CSB
Cell & Systems Biology

BIO230H/BIO255H

From Genes to Organisms

Professor Darrell Desveaux

- Section 1
- Regulation of Genome Expression

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

Professor Tony Harris

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

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

Dr. Chris Garside - Lecturer & Lab/Course Coordinator – BIO230

Dr. Melody Neumann – Senior Lecturer and Lab Coordinator – BIO255

Dr. Kenneth Yip –Evening Lecturer

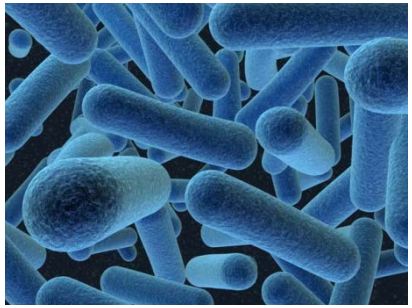
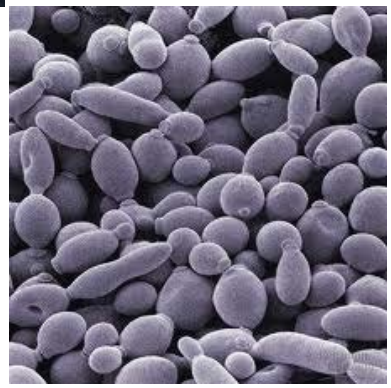
Ms. Nyla Maharaj Cabrera - Course Administrator

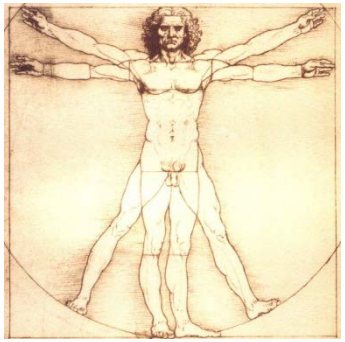
Ms. Tatjana Vasic/Karen Xu - BIO230/255 Lab Technicians

BIO230 Teaching Assistants

BIO230/255

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

Welcome to BIO230/255!

Dr. Chris Garside

Course and Lab Coordinator

Assumed Background

- Content covered in the **prerequisite** course BIO130H1.
- A detailed list of concepts and topics covered can be found on the BIO230H1 Blackboard site.
  - Very little lecture time will be spent reviewing this background material.
  - It is therefore essential that you have a firm understanding of this content.
  - We therefore strongly recommend that you review this material.

Evaluation

30% Laboratories, Annotated Bibliography, and Quizzes

Labs

13 % lab work

Quizzes

6 % lab quizzes

2 % textbook reading on-line quizzes

Assignments

4 % annotated bibliography

5 % revised annotated bibliography

70% Exams

30% Mid-term (Mon. Oct. 26: 5:30 – 7:15) covering Section 1 of the lectures*

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

** Note: the BIO230 midterm is in the evening. If this time conflicts with a lab/lecture/tutorial from a different course, come to the BIO230 office and we will provide a make-up time. However, if any other course schedules a midterm exam during your BIO230 lab time, you must go to that course office to obtain permission to write a make-up.*

Your BIO230 Learning Tools…

Course Website

Blackboard (Bb) site on Portal

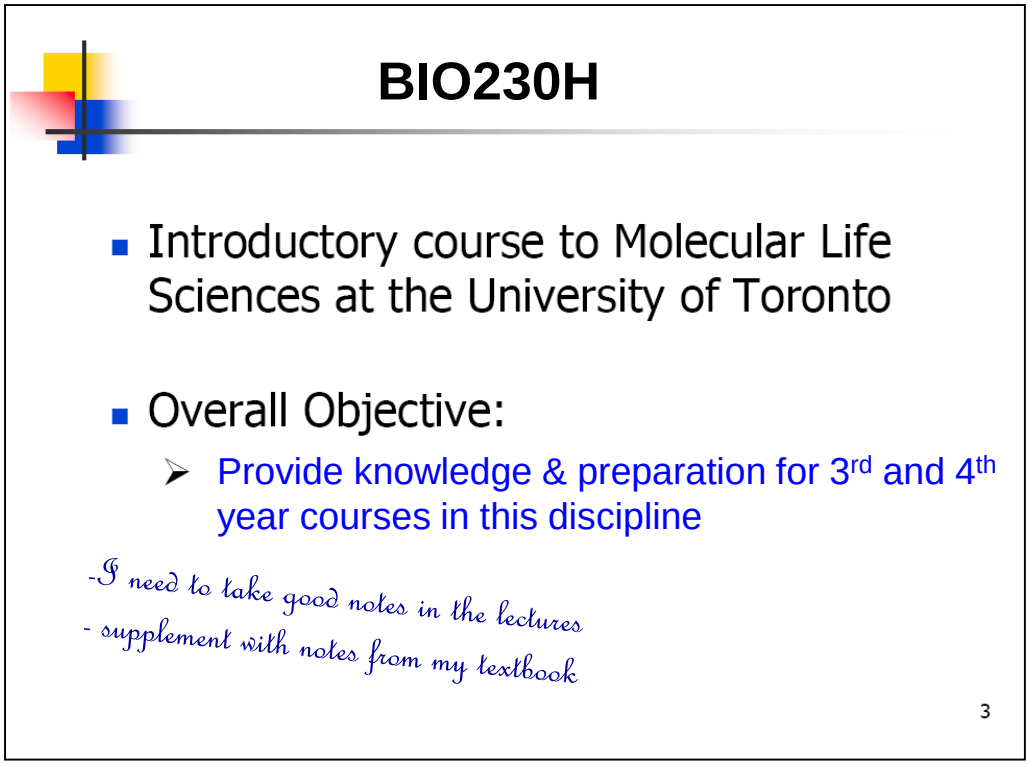
- Course News
- Lecture Notes
- E-Textbook Reading Quizzes
- mp3 Lecture Recordings
- Extra Lab Notes
- Electronic Discussion Board for Questions
  - Lecture questions answered by Dr. Ken Yip

- **Visit frequently**

Lecture Notes

Guided notes

- Fill-ins of key points (provided in lectures)
- You will need to add additional notes on your own
- Supplement and clarify with custom textbook readings

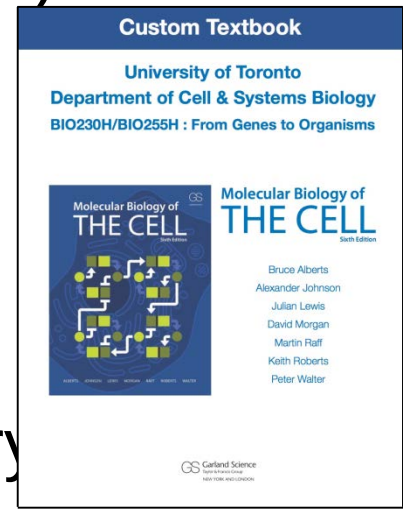


iClickers

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

BIO230 Custom Textbook

- Derived from: Molecular Biology of the Cell, 6th ed. Alberts *et al*. 2015. Garland Science, New York.
- This is an e-text with a 6 month rental (30 usd)
  - Download here www.garlandscience.com/bio230
- Midterm test and final exam will include material from the custom text and lecture material
  - Note: final exam will also include laboratory material
- Associated student resources are available from: www.garlandscience.com/MBOC6-students



Why custom e-text

- Limit material to what we want you to know
- Supplement lectures/different presentation
- Opportunity to deepen learning

