


Negative control: A repressor inhibits transcription.



Positive control: An activator promotes transcription.

- Transcriptional regulation also involves small effector molecules
- Binds to regulatory transcription factor and causes conformational change
- Determines whether or not regulatory transcription factor can bind to DNA
- 2 domains in regulatory transcription factor that respond to small effector molecules
  - Site where protein binds to DNA
  - Site for small effector molecule



(b) Action of a small effector molecule on a repressor protein

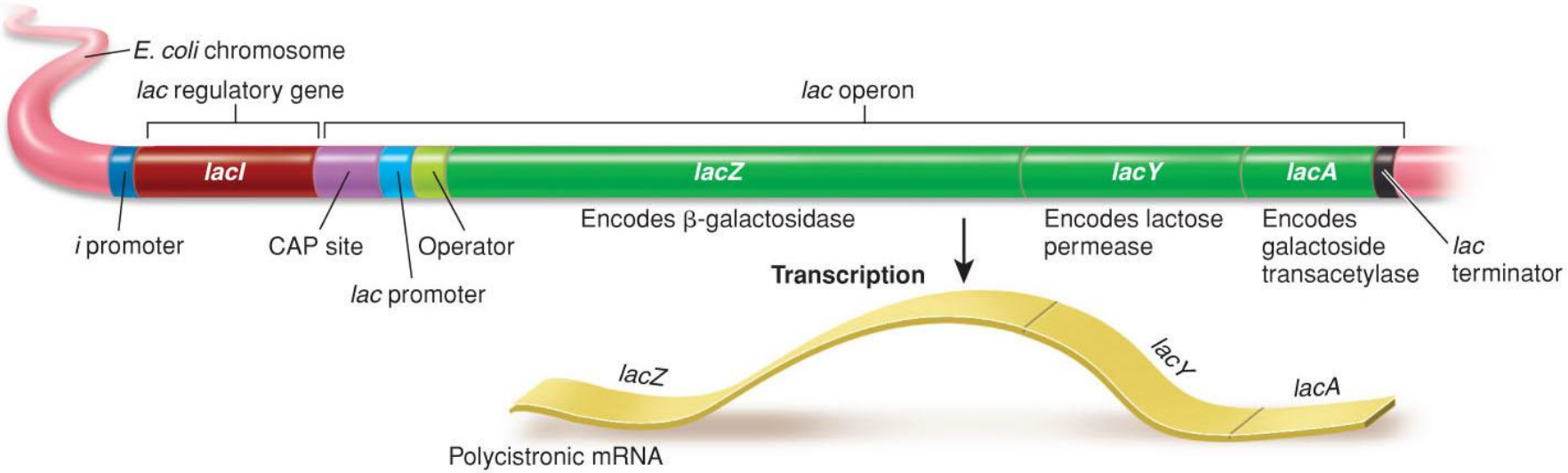
Operon

- Operon in bacteria is a cluster of genes under transcriptional control of one promoter
  - Regulatory region called operator
- Transcribed into mRNA as polycistronic mRNA – encodes more than one protein
- Allows regulation of a group of genes with a common function

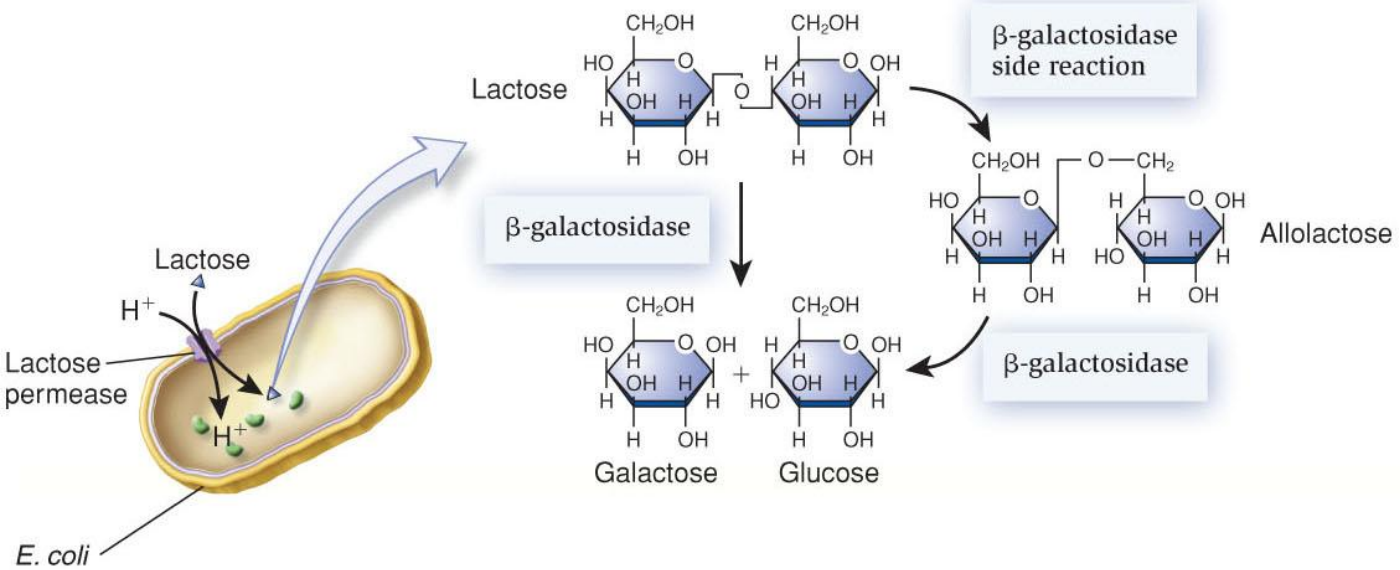
lac operon

- In *E. coli* contains genes for lactose metabolism
- *lacP* - promoter
- 3 structural genes
  - *lacZ* – β-galactosidase
    - Allolactose important in *lac* operon regulation
  - *lacY* – lactose permease
  - *lacA* – galactosidase transacetylase

- Near the *lac* promoter are 2 regulatory sites
  - *lacO* – operator – provides binding site for repressor protein
  - CAP site – activator protein binding site
- *lacI* gene - codes for *lac* repressor
  - Considered a regulatory gene since its sole function is to regulate other gene's expression
  - Has its own promoter (not part of *lac* operon)

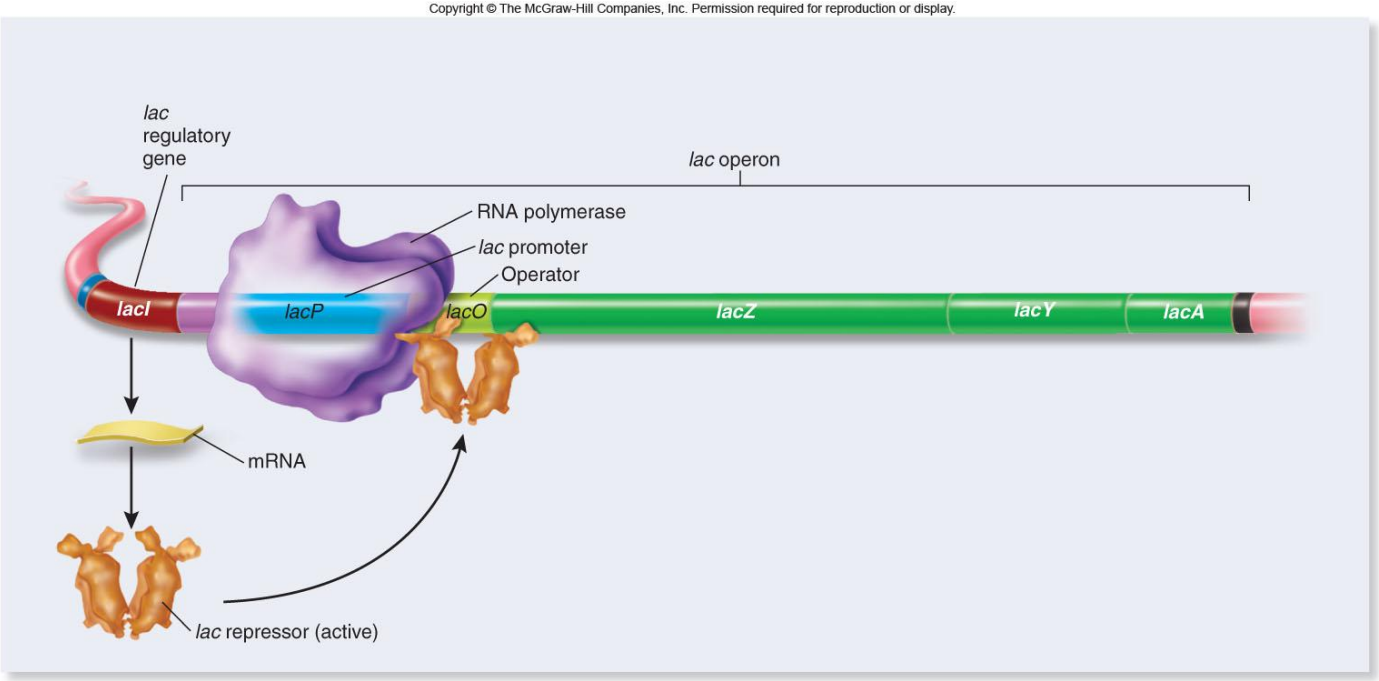


(a) Organization of DNA sequences in the *lac* region of the *E. coli* chromosome

