

BIO230H/BIO255H

From Genes to Organisms



- Section 1
- Regulation of Genome Expression

- Department of Cell and Systems Biology, Earth Sciences Centre.

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Professor Tony Harris



- Section 2
- Cell signaling, molecular basis of development and cancer
- Department of Cell and Systems Biology, Ramsay Wright Laboratories

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The rest of the BIO230/255 Teaching Team.....

Dr. Melody Neumann—Senior Lecturer & Lab/Course Coordinator

Dr. Kenneth Yip –Evening Lecturer

Ms. Nyla Maharaj Cabrera —Course Administrator

Ms. Tatjana Vasic/Karen Xu—BIO230/255 Lab Technicians

BIO230 Teaching Assistants

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- Regulation of Genome Expression
 - How can the genome create and maintain a living organism?

30% Laboratories, Bibliography, and Quizzes

Labs

11.5% lab work

Quizzes

7.5% lab quizzes

2 % textbook reading on-line quizzes

Assignments

4% annotated bibliography

5% revised annotated bibliography

70% Exams

30% Mid-term (October) covering Section 1 of the lectures

40% Final exam (December) covering Section 2 of the lectures and all labs

Course Website

Your BIO230 Learning Tools...

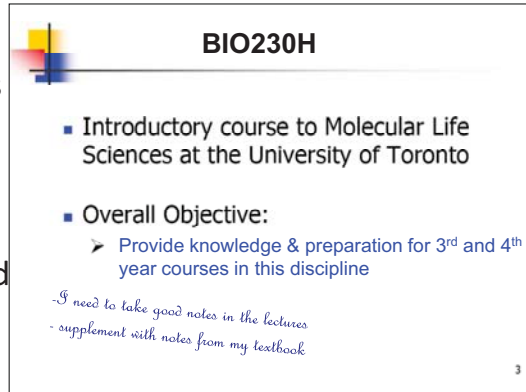
Blackboard (Bb) site on Portal

- Course News
- Lecture Notes
- Textbook Reading Quizzes
- MP3 Lecture Recordings
- Extra Lab Notes
- Electronic Discussion Board for Questions

Lecture Notes

Guided notes

- Fill-ins of key points (provided in lectures)
- You will need to add additional notes on your own



BIO230H

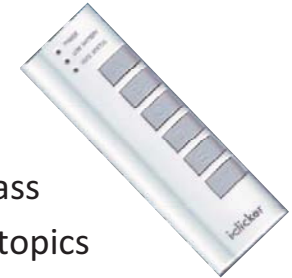
- Introductory course to Molecular Life Sciences at the University of Toronto
- Overall Objective:
 - Provide knowledge & preparation for 3rd and 4th year courses in this discipline

*- I need to take good notes in the lectures
- supplement with notes from my textbook*

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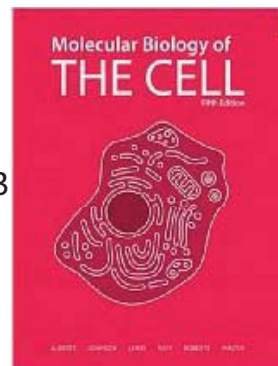
iClickers

- Not for marks
- Encourage active learning in class
- Exploration of a wide range of topics
- Available at UofT Bookstore—check Bb for details



Textbook

- Molecular Biology of the Cell, 5th ed. Alberts *et al.* 2008. Garland Science, New York.
- Same text as for BIO130
- UofT Bookstore also has textbook rental programme
- 11 copies on Reserve at Gerstein, 3 at Noranda Library (ESC)



Textbook Reading Quizzes

- 5 questions on Bb for each week's readings
- Objectives of quizzes are to help you to:
 - learn course materials
 - gauge depth required
 - keep up with lecture materials
- First quiz is for practice

BIO230/255 Bulletin Board

- Lecture, Lab and Administration Forums
- For lectures, clearly label your thread with the slide # and put in right place
- Check to make sure your question is a new one

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Bonus features.....

- High quality mp3s posted after lectures
- Bulletin board questions answered regularly
- 11 copies of textbook on reserve in Gerstein; 3 in Noranda (Earth Sciences)
- 3 hours of lecture time per week
 - Extra hour used in a variety of ways

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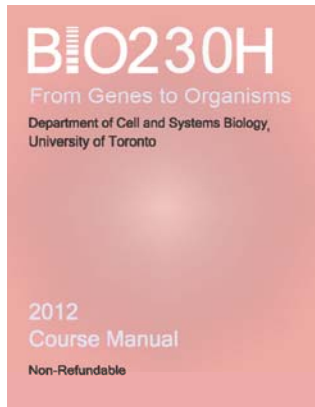
Welcome to BIO230/255!