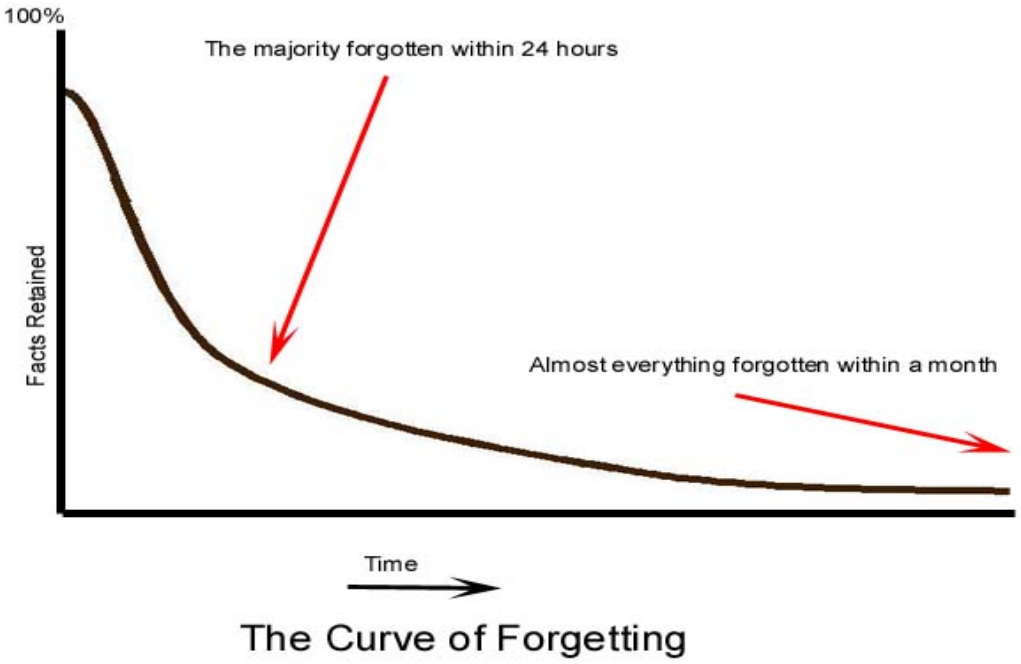


Figure 1. Curve of Forgetting

Textbook

- BIO230 Custom e-text 6 month rental derived from: Alberts B, Johnson A, Lewis J, Raff M, Roberts K, and Walter P. Molecular Biology of the Cell 6th Ed. New York: Garland Science; 2014. p 1464
- Download the etext by following this link: www.garlandscience.com/bio230
- Associated student resources are available from: www.garlandscience.com/MBOC6-students
- 4 copies of Molecular Biology of the Cell 6th Ed. New York: Garland Science; 2014. p 1464 on Reserve at Gerstein library

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 Discussion Board

- Lecture, Lab, Annotated Bibliography, and Administration Forums

- For lectures, clearly label your thread with the slide # and the correct lecture thread
  - Before posting, please check to make sure your question has not already been addressed

Bonus features…..

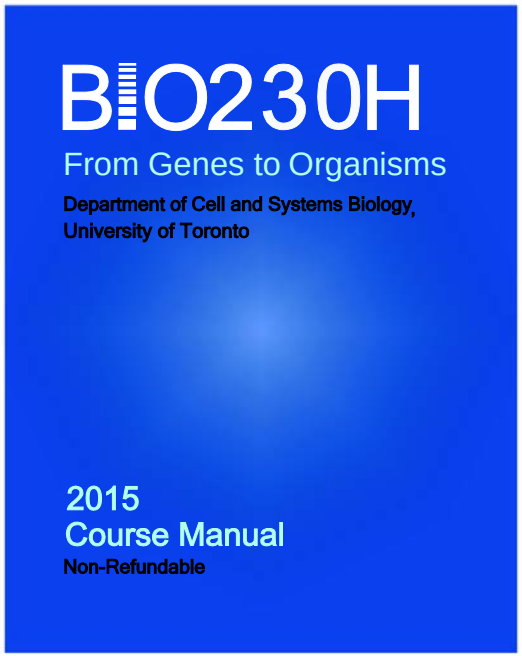
- High quality mp3s posted after lectures
- Discussion board questions answered regularly
- 4 copies of complete textbook on reserve in Gerstein
- 3 hours of lecture time per week
  - Extra hour used in a variety of ways
    - Tutorial/Q and A
    - Lecture spillover

Labs

- Lab topics:
  - Gene regulation Part I.
  - Gene regulation Part II.

  Annotated Bibliography
  Introduction and Exercise
  - RNA isolation and electrophoresis (*E. coli*).
  - Live cell imaging.
  - Role of the cytoskeleton in development (transgenic *D. melanogaster*).

What you need for labs…



2015 Course Manual
U of T Bookstore
~ $13

Safety goggles
Available at
bookstore

Lab coat
$16

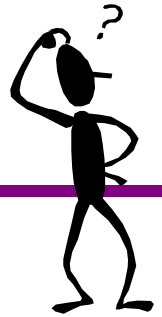
- Sold by CSBGU
  - 15/09 2:00 - 2:20 pm @ CH
  - 15/09 5:40 - 6:00 pm @ ES1050
  - 17/09 2:00 - 2:20 pm @ CH
  - 22/09 2:00 - 2:20 pm @ CH
  - 22/09 5:40 - 6:00 pm @ ES1050
  - 24/09 2:00 - 2:20 pm @ CH

When do labs start?

- Lab section Pxx**01**x – cycle 1 – week of **Sept. 21st**
- Lab section Pxx**02**x – cycle 2 – week of **Sept. 28th**
- All labs start at 10 minutes after the hour
- No Quiz in Lab 1!
  - **but**, there is an assignment due at the beginning of the lab…see course manual and complete

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
  - Bring your ROSI timetable



Where can I get help?

- Discussion Board - monitored by course staff
- Tuesday lecture tutorials with course Professors
- Ms. Nyla Maharaj Cabrera - administrative issues
- Dr. Chris Garside - lab content and other academic issues
  - Drop-in Hours: Tuesdays 2:00 pm – 4:00 pm

Final Reminders

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

Again, welcome to BIO230/255!!!

Cell & Systems Biology

facebook

https://www.facebook.com/csbundergrad

-research news-

-undergrad events-

-career advice-

Join our community!

BIO 230

Section 1:

Regulation of Genome Expression

BIO 230

Lecture 1 :

Review and Overview

1) Review of Cell Types

2) Genomes

3) Regulation of Genome Expression

Readings (Alberts *et al.* custom text)

Pages 1-14; 93-97

…first a little review

Divided according to cell types

- The tree of life has three primary branches

Prokaryotes: eubacteria (bacteria) & archea bacteria (live in extreme environment)