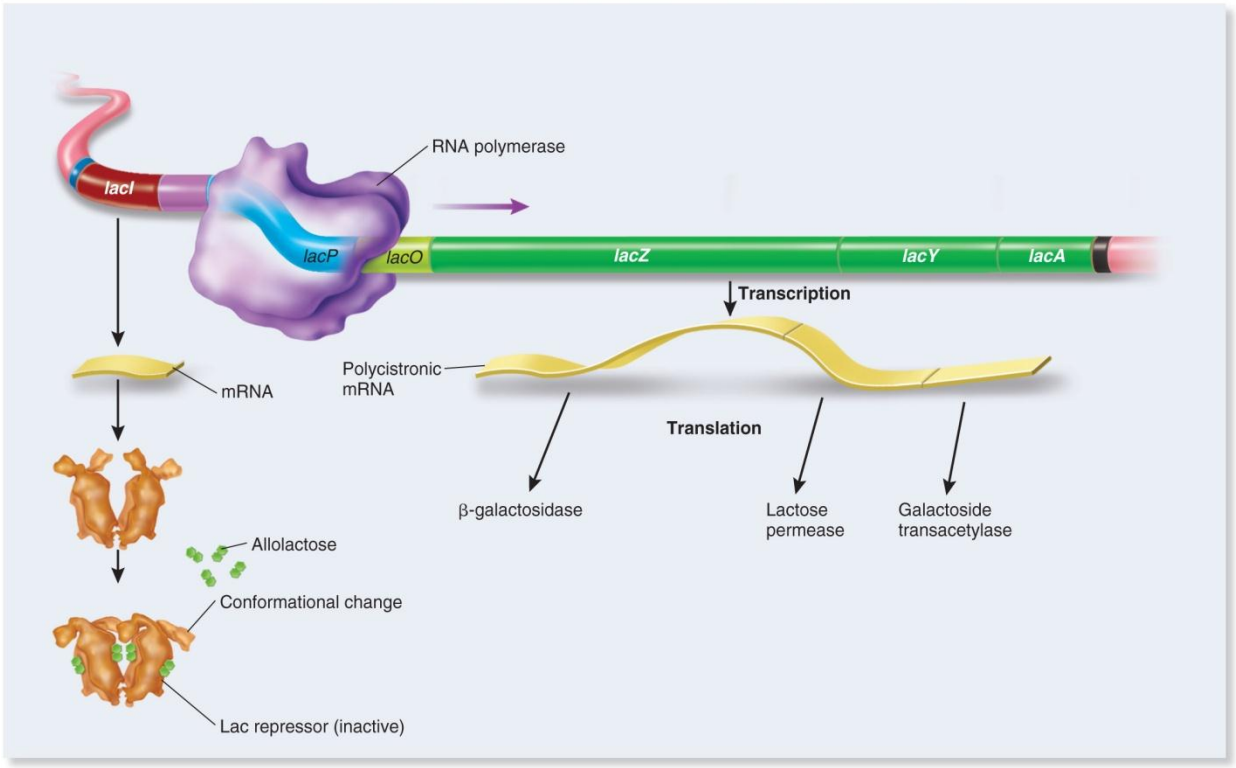


(b) Functions of lactose permease and β-galactosidase

- When lactose is absent
  - *Lac* repressor protein binds to nucleotides of *lac* operator site preventing RNA polymerase from transcribing *lacZ*, *lacY* and *lacA*
  - RNA polymerase can bind but not move forward



- When lactose is present
  - Allolactose is a small effector molecule
  - 4 allolactose molecules binding to *lac* repressor prevents repressor from binding
  - Process called induction and *lac* operon is inducible

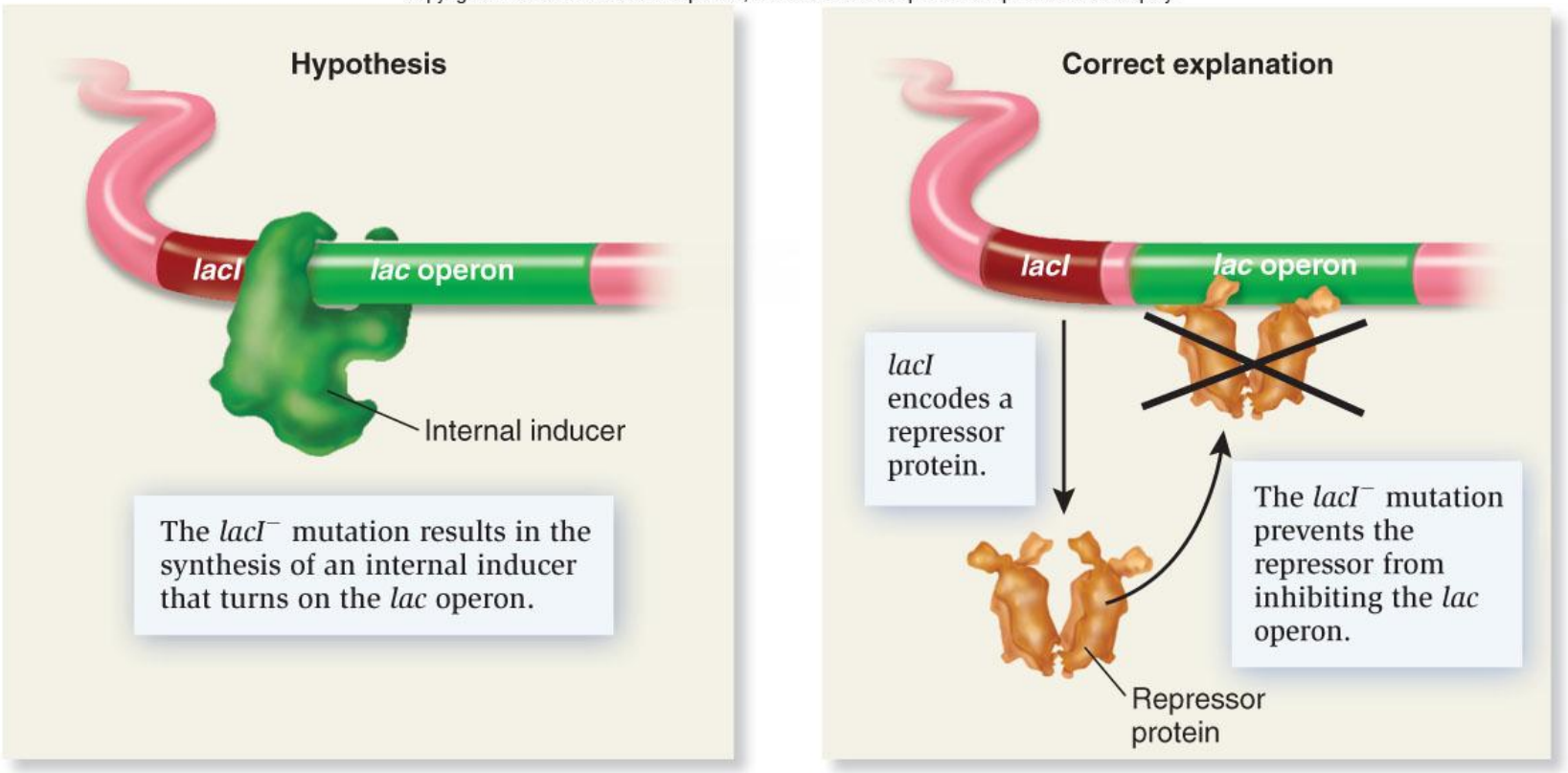


(b) Lactose present

Jacob, Monod and Pardee studied a constitutive bacterial mutant to determine the function of the *lac* repressor

- Found rare mutants with abnormal lactose use
- Expressed genes of *lac* operon constitutively
- Some mutations were in the *lacI* region
  - Strains were called *lacI*$^{-}$ (normal strains are *lacI*$^{+}$)
- 2 different functions for *lacI* region proposed