Dr. Melody Neumann
Course and Lab Coordinator

Labs

- Lab topics:
 - RNA isolation and electrophoresis
 - Gene Regulation Part 1 and Annotated Bibliographies
 - Gene Regulation Part 2
 - Live Cell Imaging
 - Cytoskeleton and Development (transgenic fly)

What you need for labs...



Safety goggles Lab coat
\$12 \$16
Both: \$25

2012 Course Manual
U of T Bookstore \$ 12.75

When do labs start?

- Lab section Pxx01x – cycle 1 – week of **Sept. 17th**
- Lab section Pxx02x – cycle 2 – week of **Sept. 24th**
- All labs start at 10 minutes after the hour
- No Quiz in Lab 1!

Lab conflict? No lab?

- All lab enrollments and changes have to be requested through the BIO230 office (ES3053) this week
- Mon 2-4, Tues – Thurs 10-12 am and 2-4 pm
- You should have 2 potential lab times



Where can I get help?

- Discussion Board--monitored by course staff
- Tuesday lecture tutorials with course Professors
- Ms. Nyla Maharaj Cabrera--administrative issues
- Dr. Melody Neumann--lab content and other academic issues

Final Reminders

- Course manual and i-clickers at U of T bookstore
- Textbook
- Check Blackboard site regularly
- Have lab coat and goggles for wet labs
- Check lab group on PORTAL/Blackboard to get room # for lab

Again, welcome to BIO230/255!!!

BIO 230

Lecture 1 :

Review and Overview

- 1) Review of Cell Types
- 2) Genomes
- 3) Regulation of Genome Expression

Readings (Alberts *et al.*, 5th ed.)

Chapter 1: pages 1-8; 14-16

BIO 230

Section 1: Regulation of Genome Expression

...first a little review

- The tree of life has three primary branches

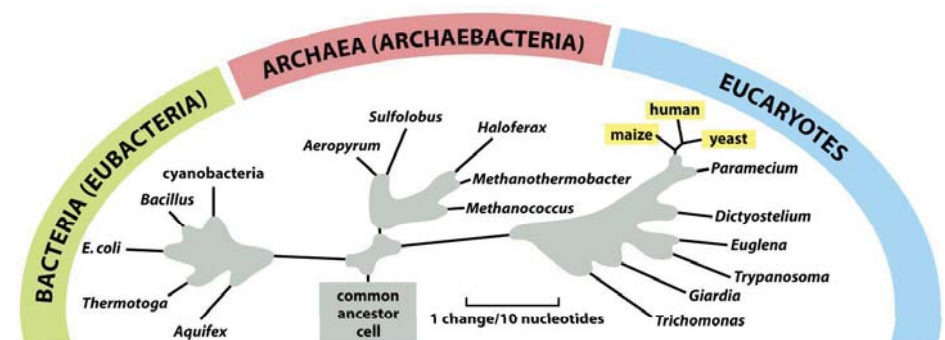


Figure 1-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Two Main Cell Types

1) Prokaryotic Cells

- Eubacteria, and archaea



2) Eukaryotic Cells

- Plants, fungi, animals, humans



Prokaryotic Cell

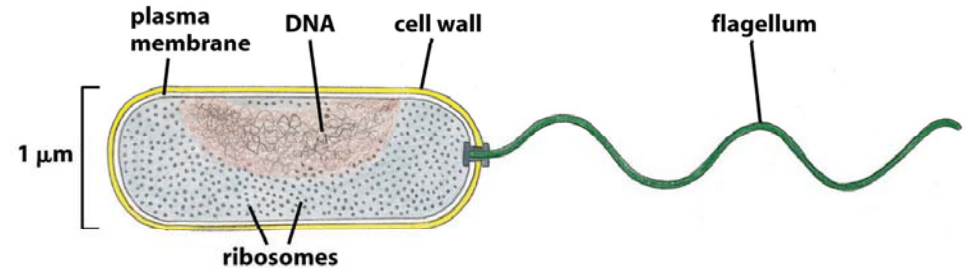


Figure 1-18a Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Eukaryotic Cell

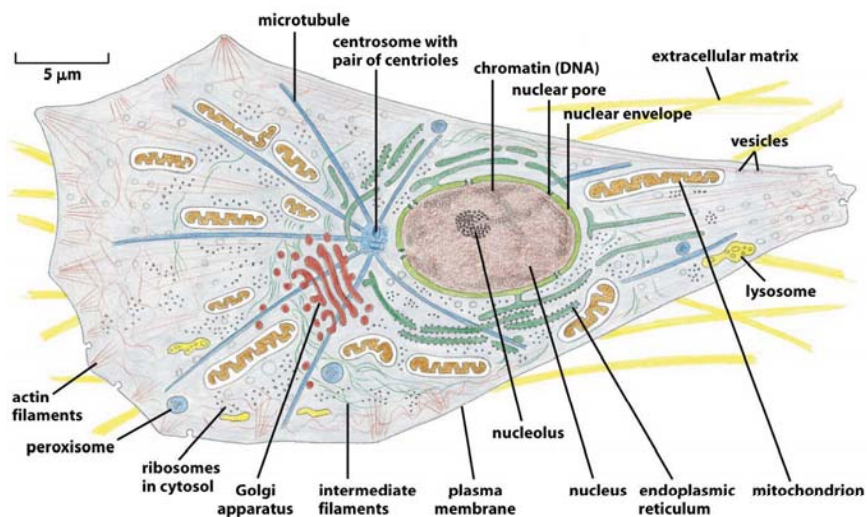


Figure 1-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Animation

- <http://aimediaserver.com/studiodaily/videoplayer/?src=harvard/harvard.swf&width=640&height=520>