Eukaryotes: animals, plants, fungi etc.,

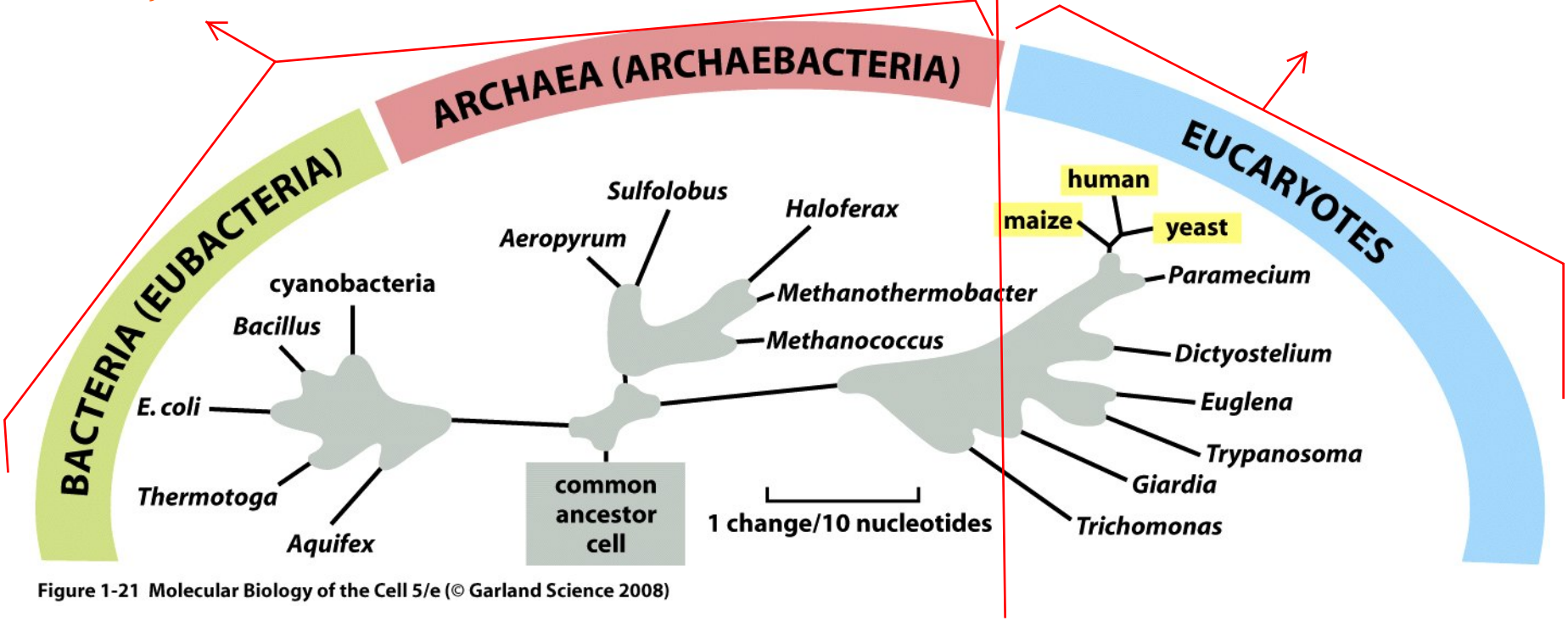


Figure 1-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

singled-cells,
no nucleus or organelles

singled or muticellular,
have nucleus and organelles

archaea live in extreme environments, are missing a nucleus, BUT are very similar to eukaryotes; especially in regards to gene expression

Two Main Cell Types

Archaea bacteria: slightly different; live in places we don't want to live in, e.g. hot springs, sewage treatment plant. Although they look like eubacteria and have similar prokaryotic structure, the way the deal with genetic information is very different from eubacteria, actually closer to eukaryotes.

1) Prokaryotic Cells

Eubacteria: typically what we refer to as "bacteria".

- Eubacteria, and archaea
- Single-celled
- Lack nucleus and organelles

Archaea bacteria are thought to have diverged from a common ancestor, from the eubacteria and at the same time as eukaryote did. Archaea bacteria is a distinct branch on the tree of life. The cellular structure and single-celled feature is what unites them with eubacteria as prokaryotes.

Biofilms are notorious at the hospital for being safe haven for bacteria hiding inside, making it difficult to clean and sanitize. Biofilms are protective coverance for bacteria communities.

Prokaryotic organisms often can be stuck together by sugar molecules and form biofilm.

A prokaryotic cell is just a cytosol package by a plasma membrane. Most of them have a bi-layer that encases the organism. Their sugar-coating for their cell-wall. Their cell-wall is at the outside of their plasma-membrane that protects bacteria from environmental condition.

2) Eukaryotic Cells

- Plants, fungi, animals, humans
- Single-celled or muticellular

e.g. yeast

e.g. human, some fungi

- Have nuclei and organelles

Nucleus houses genetic materials

membrane bound organelles can carry out specific functions.

Prokaryotic Cell

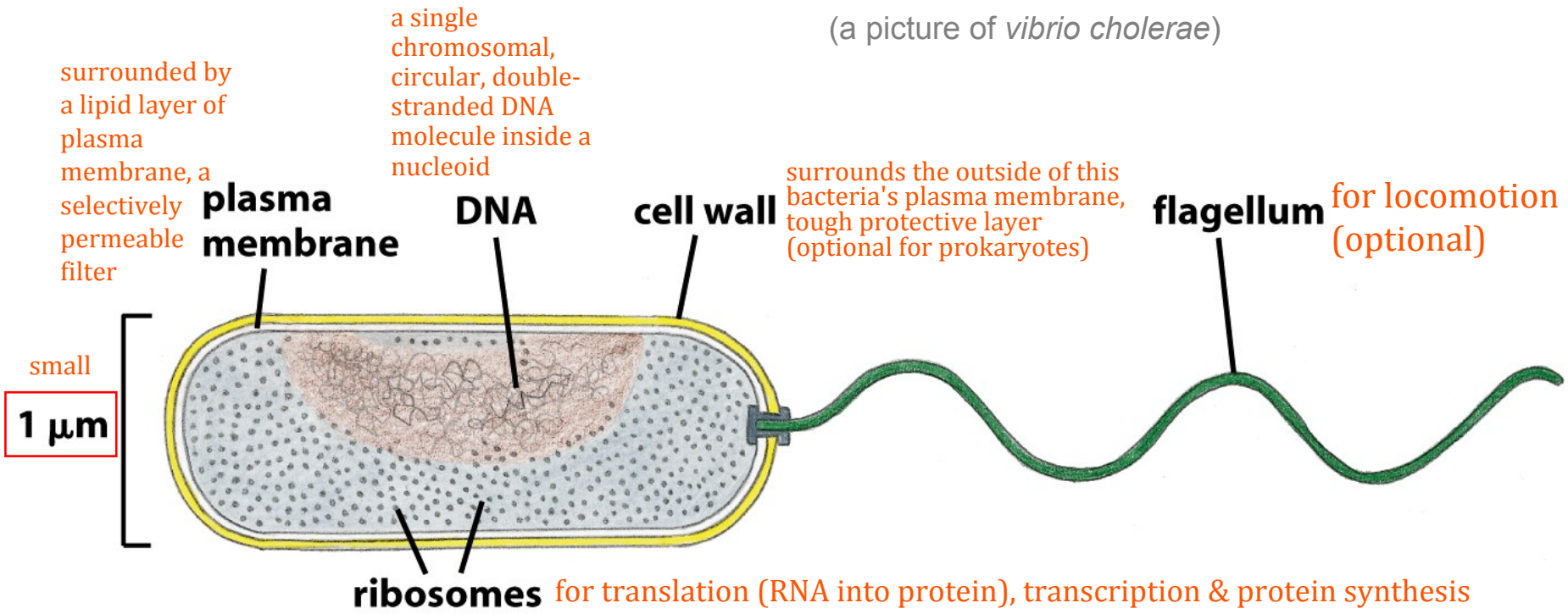


Figure 1-18a Molecular Biology of the Cell 5/e (© Garland Science 2008)

- One compartment houses all the genetic material;
- Transcription, translation, replication, all happen in the same compartmet for prokaryotes;
- Prokaryotes have the most biochemical diversity, with tons of reactions producing different compounds much more than eukaryotic organisms, e.g. vibrio cholerae can produce toxins. They may look simple, but actually very complicated in biochemical context.