Feature Investigation

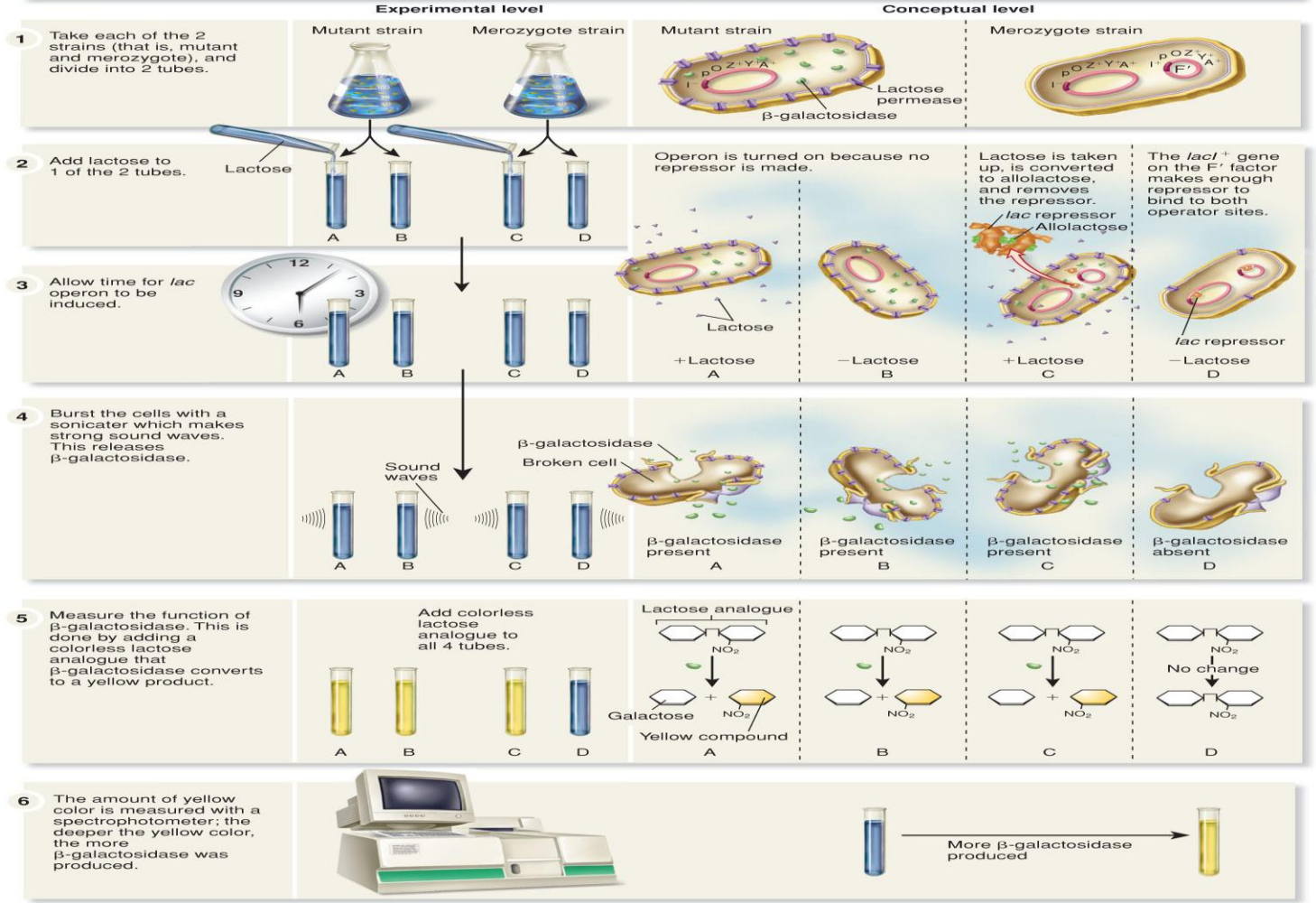


- Applied genetic approach using merozygotes
  - Strain of bacteria containing F' factor genes
    - Contain circular segments of DNA that carry additional copies of genes
    - Some carry *lac* operon and *lacI* gene
- If hypothesis 2 had been true, the *lacI*$^{+}$ region on the F' factor would not have been able to control the mutant *lacI*$^{-}$
- *Trans*-effect – form of genetic regulation that can occur even though 2 DNA segments are not physically adjacent
  - Action of *lac* repressor on *lac* operon
- *Cis*-effect or *cis*-acting element – DNA segment that must be adjacent to the gene(s) that it regulates

Feature Investigation

HYPOTHESIS The *lacI*$^-$ mutation causes the synthesis of an internal inducer.

STARTING MATERIALS A constitutive *lacI*$^-$ mutant strain was already characterized. An F′ factor carrying a normal *lacI*$^+$ gene and *lac* operon as introduced into this strain to produce a merozygote strain.



7 THE DATA

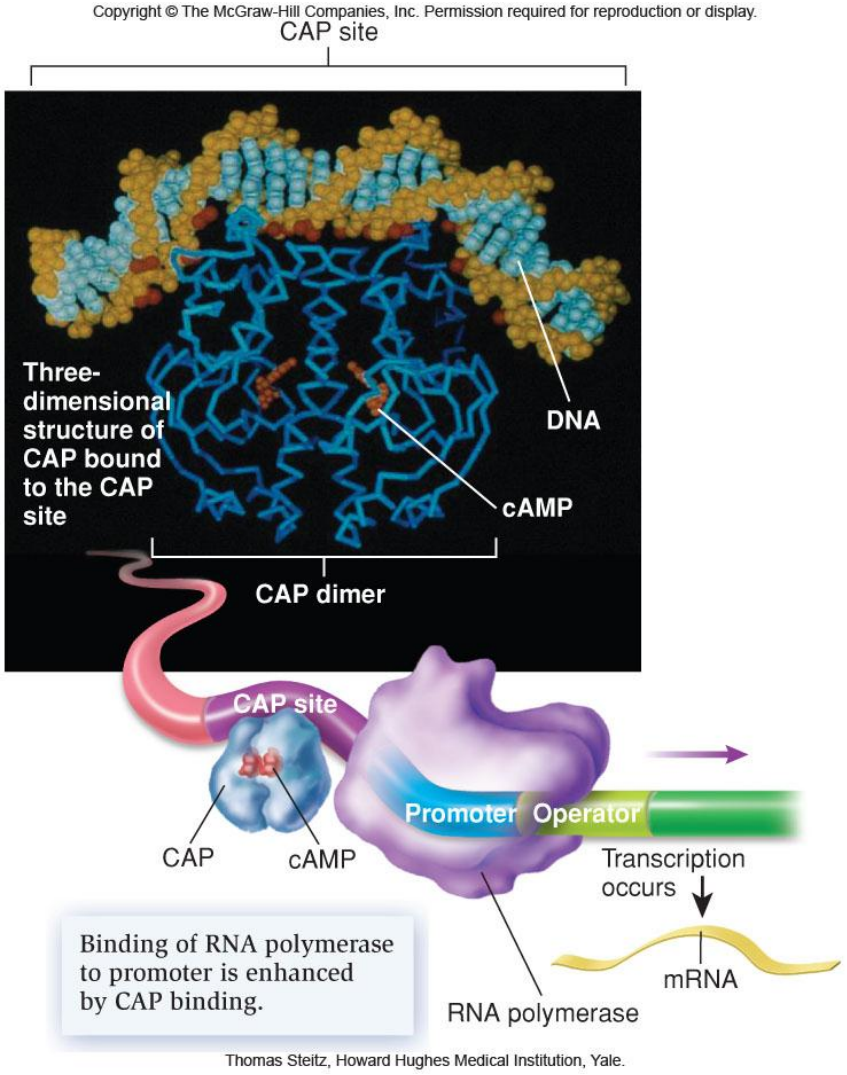
Results from step 6:

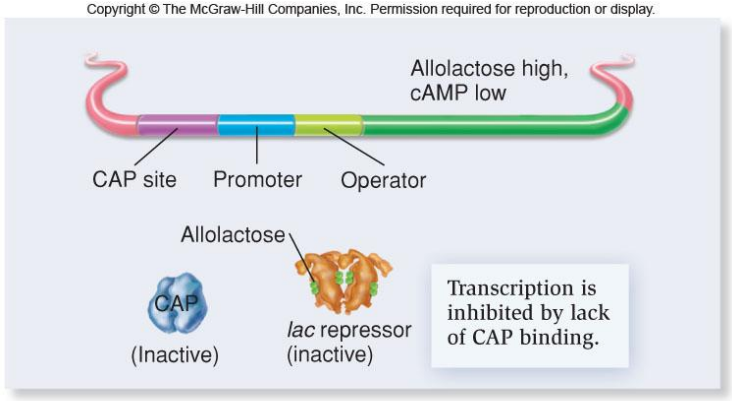
	Expression of the *lac* operon	
	With lactose	Without lactose
Mutant strain	100%	100%
Merozygote strain	220%	<1%

Lac operon also under positive control by activator protein

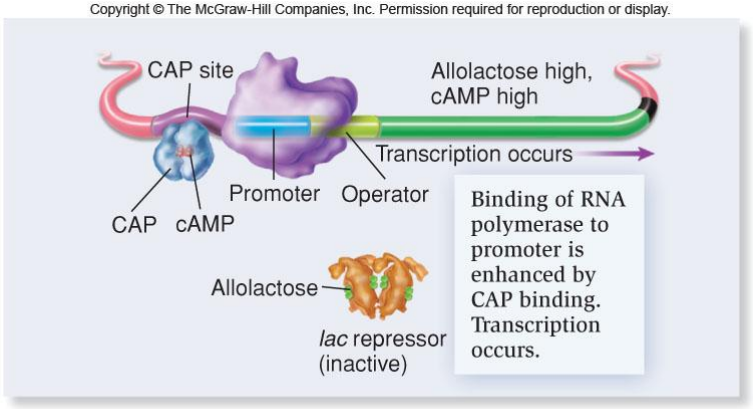
- CAP (catabolite activator protein) is an activator
- Catabolite repression – glucose, a catabolite, represses *lac* operon
- Small effector molecule, cAMP, binds to activator protein called catabolite activator protein (CAP) or cAMP receptor protein (CRP)
- Operon is turned off when CAP is not bound
- Glucose inhibits production of cAMP and so prevents binding of CAP to DNA

- Gene regulation involving CAP and cAMP an example of positive control
- When cAMP binds to CAP, complex binds to CAP site near *lac* promoter
- Resulting bend in DNA enhances RNA polymerase binding which increases transcription



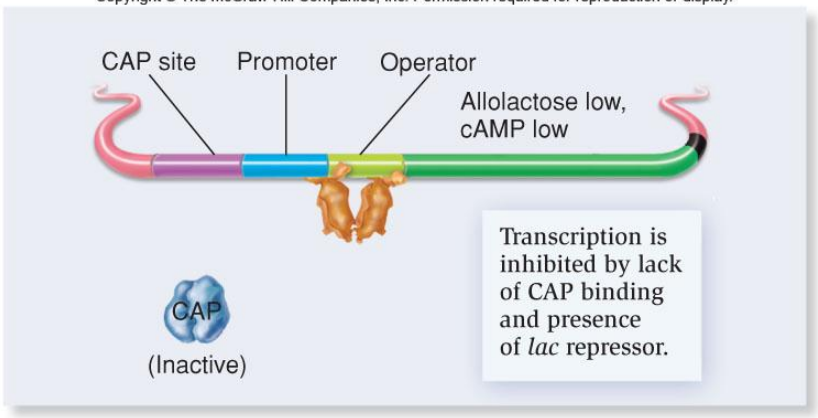


(a) Lactose high, glucose high

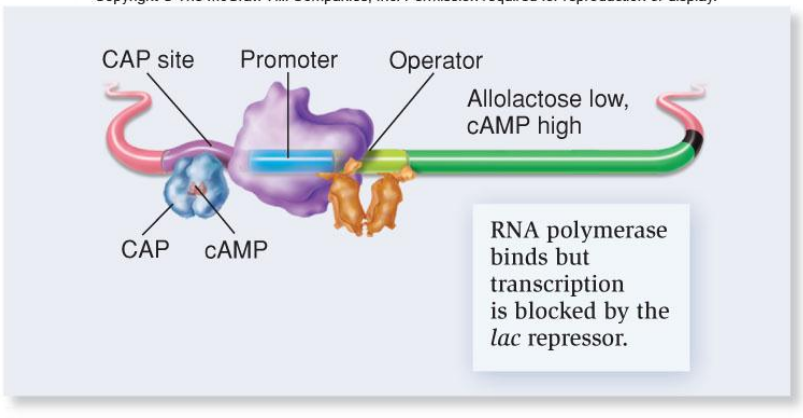


(b) Lactose high, glucose low

- When both lactose and glucose are high, the *lac* operon is shut off
  - Glucose uptake causes cAMP levels to drop
  - CAP does not activate transcription
  - Bacterium uses one sugar at a time, glucose
- When lactose is high and glucose is low, the *lac* operon is turned on
  - Allolactose levels rise and prevent *lac* repressor from binding to operator
  - CAP is bound to the CAP site
  - Bacterium uses lactose