OR

- <http://www.studiodaily.com/main/technique/tprojects/6850.html>

Inner Life of the Cell

Animation conception and scientific content by Alain Viel and Robert A. Lue.

Animation by John Liebler/XVIVO

Genomes

- All known life forms possess a genome
- Encodes the information to construct and maintain an organism
- Most genomes are made of ● (except some viruses have RNA genomes)
- Release of the biological information stored in the genome requires: ●

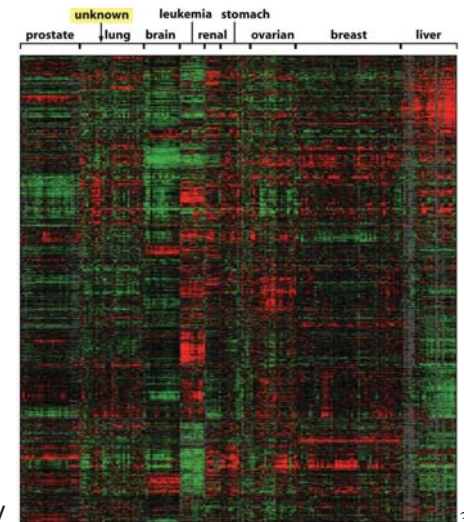
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Genome Expression

- The first product of genome expression is:



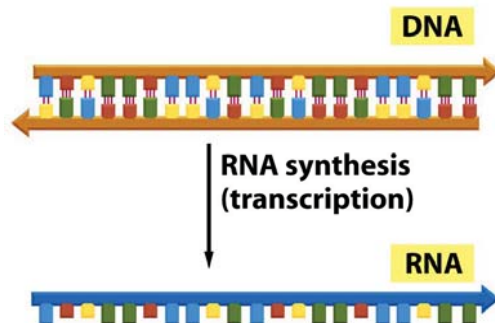
- The repertoire of RNA molecules present in a cell at a particular time



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Genome Expression

The transcriptome is maintained by the process of: ●



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Genome Expression

- The second product of genome expression is:



- The collection of proteins in a cell; define the biochemical functions of the cell

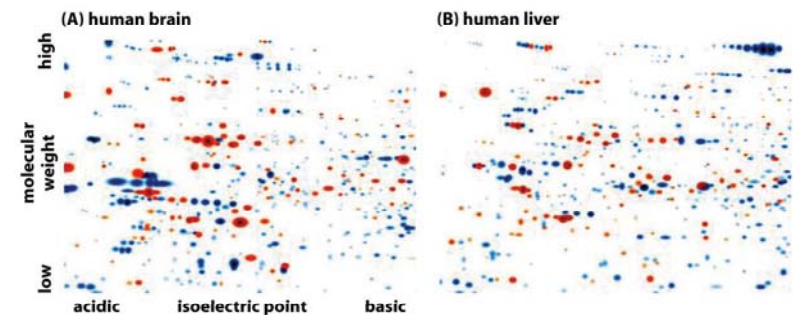


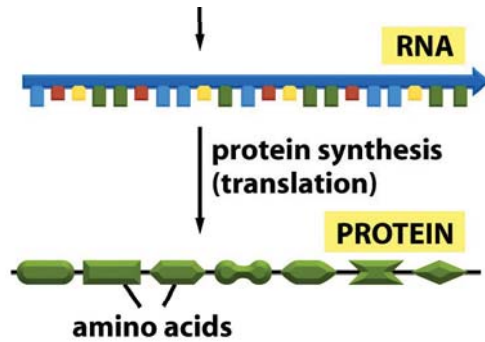
Figure 7-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

2D gel electrophoresis

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Genome Expression

The proteome is maintained by the process of:



- Genome (DNA) → Transcriptome (RNA) → Proteome (Protein) also known as:

Genome Expression

The different cell types of a multicellular organism contain

How to produce different cell types?