Eukaryotic Cell (a typical animal cell)

- Compartmentalization in eukaryotes: transcription and replication happen in the nucleus; translation happens in cytosol.

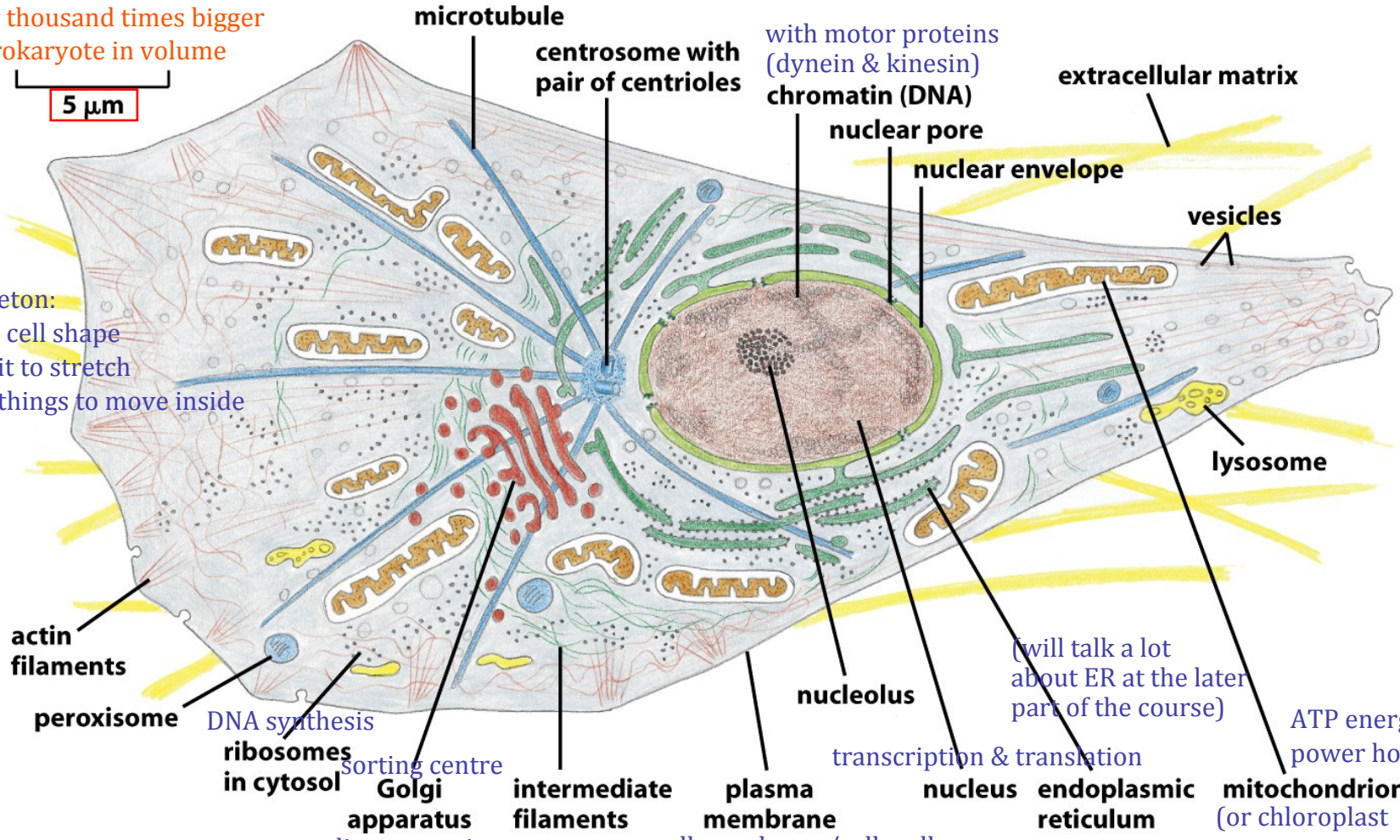


Figure 1-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Video

Endosymbiosis: ancestral eukaryotic cell was a predator, and evolved by engulfing a prokaryotic cell that later became mitochondria/chloroplast. The ancestral eukaryotic cell might look like a neutrophil in your immune system.

Phagocytosis

- E.g.. phagocytic cells
- **Video**: Alberts 5th edition
  - 16.2 Neutrophil Chase

Neutrophil: a white blood cell, hunting and eating bacteria;
Those cells are constantly moving in fluid and not static at all, with constantly changing shape, due to the fluidity of phospholipid membrane.

Animation

- http://www.xvivo.net/the-inner-life-of-the-cell/
- OR
- http://www.studiodaily.com/main/technique/tprojects/6850.html

- Appreciate how dynamic cells can be in fluid.
- Questions to focus for the 1st section of class:
How does a genome get expressed?
How does genome expression get regulated?
What are the detrimental effects if it goes wrong?

Inner Life of the Cell
Animation conception and scientific content by Alain Viel and Robert A. Lue.
Animation by John Liebler/XVIVO

Genomes

"genome" = the blueprints

-All known life forms possess a genome

When genome is not properly regulated, things can go badly, e.g. cancer, uncontrolled proliferation and cell growth. Regulation has to be kept tightly to avoid detrimental effect.

-Encodes the information to construct and maintain an organism

Human genome has 3 billion nucleotides;
nucleotides are packaged into 23 pairs of chromosomes;
chromosomes encodes 25,000-35,000 genes in each cell;
Only about 30-60% of the genes get expressed;
Depends on the cell type, different genes get expressed.

-Most genomes are made of ● DNA
(except some viruses have RNA genomes)

Viruses aren't cells, aren't alive;
but they do store genetic information

-Release of the biological information stored in the genome requires: ● genome expression

We have nuclear genome & mitochondrial genome. Plants have chloroplast genome.
Our mitochondrial genome has about 37 genes, very important, look very prokaryotic, with circular shape.
Mitochondria shows a lot of reminiscing traits of a prokaryote, as proof of endosymbiotic theory.

DNA by itself can't lead to genome expression.

Genome Expression

= the process of releasing the info stored in DNA

- The first product of genome expression is:

- **transcriptome**

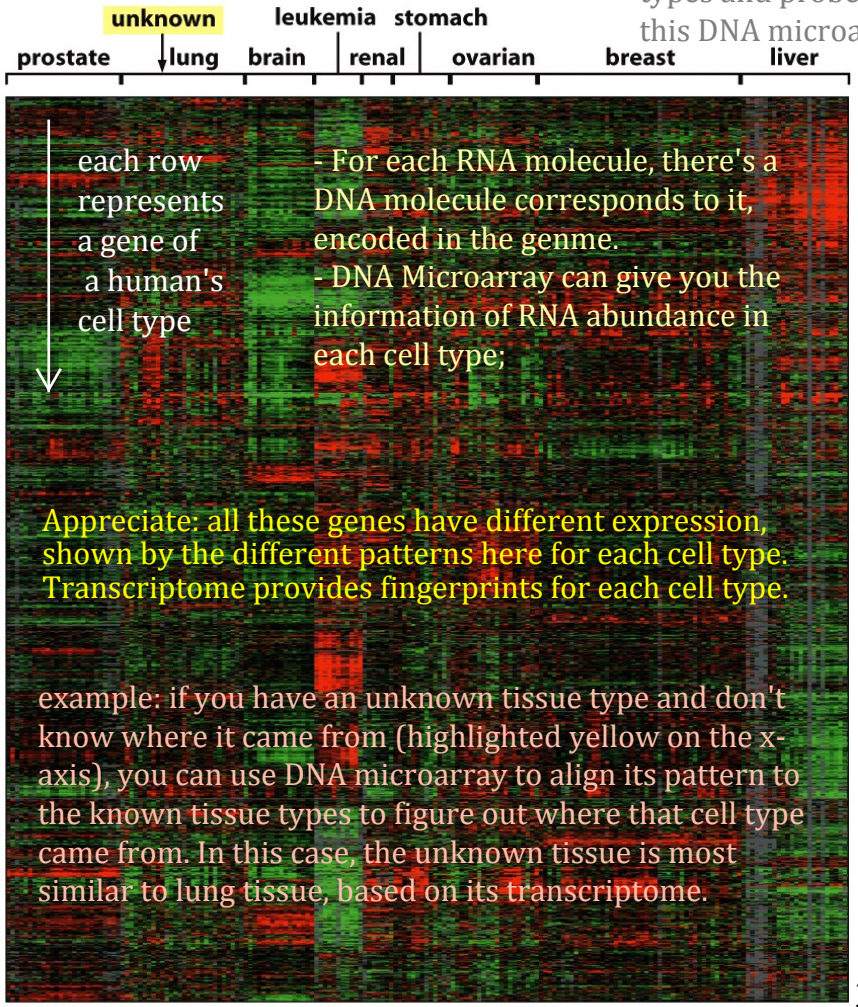
- 1st step of genome expression is transcription
- transcriptome is all of those RNA molecules produced when the cell tries to express the genes it wants to express