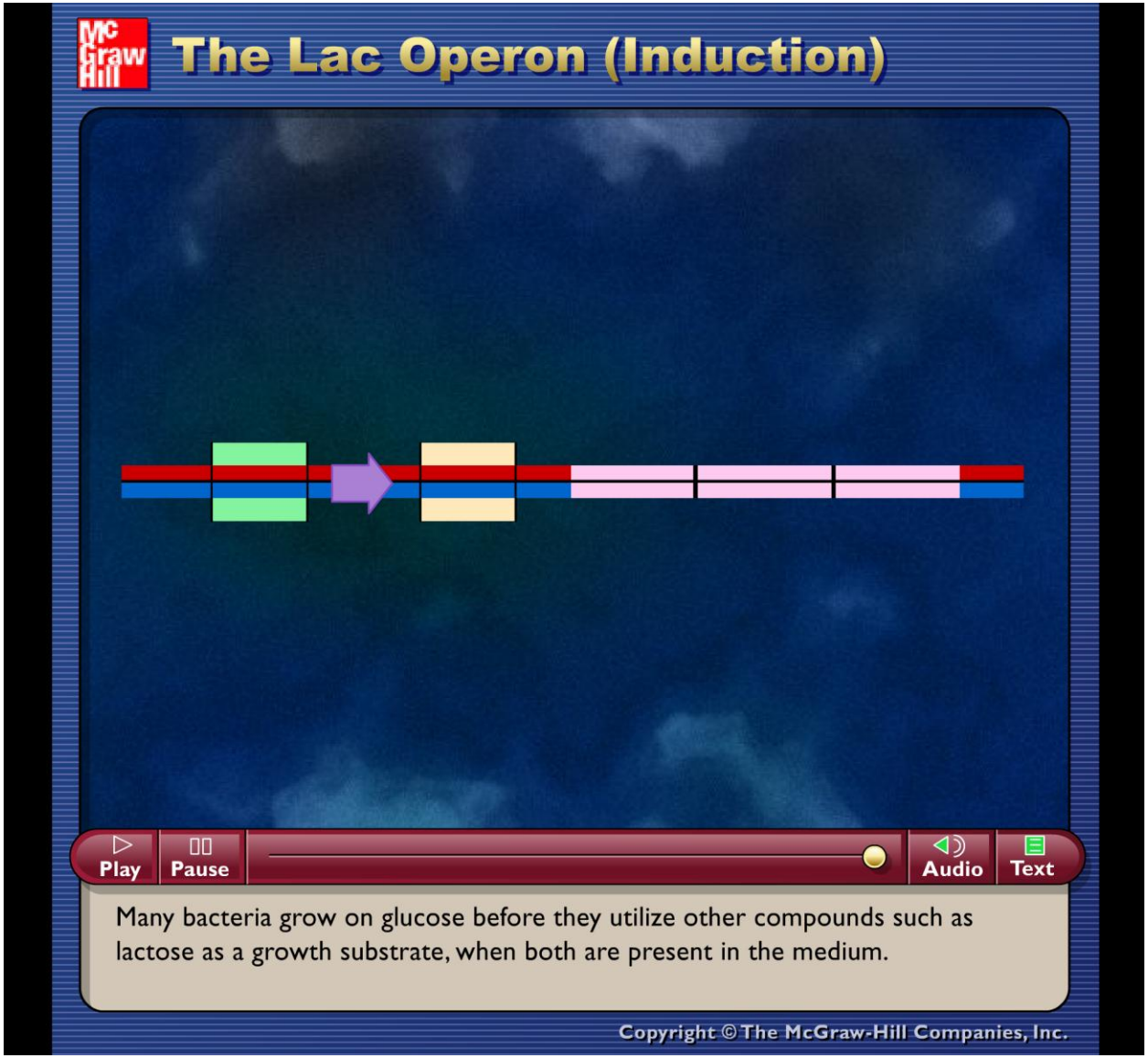


(c) Lactose low, glucose high



(d) Lactose low, glucose low

- When lactose is low and glucose is high or low, the *lac* operon is shut off
  - Under low lactose conditions, *lac* repressor prevents transcription of *lac* operon


The Lac Operon (Induction)
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Many bacteria grow on glucose before they utilize other compounds such as lactose as a growth substrate, when both are present in the medium.
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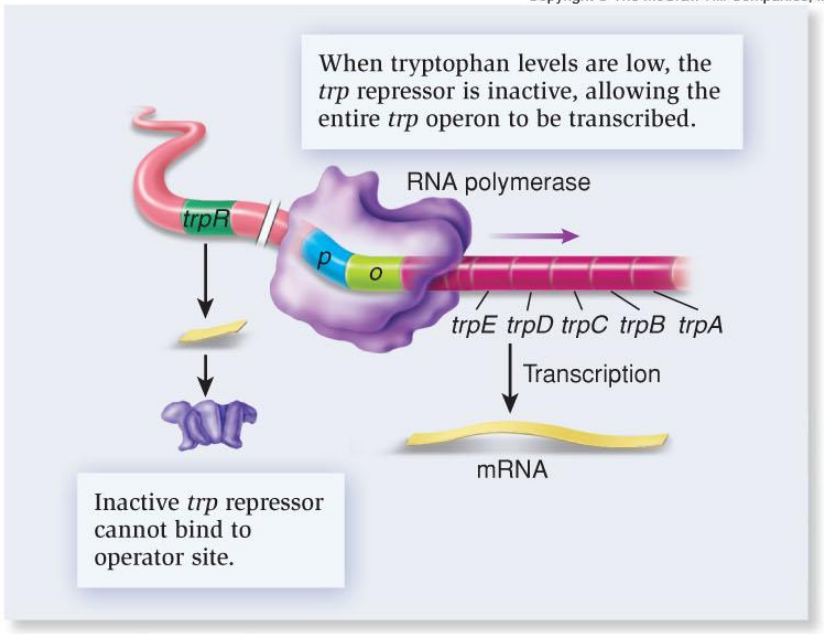
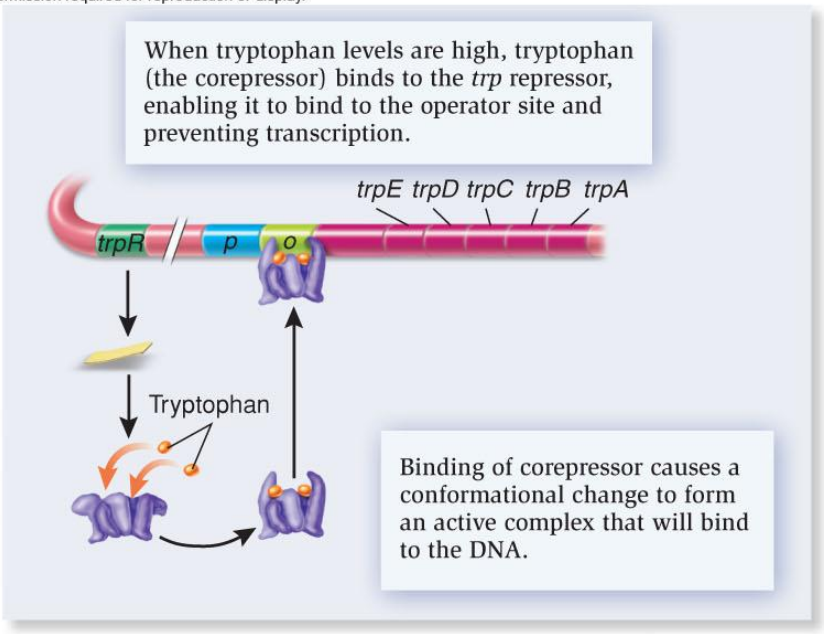
trp operon

- In *E. coli*, encodes enzymes required to make amino acid tryptophan
- Regulated by a repressor protein encoded by *trpR* gene
- Binding of repressor to *trp* operator site inhibits transcription
- When tryptophan levels low, *trp* repressor cannot bind to operator site and operon genes transcribed
- When tryptophan levels are high, tryptophan turns off the *trp* operon
- Tryptophan acts as a small repressor molecule or corepressor



(a) Low tryptophan



(b) High tryptophan

- *lac* repressor binds to its operator in the absence of its small effector molecule
  - Inducible- allolactose induces transcription
  - Operons for catabolism are often inducible
  - Genes turned off unless appropriate substance available
- *trp* repressor binds to its operator only in the presence of its small effector molecule
  - Repressible – tryptophan represses transcription
  - Operons for anabolism are often repressible
  - When enough of product present, genes are turned off to prevent overproduction