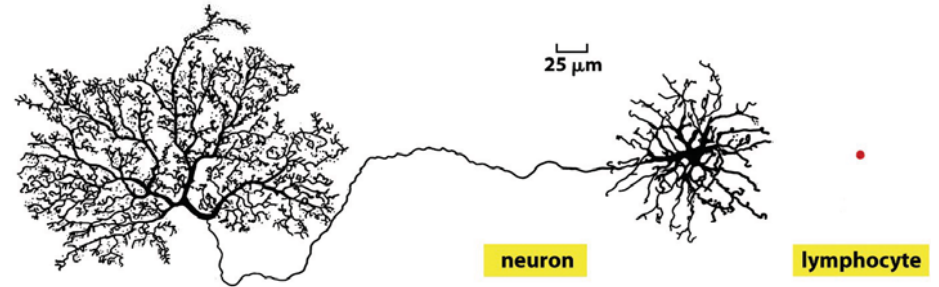


Figure 7-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Differences in

Genome Expression

Human genome ~25 000 genes

At any one time only 30-60% of genes expressed

Expression of almost all genes varies from one cell type to another

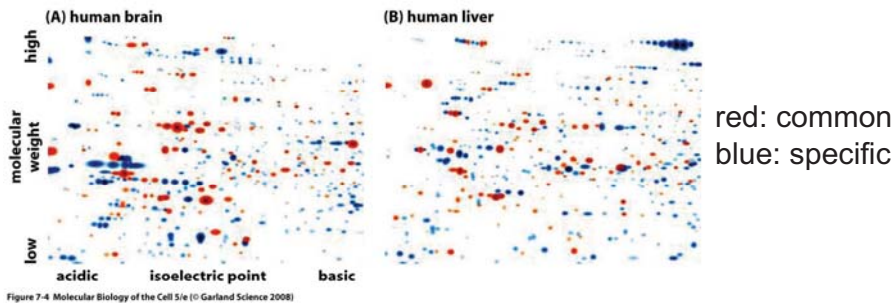
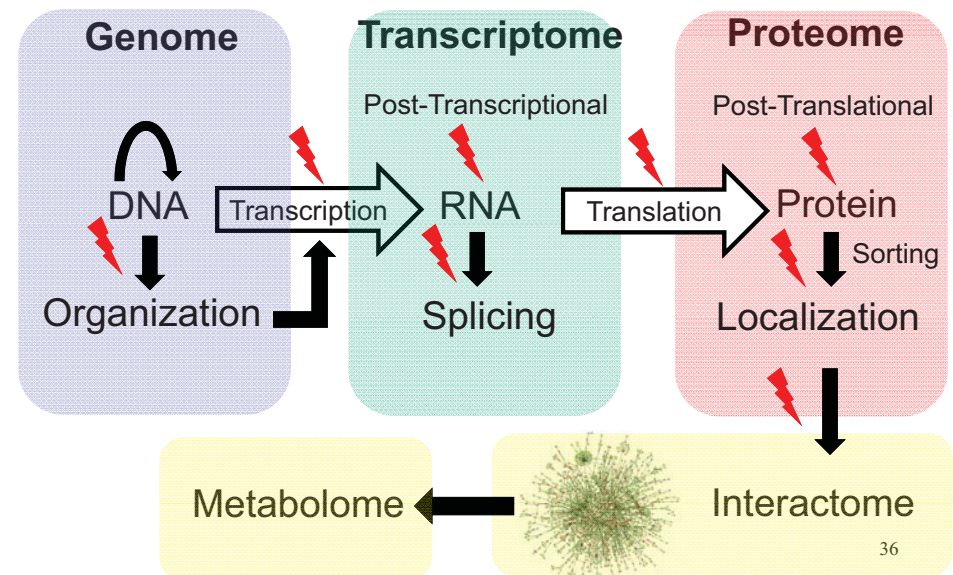


Figure 7-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Genome expression is regulated at many steps from DNA to protein