Transcriptome is

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

- Transcriptome varies among different cell types;
- It gives distinct properties to different cell types;
- Scientists use various techniques to visualize all the RNA molecules present in a cell at certain type; DNA microarray is one of those techniques, a bit outdated though.

take RNA molecules out of these different cancer cell types and probe them onto this DNA microarray



red: highly expressed genes

green: lowly expressed genes

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

DNA Microarray

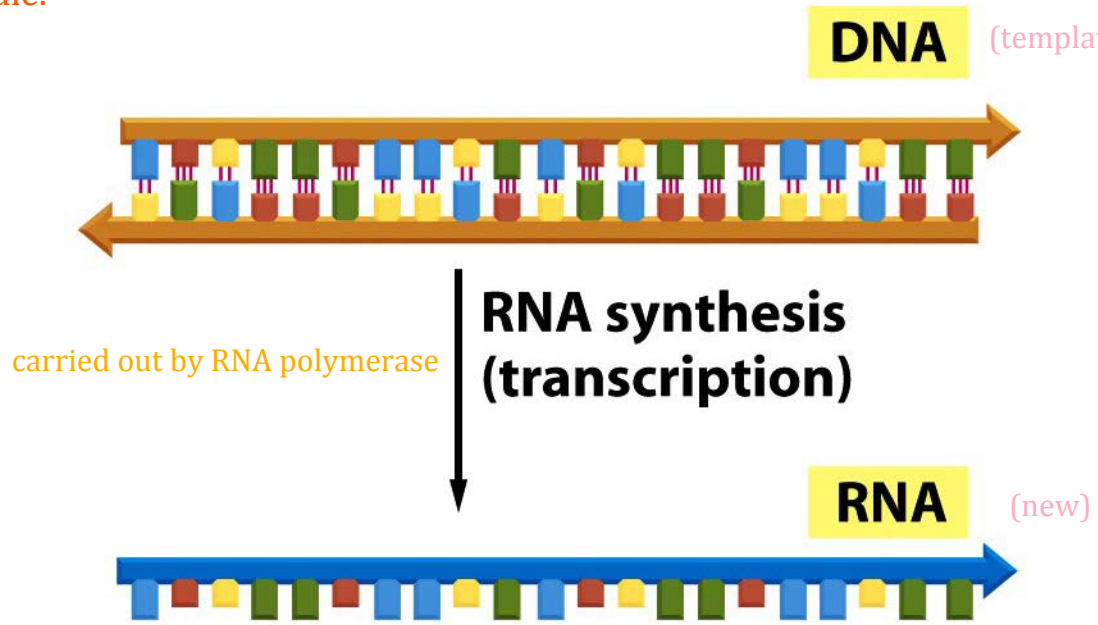
represents most of the genes of an organism

(more visual than current techniques used nowadays)

Genome Expression

The transcriptome is maintained by the process of: • transcription

transcriptome is the first product of genome expression, generated by transcription, whereby RNA is synthesized from an DNA molecule.



RNA polymerase transcribes DNA into RNA. Transcription steps include (in prokaryotes):
- initiation: promoters of DNA signaling sequence
- elongation: 5' ---> 3'
- termination: the release of the nascent RNA and the enzyme from the template, recognition of terminal signal

Genome Expression

- The second product of genome expression is:
  - the proteome
    - important for biochemical functions of cells
    - proteins responsible for metabolic reactions
- The collection of proteins in a cell; define the biochemical functions of the cell

proteome = all proteins in a cell

each dot is a protein in a cell type

some of the spots are same;
some are the same, meaning having the same proteins

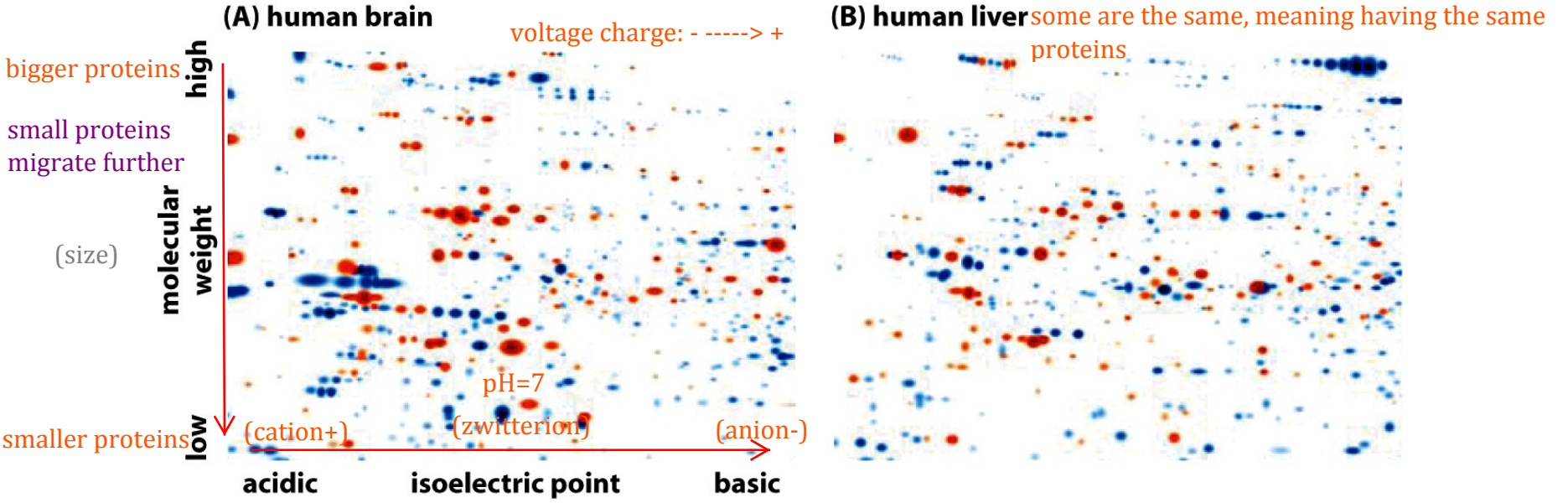


Figure 7-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

2D gel electrophoresis

- visualization of proteins in cells
- sort by size and charges based on amino acids substrates "-R" (acidic/basic/amphipathic etc.,)