Regulation of transcription in eukaryotes

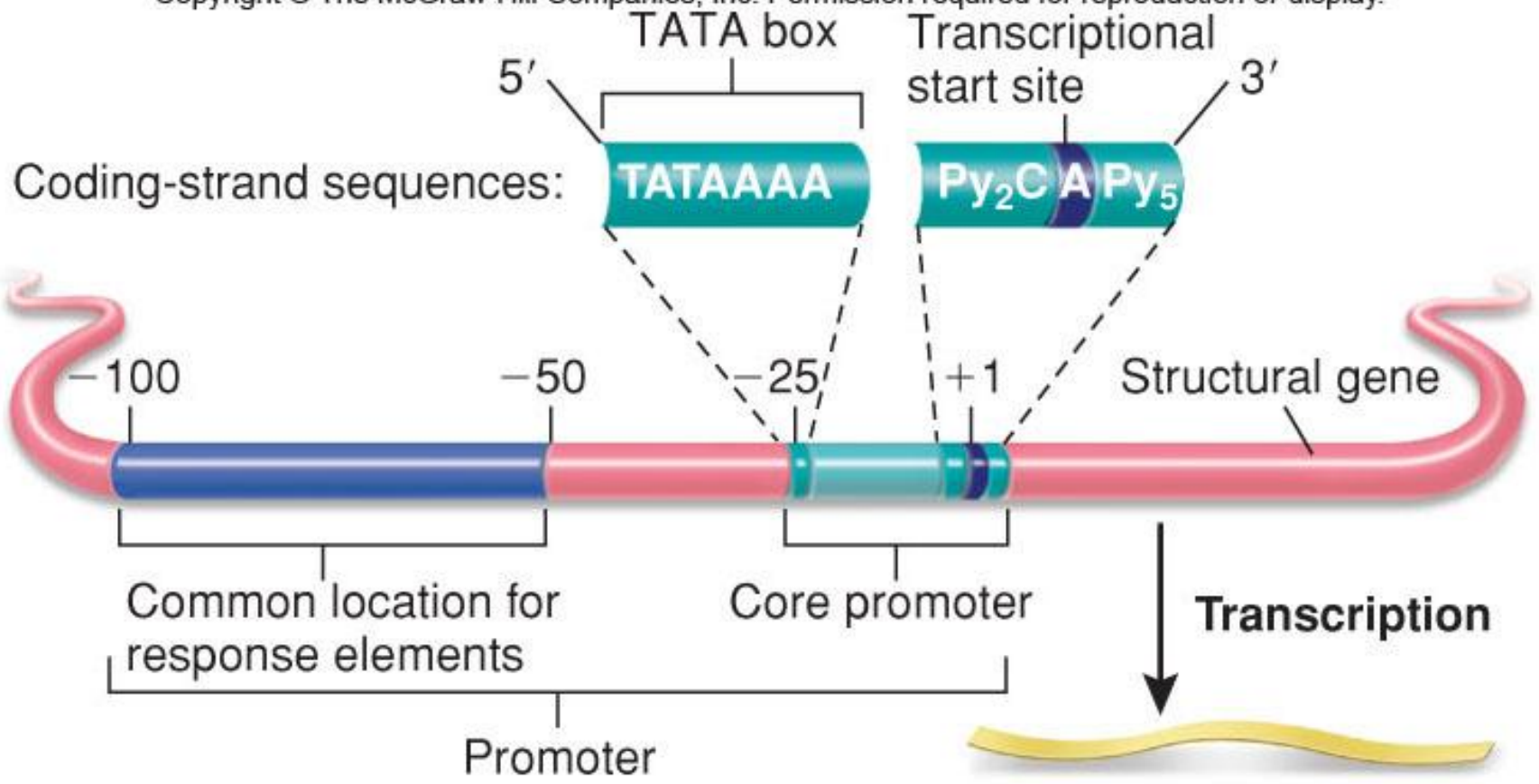
- Follows some of same principles found in prokaryotes
  - Activator and repressor proteins influence ability of RNA polymerase to initiate transcription
  - Many regulated by small effector molecules
- Many important differences
  - Genes almost always organized individually
  - Regulation more intricate

Combinatorial control

1. One or more activator proteins may stimulate the ability of RNA polymerase to initiate transcription.
2. One or more repressor proteins may inhibit the ability of RNA polymerase to initiate transcription.
3. The function of activators and repressors may be modulated in several ways. These include the binding of small effector molecules, protein–protein interactions, and covalent modifications.
4. Activator proteins are necessary to promote the loosening up of the region in the chromosome where a gene is located, thereby making it easier for the gene to be recognized and transcribed by RNA polymerase.
5. DNA methylation usually inhibits transcription, either by preventing the binding of an activator protein or by recruiting proteins that cause the DNA to become more compact.

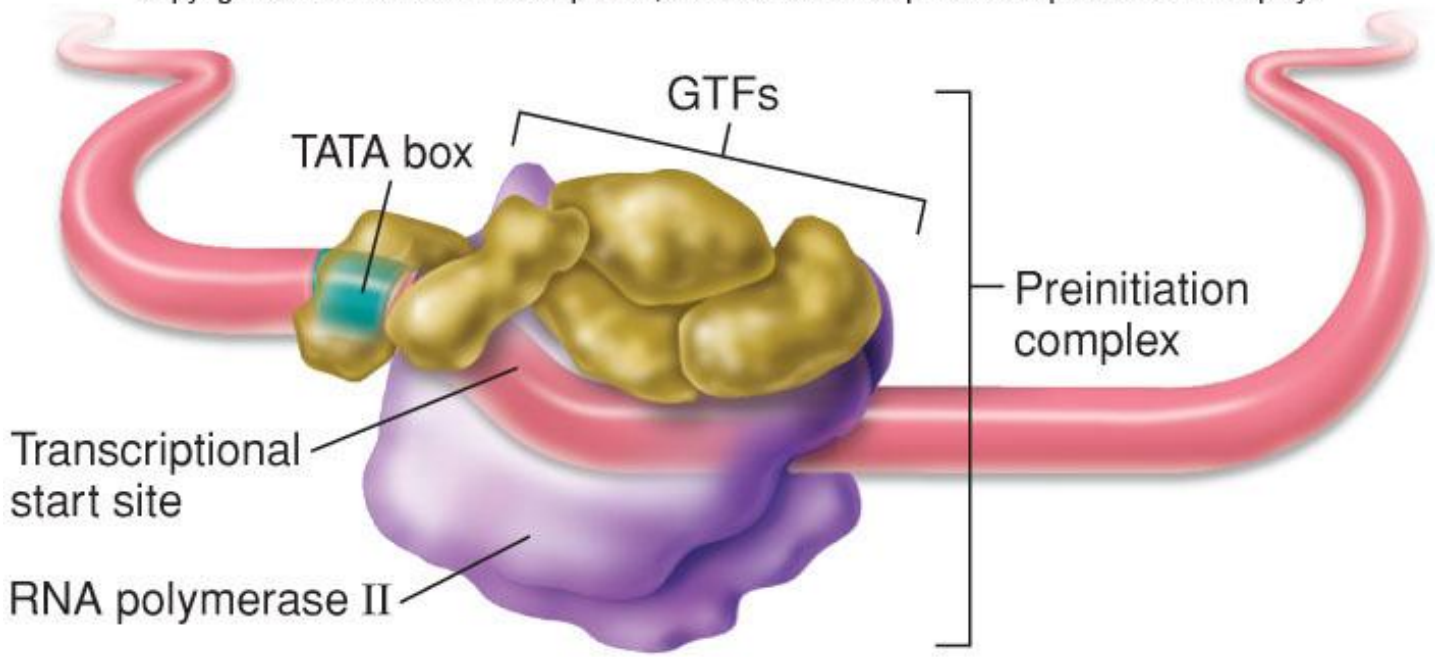
Eukaryotic structural genes

- 3 features found in most promoters
  - TATA box
    - 5’ – TATAAAA – 3’
    - 25 base pairs upstream from transcriptional start site
    - Determines precise starting point for transcription
  - Transcriptional start site
    - Where transcription begins
    - With TATA box forms core promoter
      - By itself results in low level basal transcription
  - Regulatory or response elements
    - Recognized by regulatory proteins that control initiation of transcription
    - Enhancers and silencers