Regulation of Genome Expression



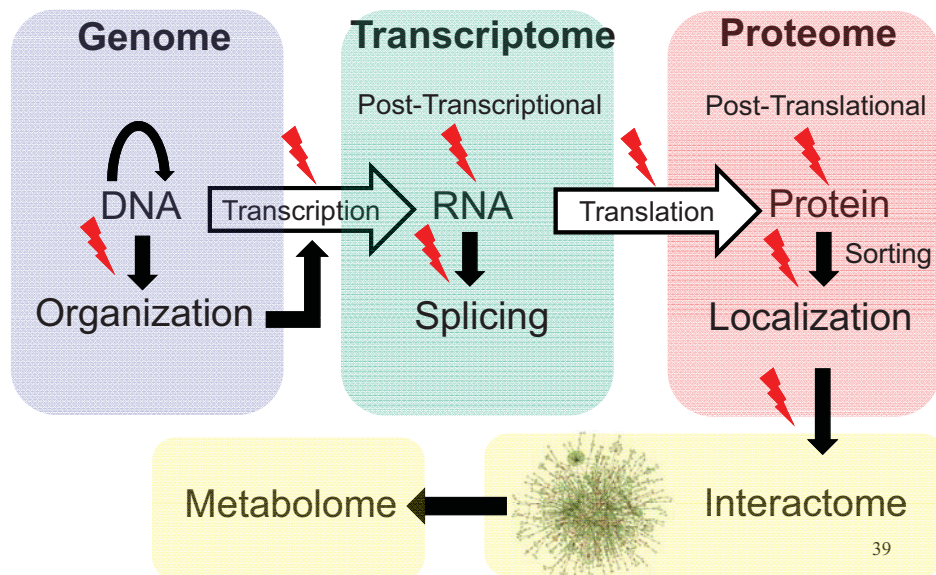
Question

Question 1: The bacterial toxin cyclohexamide inhibits eukaryotic translation. Which type of molecule would you expect to accumulate to high levels in human cells treated with cyclohexamide:

- A) DNA
- B) RNA
- C) Protein

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Regulation of Genome Expression



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BIO 230

Lecture 2 :

Prokaryotic Transcriptional Regulation

- 1) Genetic Switches
 - a) *Trp* Operon
 - b) *Lac* Operon

Readings (Alberts *et al.*, 5th ed.)
Chapter 4: pages 411-416; 432-438

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Transcriptional Regulation

Regulation of gene expression is crucial for:

-
-

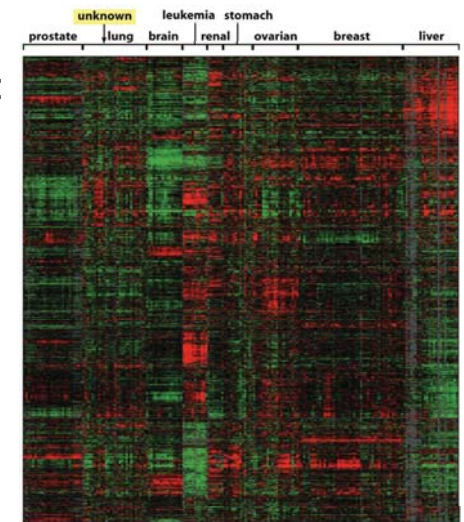


Figure 7-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Transcriptional Regulation

Recall that DNA is transcribed into RNA by the enzyme

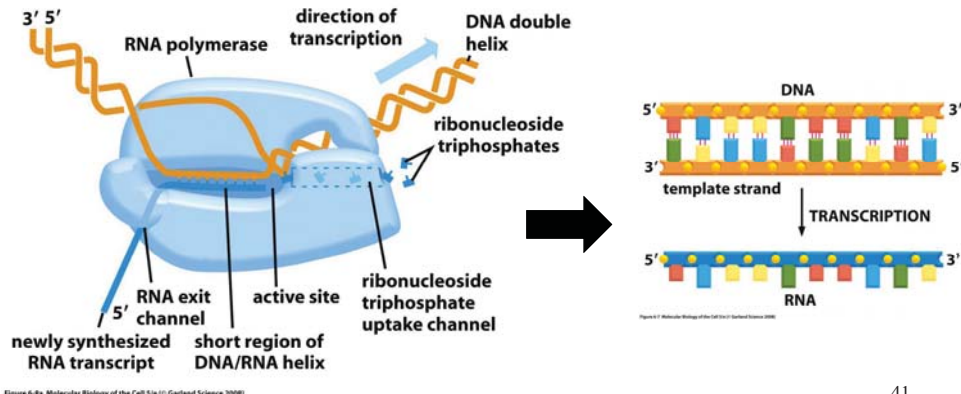


Figure 6-8a Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Transcriptional Regulation

Recall prokaryotic transcription

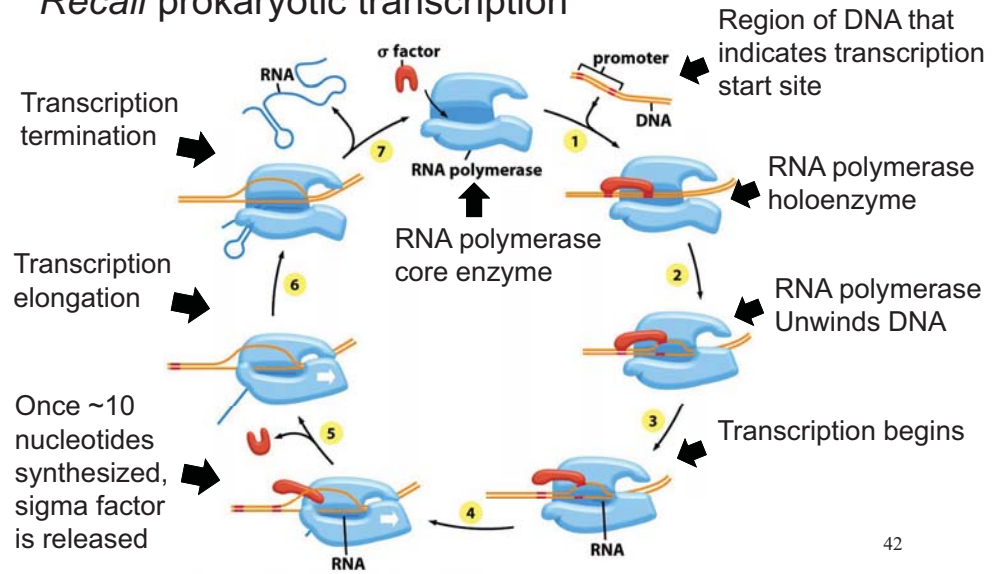


Figure 6-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Transcriptional Regulation