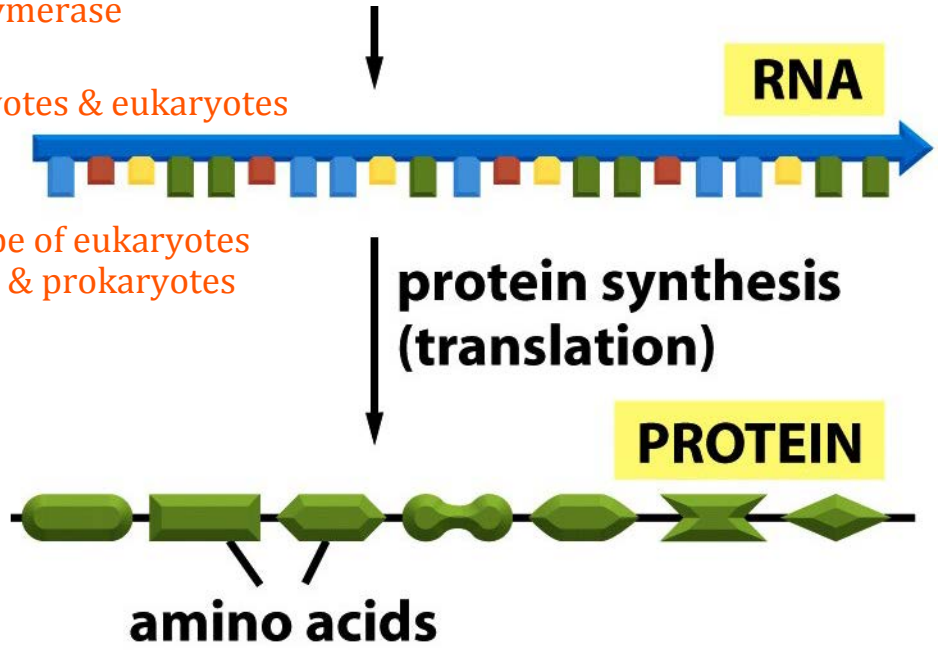
Genome Expression

The proteome is maintained by the process of:

- translation (RNA molecule gets translated into a protein by a ribosomes)

trancription by RNA polymerase
translation by ribosome
both common in prokaryotes & eukaryotes

specifics might be a bit
different for each cell type of eukaryotes
and between eukaryotes & prokaryotes



- Genome (DNA) ➔Transcriptome (RNA) ➔Proteome (Protein) also known as: the central dogma

Genome Expression

The different cell types of a muticellular organism contain ● the same genome

How to produce different cell types?

fundamental differences in structure and function of what the cells can do are decided from the regulation of genome expression.

a neuron and lymphocyte can have the same sets of genes but expressed differently then develop into two completely different cell types

their structures look completely different

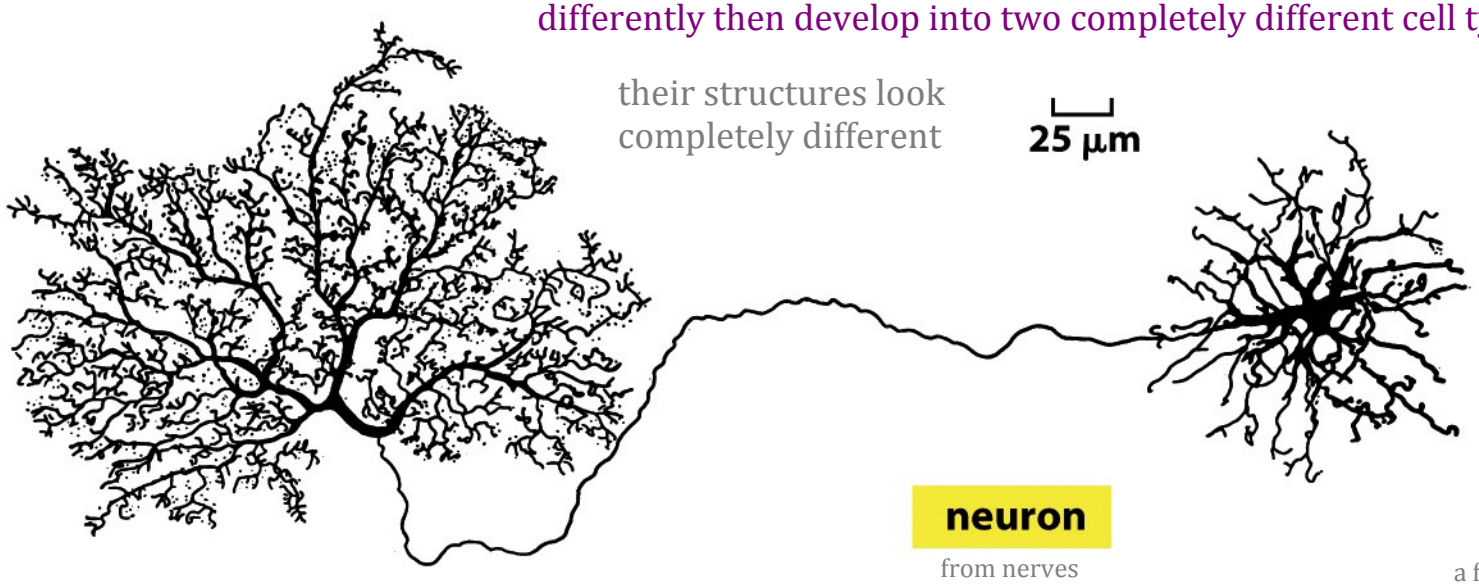


Figure 7-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Differences in ● genome expression without any significant rearrangement of the genome

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

the level of expression is different for those genes

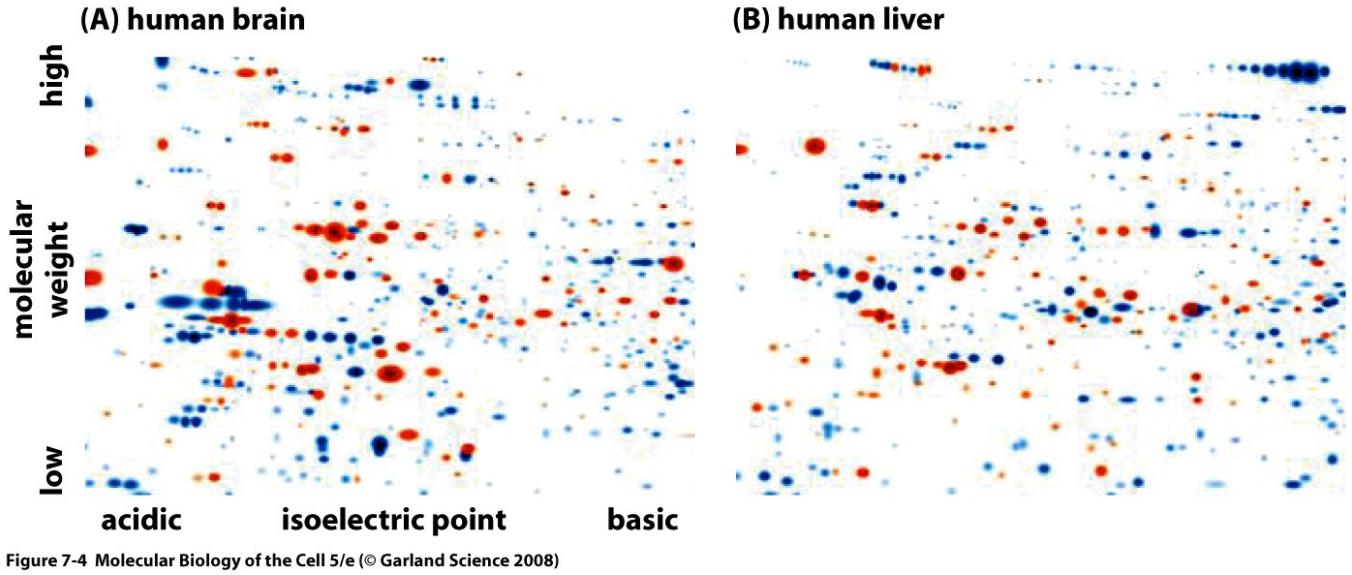


Figure 7-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

proteins:
red: common
blue: specific

even between these two tissues they have common proteins and the ones specific to their own. The specific ones are contributing to the differences of their functions