3 proteins needed for transcription

1. RNA polymerase II
2. 5 different general transcription factors (GTFs)
3. Large protein complex called mediator

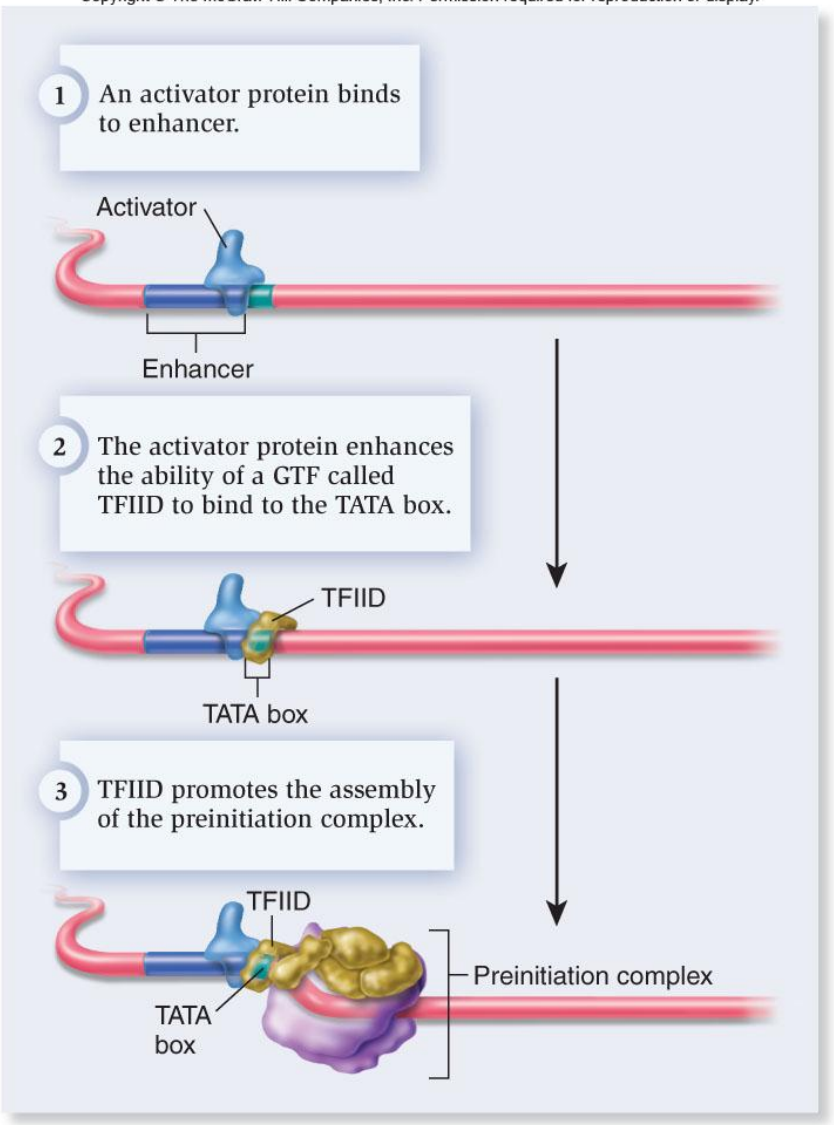


- GTFs and RNA polymerase II must come together at core promoter before transcription can be initiated
- Preinitiation complex – assembled GTFs and RNA polymerase II at the TATA box
  - Form basal transcription apparatus
- Mediator composed of several proteins
  - Partially wraps around GTFs and RNA polymerase II
  - Mediates interactions with activators or repressor that bind to enhancers or silencers
  - Controls rate at which RNA polymerase can begin transcription

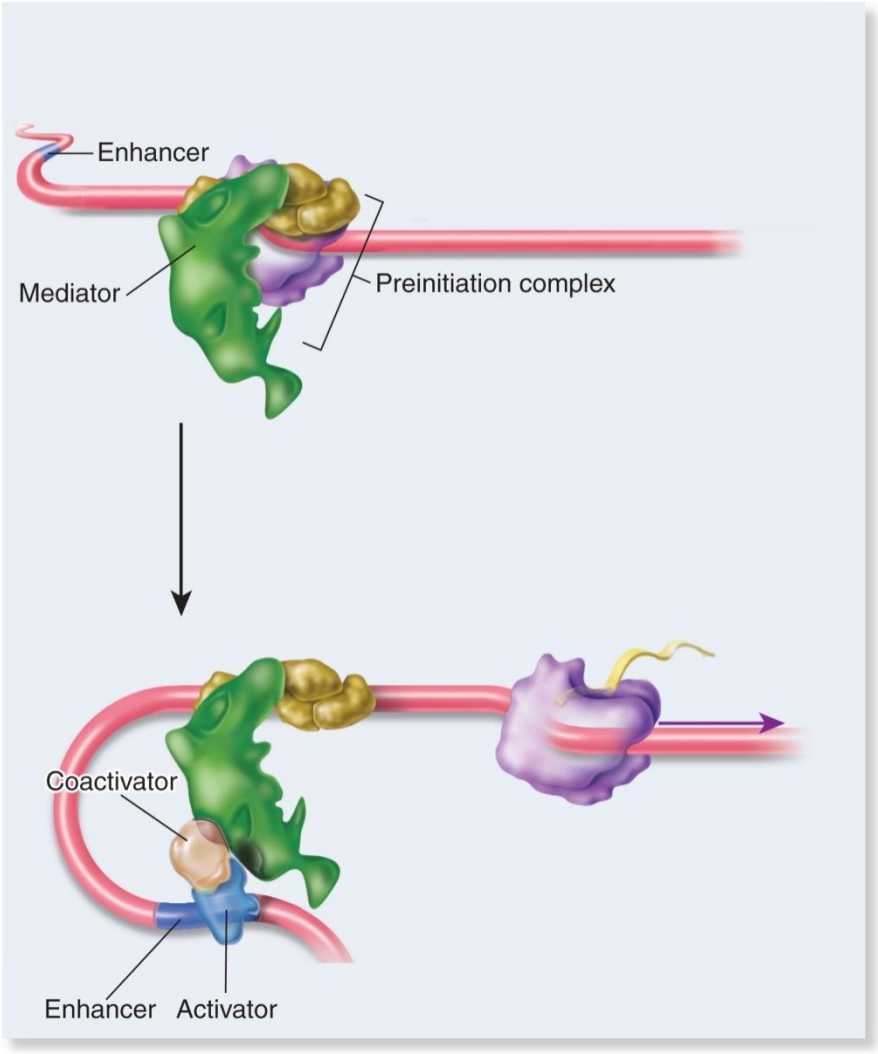
- Activators bind to enhancers
- Repressors bind to silencers
- Regulate rate of transcription of a nearby gene
- Most do not bind directly to RNA polymerase II

- 3 ways to control RNA polymerase II

1. Activators and repressors commonly regulate function of RNA polymerase II by binding to GTFs (including TFIID) or mediator



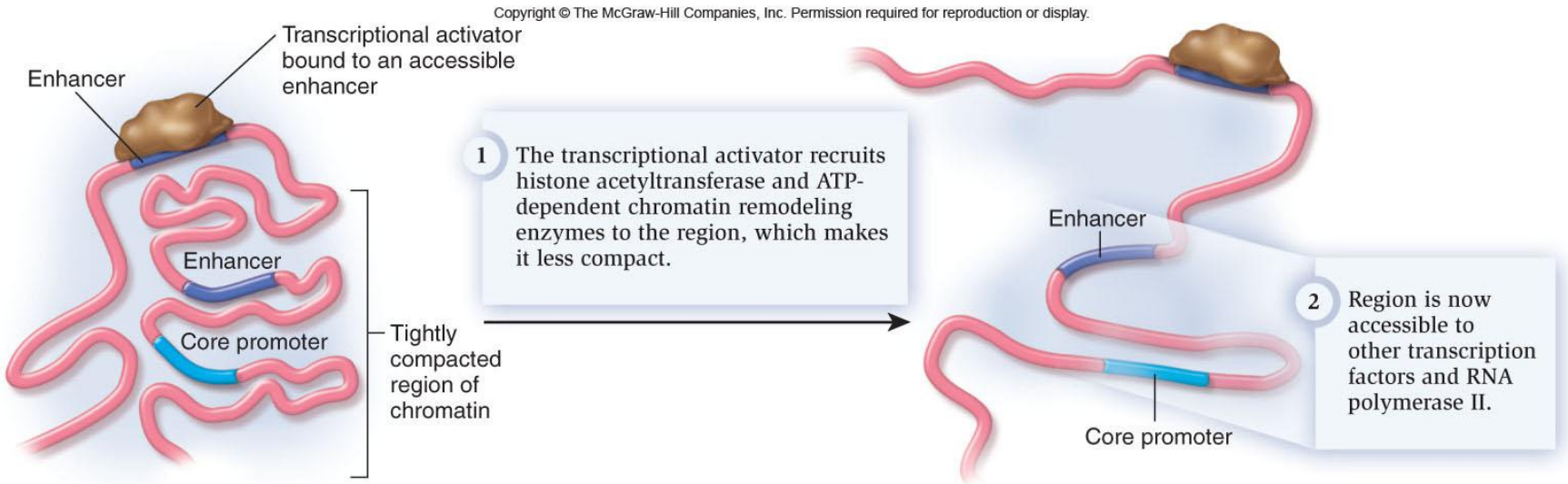
2. Control RNA polymerase II is via mediator
   - Activators stimulate the function of mediator by allowing faster initiation
   - Repressors inhibit mediator so RNA polymerase II cannot progress to elongation
3. Recruit proteins that influence DNA packing