Transcription initiation in eukaryotes requires many proteins:

Help position RNA polymerase at eukaryotic promoters

Table 6-3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSCRIPTION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Table 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

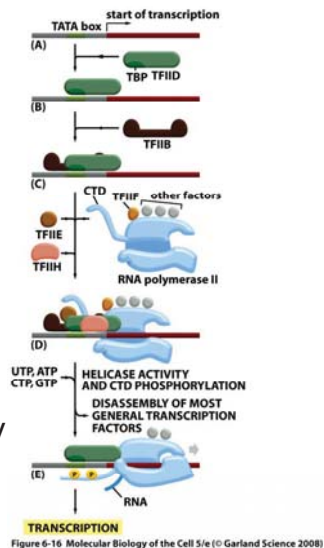


Figure 6-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Required by nearly all promoters used by

Cells produce several types of RNA:

Different RNAs transcribed by different RNA polymerases in eukaryotes

Prokaryotes have a single type of RNA polymerase

Table 6-1 Principal Types of RNAs Produced in Cells

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
scaRNAs	small cajal RNAs, used to modify snoRNAs and snRNAs
miRNAs	microRNAs, regulate gene expression typically by blocking translation of selective mRNAs
siRNAs	small interfering RNAs, turn off gene expression by directing degradation of selective mRNAs and the establishment of compact chromatin structures
Other noncoding RNAs	function in diverse cell processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

Table 6-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Table 6-2 The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

Table 6-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcriptional Regulation

Genes can be transcribed at different efficiencies

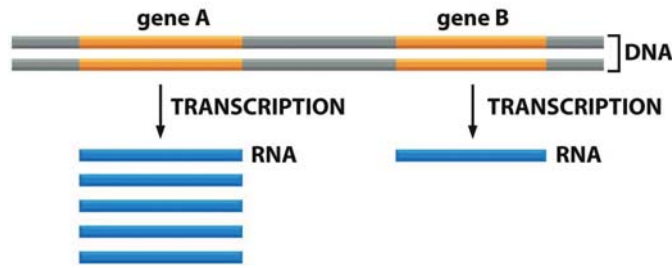


Figure 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

How?

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Transcriptional Regulation

Gene expression in both prokaryotes and eukaryotes is regulated by:

●
Which bind specifically to:

●
Gene regulatory proteins can turn genes:
-ON; Positive regulators; ●

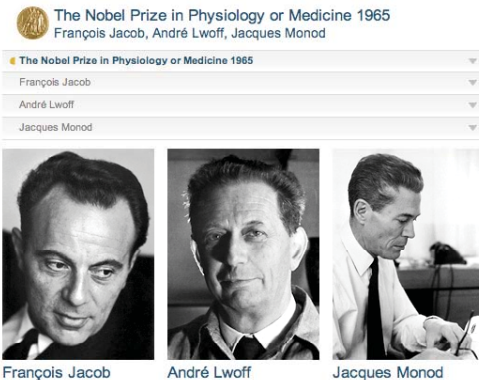
●
-OFF; Negative regulators; ●
(eg. *Trp* operon)

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Transcriptional Regulation

Gene regulatory proteins were discovered using bacterial genetics.

*“Anything found to be true of *E. coli* must also be true of elephants.”*
Jacques Monod



The Nobel Prize in Physiology or Medicine 1965 was awarded jointly to François Jacob, André Lwoff and Jacques Monod "for their discoveries concerning genetic control of enzyme and virus synthesis".

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Bacterial Gene Regulation

E. coli :

- unicellular prokaryote
- one chromosome of circular DNA
- encodes about 4300 proteins
- many transcriptionally regulated by food availability

Prokaryotic feature:

- Multiple genes can be transcribed into a single RNA molecule

●

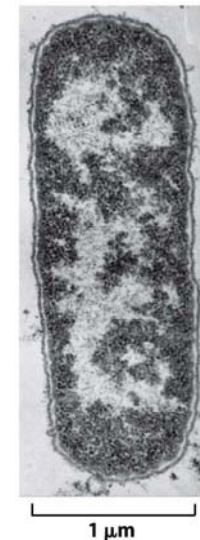


Figure 1-18b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Example 1: The Tryptophan Operon