Genome expression is regulated at many steps from DNA to protein

some specific genes are only expressed in one cell type, e.g. hemoglobin in blood

Regulation of Genome Expression

(what we will cover in our 1st part of the course: regulation that occurs at each level of the central dogma)

Genome

- directly regulated by gene regulatory protein binded to the promoter, specific to the gene
- influenced by DNA organization (compact vs. open)

DNA

Organization

Transcription

Transcriptome

- quality control in post-transcriptional regulatory mechanisms, e.g. ensuring RNA stability, translating full length RNA molecule (incompleteness is detrimental)

Post-Transcriptional

RNA

Splicing

- splicing produces different proteins;
- how to regulate alternative splicing

Translation

Proteome

Post-Translational

- how to produced proteins go to the appropiate organelles

Protein

Sorting

- how proteins manifest in functions

Localization

compartmentalization: very eukaryotic specific

keyword:
regulation

proteins produce small metabolites in the cell (won't be discussed in this course)

Metabolome

Interactome

- how proteins interact with each other
- they rarely function on their own

Question

Question 1: The bacterial toxin cyclohexamide inhibits eukaryotic translation. Which of the following would you expect to be most affected in human cells treated with cyclohexamide:

A) Genome
B) Transcriptome
C) Proteome

see the last slide
RNA molecules get translated into proteins

Question

affects proteome

Question 1: The bacterial toxin cycloheximide inhibits eukaryotic translation. If a scientist wants to compare differences in cycloheximide-treated vs. cycloheximide-untreated animal cells, which of the following techniques should she use?:

A) DNA sequencing
B) DNA microarrays
C) 2D gel electrophoresis
D) RNA sequencing

proteins get sorted by size and charges

BIO 230

Lecture 2 :

Prokaryotic Transcriptional Regulation

1) Genetic Switches

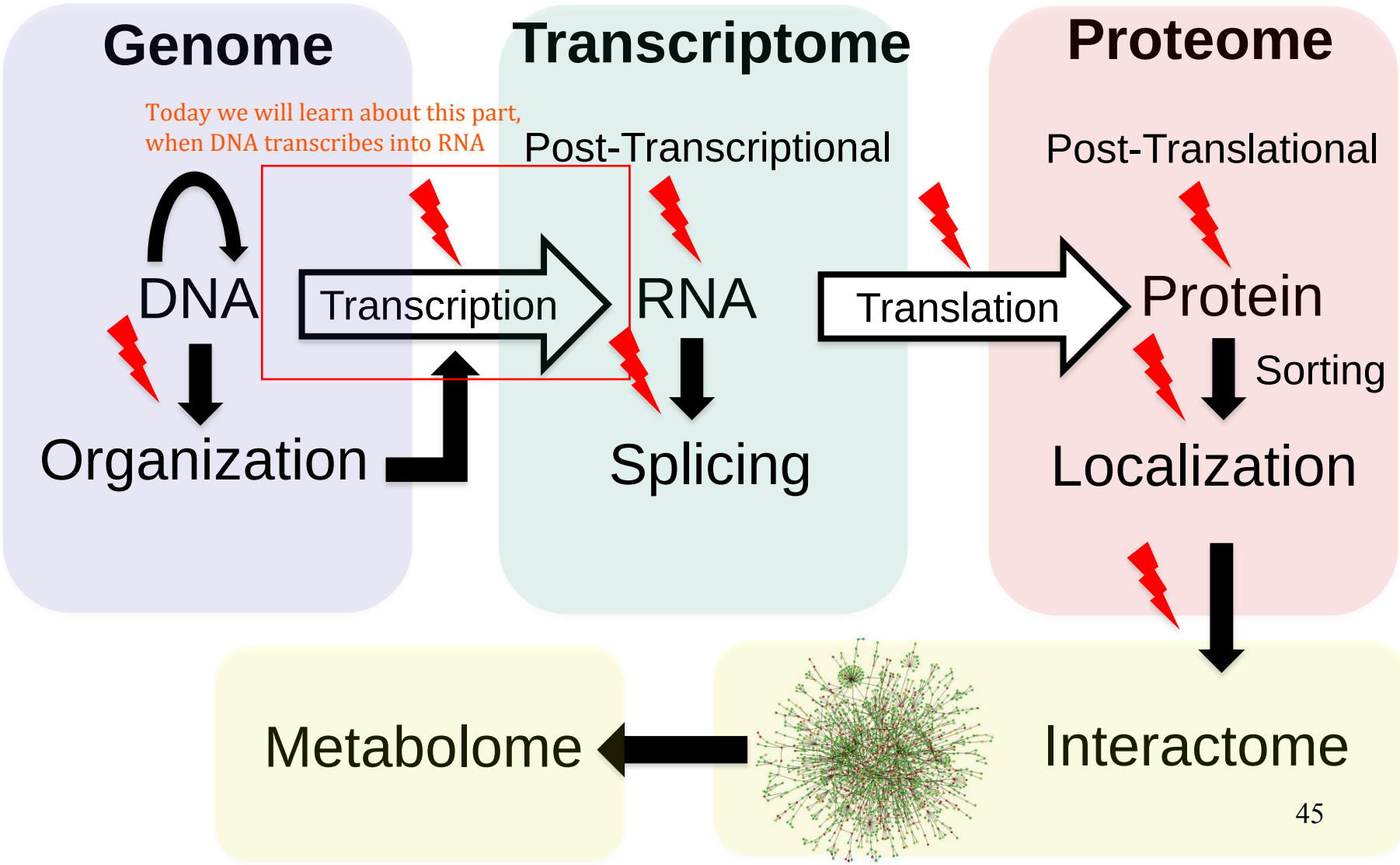
a) *Trp* Operon

b) *Lac* Operon

Readings (Alberts *et al.* custom text)

Pages 51-61; 97-102

Regulation of Genome Expression



Transcriptional Regulation

highly regulated in all organisms

Regulation of gene expression is crucial for:

Why don't organisms just transcribe all the DNA into RNA?
express them all at the same time?

- Responses to extracellular stimuli (both multicellular and unicellular organisms) Because.....