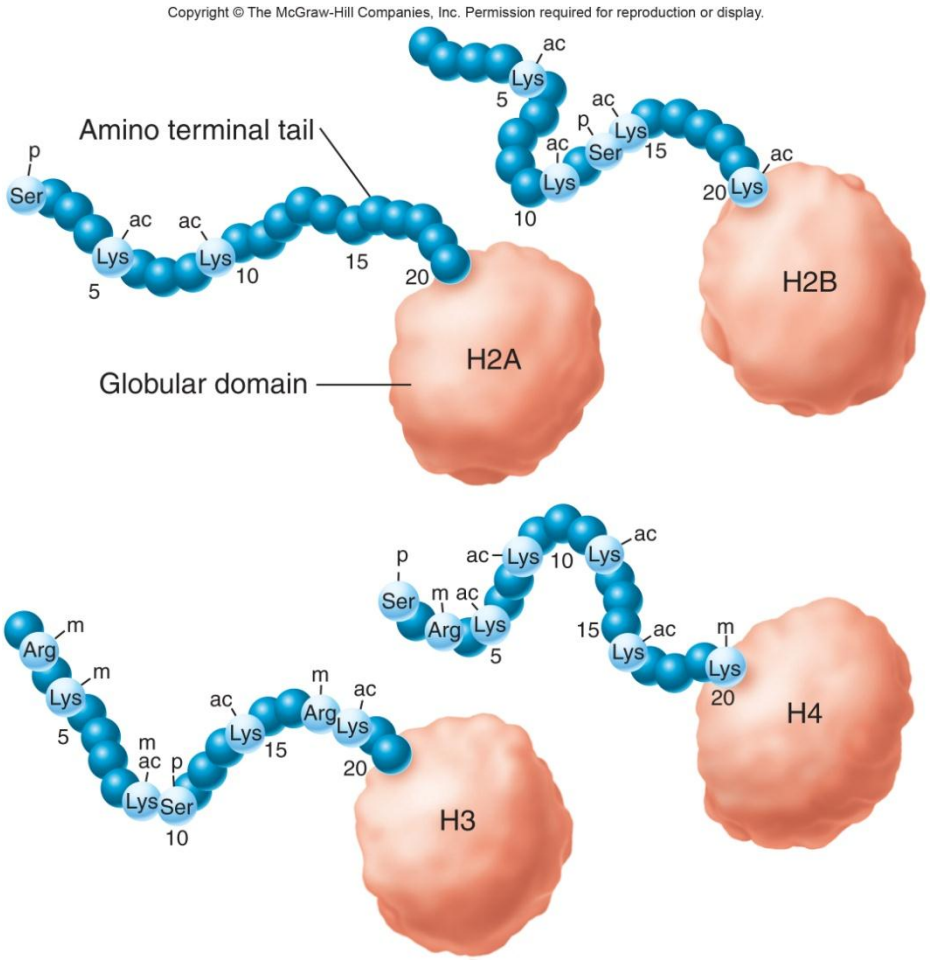
Gene accessibility

- DNA is associated with proteins to form compact chromatin
- Chromatin packing affects gene expression
- Transcription is difficult or impossible in the tightly packed chromatin in the closed conformation
- Access to the DNA is allowed in the loosely packed open conformation

- Some activators diminish DNA compaction near a gene
- Recruit proteins to loosen DNA compaction
  - Histone acetyltransferase attaches acetyl groups to histone proteins so they don't bind DNA as tightly
  - ATP-dependent chromatin remodeling enzymes also loosen DNA compaction

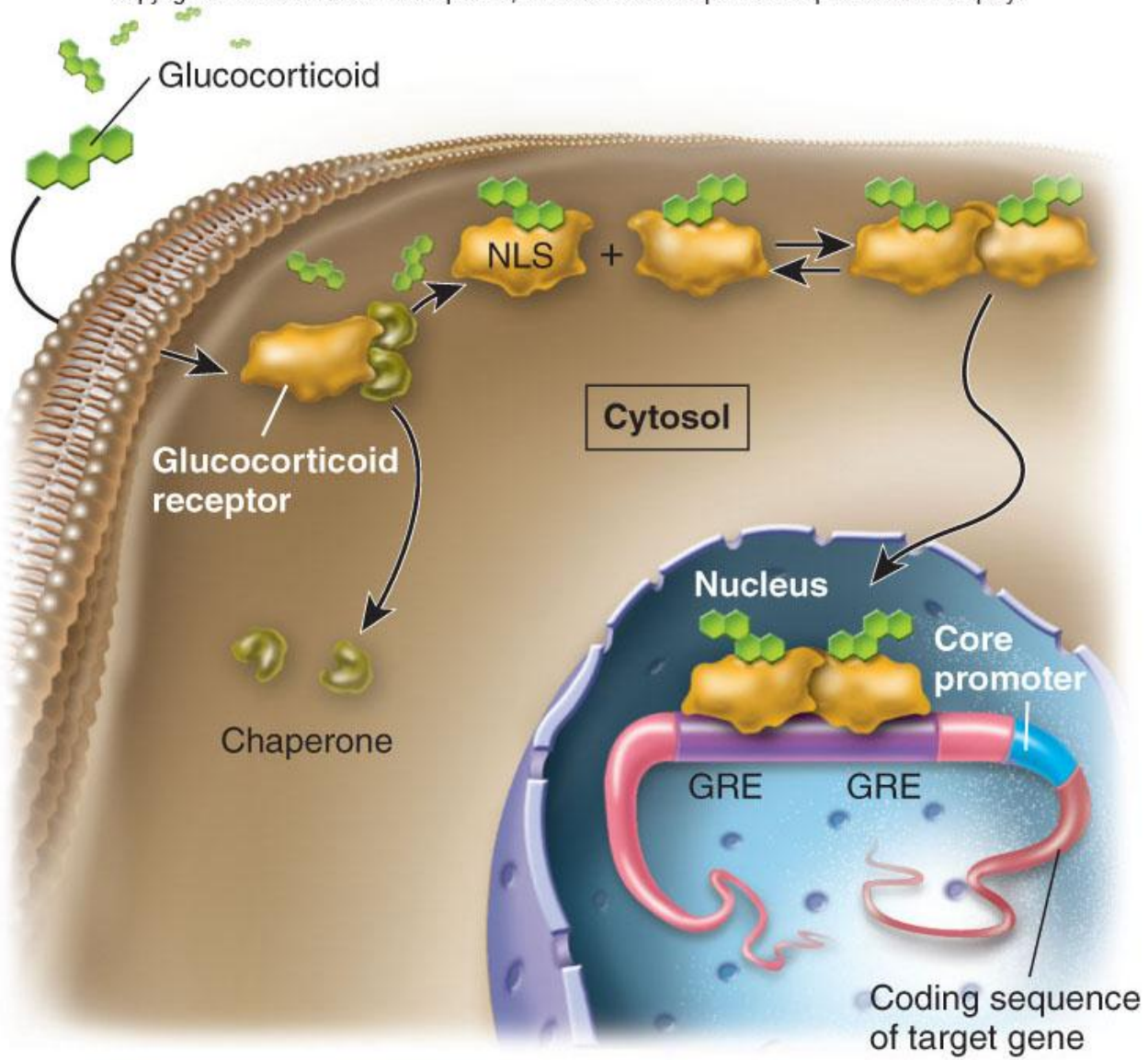


- Histone code
  - Many different amino acids in the amino terminal tails of histone proteins subject to several types of covalent modification
  - Pattern of modifications (histone code) affects degree of chromatin compaction



Steroid hormone example

- Transcriptional factor that responds to steroid hormones
  - Steroid receptor
  - Hormone is an example of a small effector molecule
- Steroid hormones made by endocrine glands and secreted in bloodstream
- Different cells respond to the hormone in different ways



DNA methylation

- DNA methylase attaches methyl groups
- Common in some eukaryotes but not all
- In mammals, 5% of DNA is methylated
- Usually inhibits transcription
- CpG islands found near promoters in vertebrates and plants
  - Cytosine and guanine connected by phosphodiester bonds
  - Unmethylated CpG islands are correlated with active genes
  - Repressed genes contain methylated CpG islands

- Methylation can inhibit transcription in 2 general ways

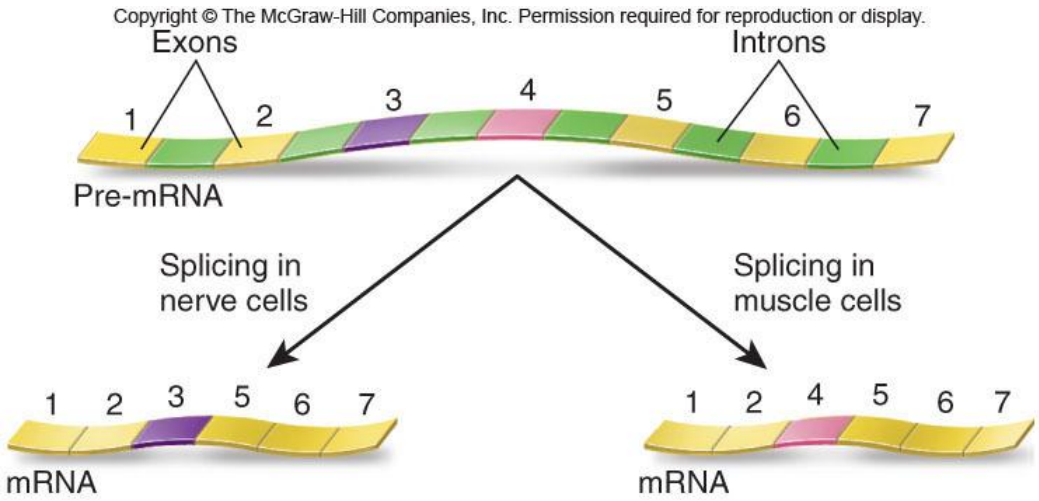
1. Methylation of CpG islands may prevent an activator from binding to an enhancer element
2. Converting chromatin from an open to a closed conformation
   - Methyl-CpG-binding proteins bind to methylated sequences and recruit proteins that condense the chromatin

Regulation of RNA processing and translation in eukaryotes

- Unlike bacteria, gene expression is commonly regulated at the level of RNA processing and translation
- Added benefits include…
  - Produce more than one mRNA transcript from a single gene (gene encodes 2 or more polypeptides)
  - Faster regulation achieved by controlling steps after RNA transcript made

Alternative splicing of pre-mRNAs

- In eukaryotes, a pre-mRNA transcript is processed before it becomes a mature mRNA
- When a pre-mRNA has multiple introns and exons, splicing may occur in more than one way
- Alternative splicing causes mRNAs to contain different patterns of exons.
- Allows same gene to make different proteins
  - At different stages of development
  - In different cell types
  - In response to a change in the environmental conditions



- Linear sequence of exons maintained in both alternates
- In most cases, the alternative versions of a protein will have similar functions, because much of their amino acid sequences will be identical to each other
- Nevertheless, alternative splicing produces differences in amino acid sequences that will provide each protein with its own unique characteristics
- Advantage of alternative splicing is that two (or more) different polypeptides can be derived from a single gene, thereby increasing the size of the proteome while minimizing the size of the genome

Increases in biological complexity are correlated with greater sizes of genomes and proteomes