Tryptophan operon

- Five genes
- Encode enzymes for tryptophan biosynthesis
- Transcription regulated by a single promoter

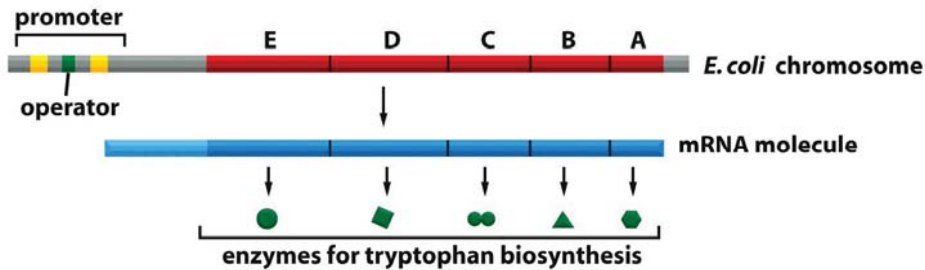


Figure 7-34 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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The Tryptophan Operon

Tryptophan (*Trp*) operon promoter

Tryptophan repressor binding blocks promoter access

-
-

BUT, Tryptophan repressor DNA-binding activity is regulated:

-

Repressor and operator provide a simple switch to control tryptophan biosynthesis according to the availability of free tryptophan

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The Tryptophan Operon

Tryptophan (*Trp*) operon promoter

Two protein-bound states:

1) Bound by RNA polymerase

-

2) Bound by a tryptophan repressor protein

-

The tryptophan repressor binds a specific DNA sequence of the promoter called an

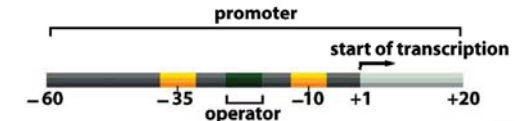


Figure 7-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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The Tryptophan Operon

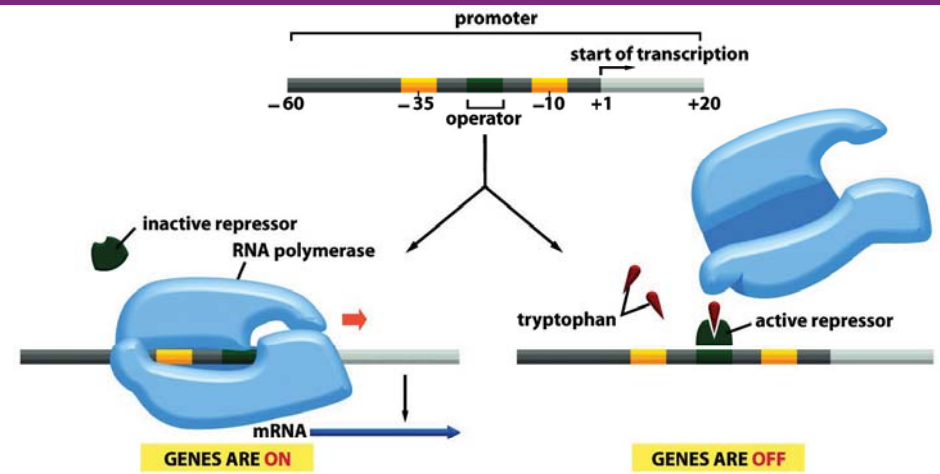


Figure 7-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

• Tryptophan Levels

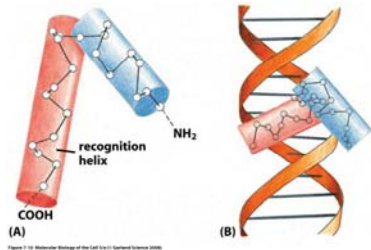
• Tryptophan Levels

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Example 1: The Tryptophan Operon

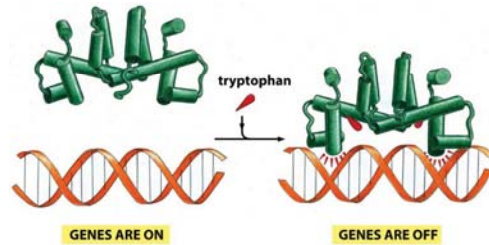
Tryptophan repressor contains a ● DNA binding motif (most common DNA-binding motif)

Helix-Turn-Helix



Binds in ● groove of DNA double helix

Tryptophan Repressor



Tryptophan binding induces ●



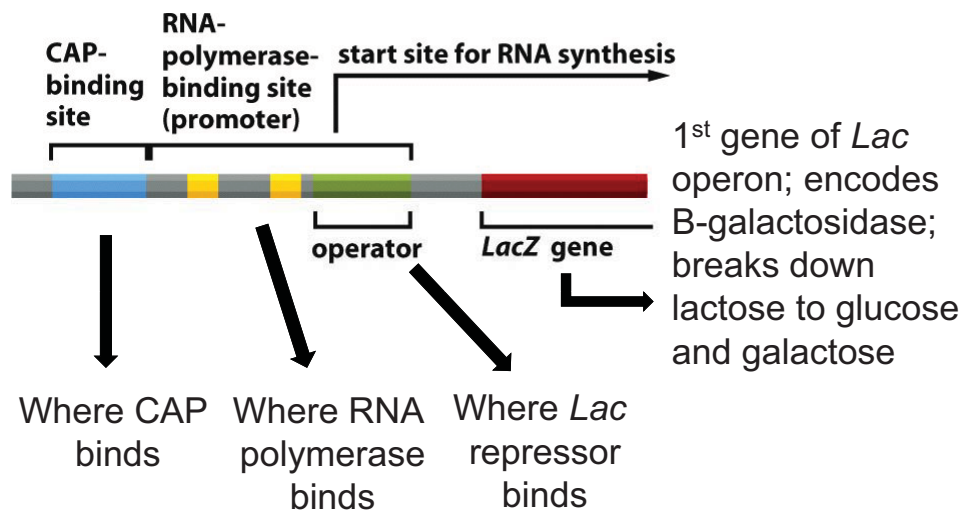
Example 2: The *Lac* Operon

E. coli *Lac* operon:

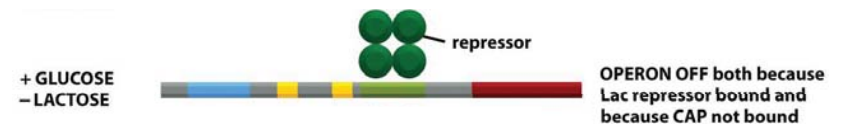
- three genes required for transport of lactose into the cell and for its catabolism
- enables use of lactose in the absence of glucose
- dual regulation: both positive and negative control

- 1) Activator: ● Promotes *Lac* expression ●
- 2) Repressor: ● Inhibits *Lac* expression ●

The *Lac* Operon



The *Lac* Operon



When lactose levels are ● Lac repressor is bound to the operator

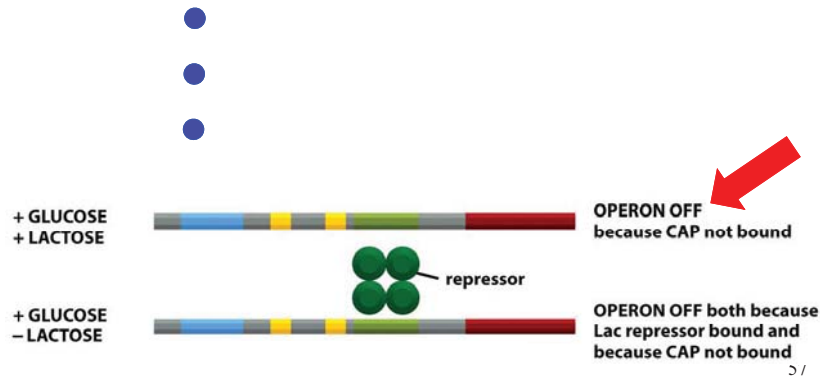
Lac operon gene expression is ●

Increased lactose removes repressor from operator- How?

The *Lac* Operon

Increases in lactose increase levels of allolactose, related to lactose; requires B-galactosidase

Allolactose binds to Lac repressor:



The *Lac* Operon

- CAP DNA-binding activity is activated by
 - glucose - How?
- Decreasing glucose levels increase the levels of a signaling molecule called •
- cAMP binds CAP protein:
 -
 -
 -
- CAP recruits RNA polymerase to the *Lac* promoter

The *Lac* Operon

Why need an activator?

- RNA polymerase binding is inefficient to *Lac* promoter
- Efficient RNA polymerase binding to *Lac* promoter requires CAP to be bound
- CAP contains a helix-turn-helix DNA binding domain



- How is CAP binding regulated?

The *Lac* Operon

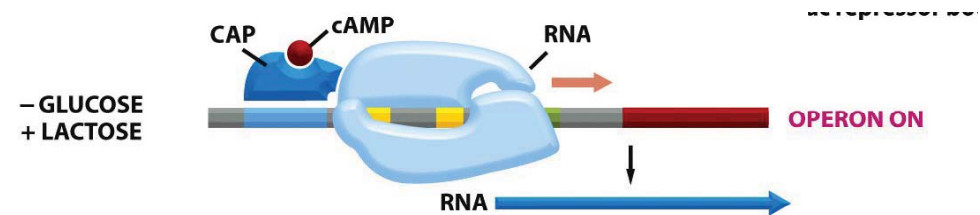


Figure 7-39 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The *Lac* Operon

Question 1: Under conditions where both glucose and lactose levels are low you would expect expression of the *Lac* operon to be:

- A) ON
- B) OFF