- Alternative splicing can increase the proteome size without increasing the total number of genes
- For organisms to become more complex, as in higher plants and animals, evolution has produced more complex proteomes
- General trend is that less complex organisms tend to have fewer genes
- Frequency of alternative splicing increases with increasing biological complexity

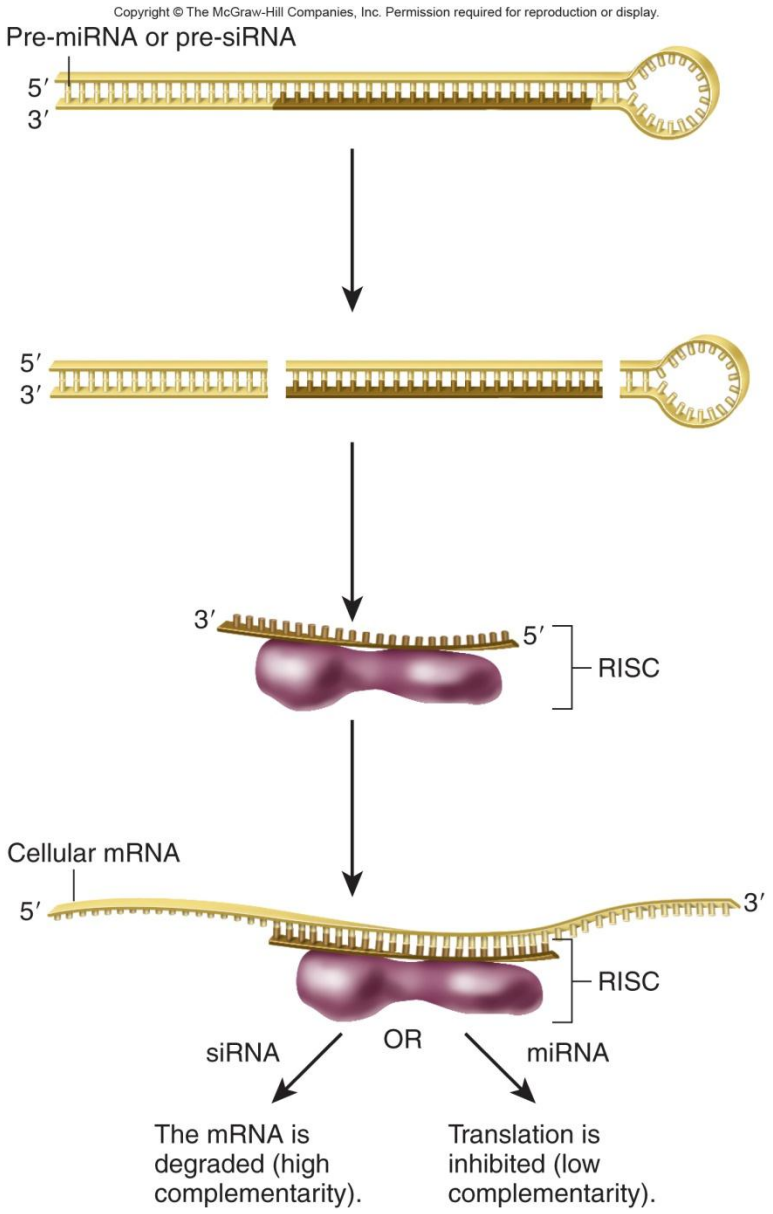
Table 13.1 Genome Size and Biological Complexity

Species	Level of complexity	Genome size (million bp)	Approximate number of genes	Percentage of genes alternatively spliced
Escherichia coli	A unicellular prokaryote	4.2	4,000	0
Saccharomyces cerevisiae	A unicellular eukaryote	12	6,000	<1
Caenorhabditis elegans	A tiny worm (about 1,000 cells)	97	19,000	2
Drosophila melanogaster	An insect	137	14,000	7
Arabidopsis thaliana	A flowering plant	142	26,000	11
Homo sapiens	A complex mammal	3,000	25,000	70

MicroRNAs

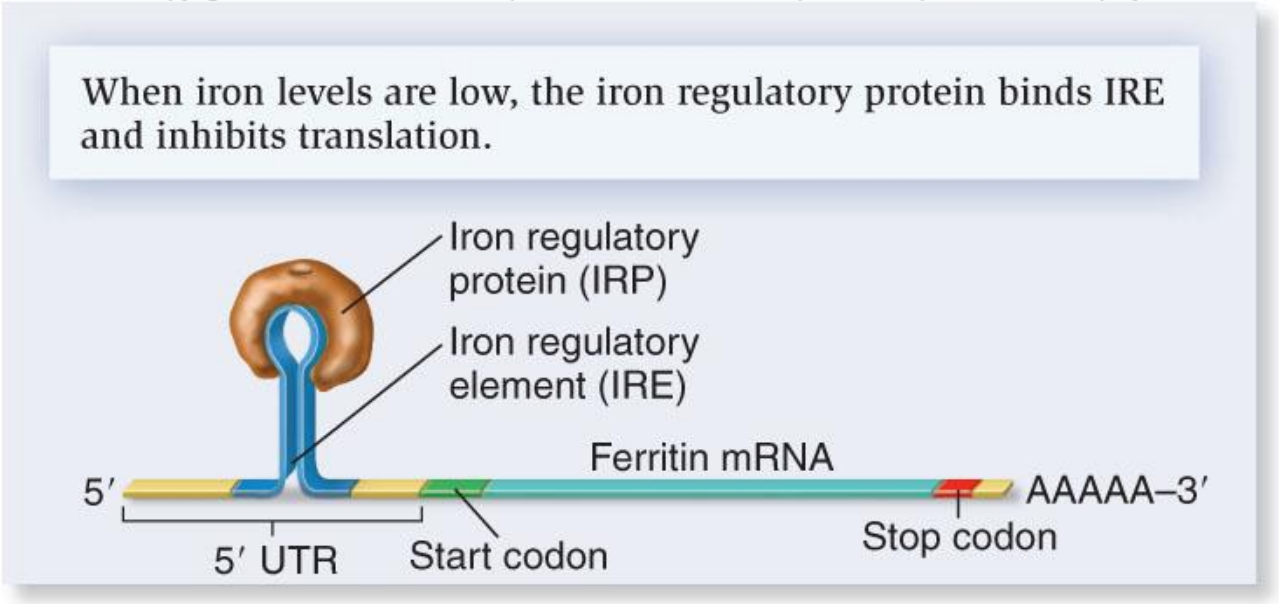
- miRNAs are small RNA molecules that silence the expression of pre-existing mRNAs
  - Formerly known as small or short interfering RNA (SiRNA)
- Widely found in animals and plants
- Important mechanism of mRNA silencing
- Effect also called RNA interference (RNAi)

- First synthesized as pre-miRNA
- Cut by dicer to release miRNA
- Associates with cellular proteins to become RNA-induced silencing complex (RISC)
- Upon binding, 2 things may happen
  - mRNA degraded
  - RISC may inhibit translation
- In either case, mRNA silenced

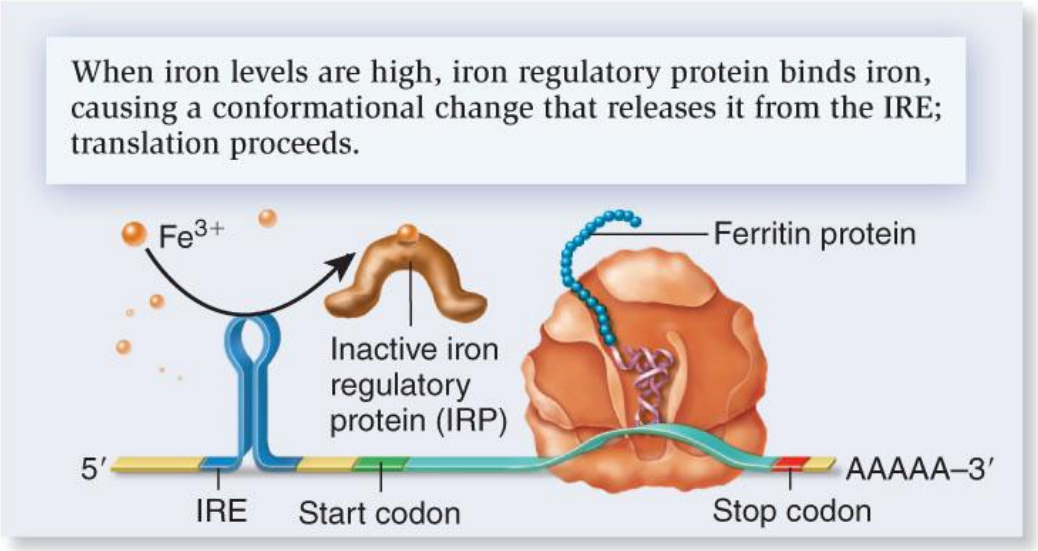


Iron toxicity in mammals

- Another way to regulate translation involves RNA-binding proteins that directly affect translational initiation
- Iron is a vital cofactor for many cellular enzymes
- However, it is toxic at high levels
- To prevent toxicity, mammalian cells synthesize a protein called ferritin, which forms a hollow, spherical complex that can store excess iron
- mRNA that encodes ferritin is controlled by an RNAbinding protein known as the iron regulatory protein (IRP)



- When iron levels in the cytosol are low and more ferritin is not needed, IRP binds to a response element within the ferritin mRNA known as the iron regulatory element (IRE)
  - Binding of IRP to the IRE inhibits translation of the ferritin mRNA



- When iron is abundant in the cytosol, the iron binds directly to IRP and prevents it from binding to the IRE
  - Ferritin mRNA is translated to make more ferritin protein
- Faster than transcriptional regulation, which would require the activation of the ferritin gene and the transcription of ferritin mRNA prior to the synthesis of more ferritin protein