- cells need to cope with environental changes and stress
e.g. drastic temperature difference, loss of nutrient source
- they can't just waste their energy without purpose.
e.g. glucocorticoids during starvation; insulin signaling in liver and pancreatic beta cells to release glucose. Special hormones only secrete at certain situation;

- Defining cell types (multicelluar organisms)

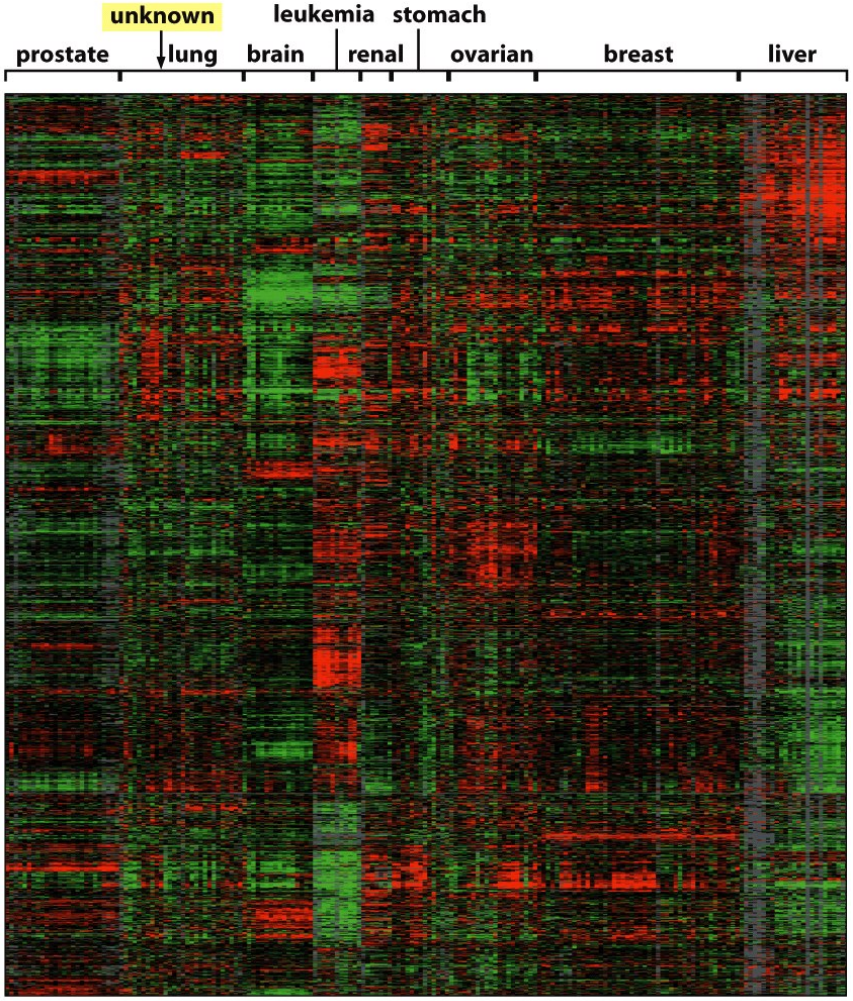


Figure 7-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcriptional Regulation

Recall that DNA is transcribed into RNA by the enzyme ● RNA polymerase (splits the DNA double helix)

RNA polymerase reads 3' to 5' but TRANSCRIBES 5' to 3'

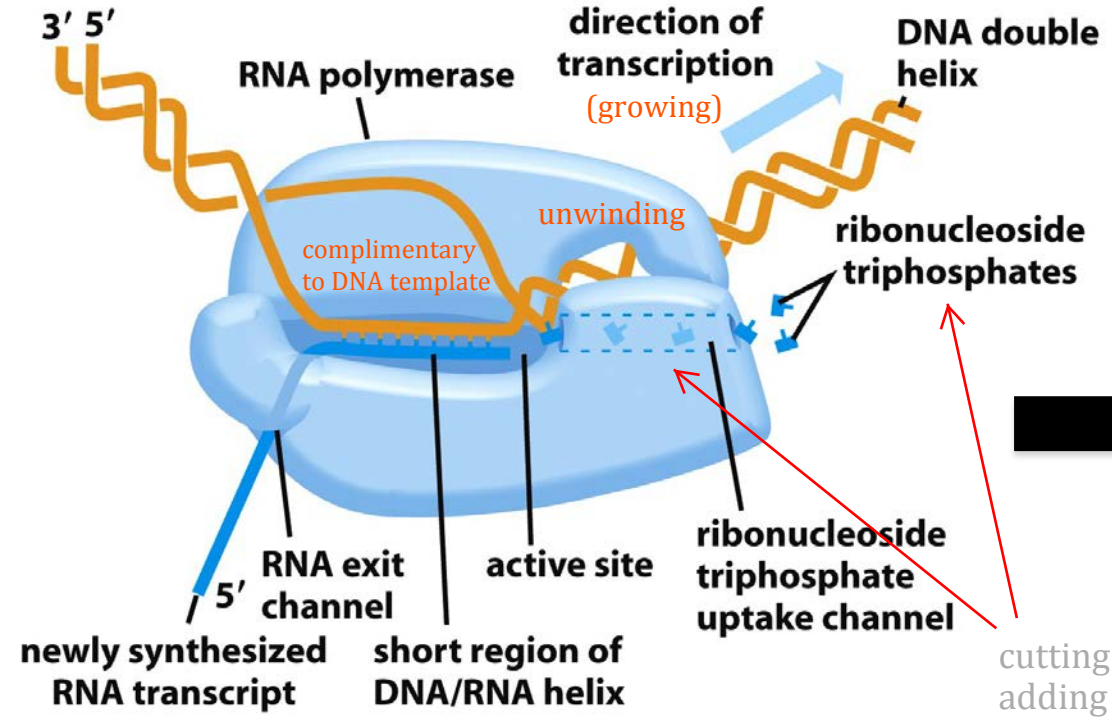


Figure 6-8a Molecular Biology of the Cell 5/e (© Garland Science 2008)

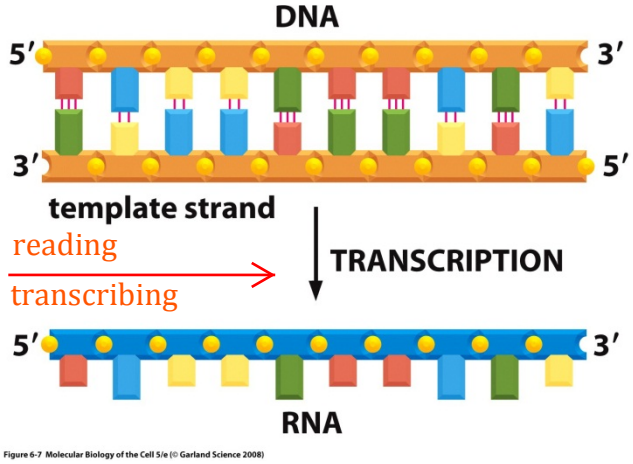


Figure 6-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

cutting off 2 phosphates, adding the rest onto RNA, 5' --> 3' growth

Transcriptional Regulation

Recall prokaryotic transcription

promoter: the region of DNA that indicates the start site of transcription.

7. RNA is released along with RNA polymerase. The process starts over again when RNA polymerase finds another promoter.

6. Transcription termination

termination signal comes from either DNA sequence or synthesized RNA structure

5. Transcription elongation

RNA polymerase works efficiently, 5' -> 3' growth

4. Once ~10 nucleotides synthesized, sigma factor is released

1. RNA polymerase holoenzyme

= sigma factor + RNA polymerase; together will find the promoter

2. RNA polymerase Unwinds DNA

Once sigma is bound to promoter

"abortive initiation": inefficient stop/start over and over again

3. Transcription begins

overcome the initiation step, release sigma factor, elongation occurs

transcription start site

RNA polymerase core enzyme

(promoter may not actually contain transcription start site). Transcription begins after promoter here.

eg. differences between neuron and lymphocyte

e.g. matching cancer cell type to tissue cell type via genome expression like fingerprints

single-cell organism only got one cell so they don't worry about this.

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

Transcriptional Regulation

Genes can be transcribed at different efficiencies

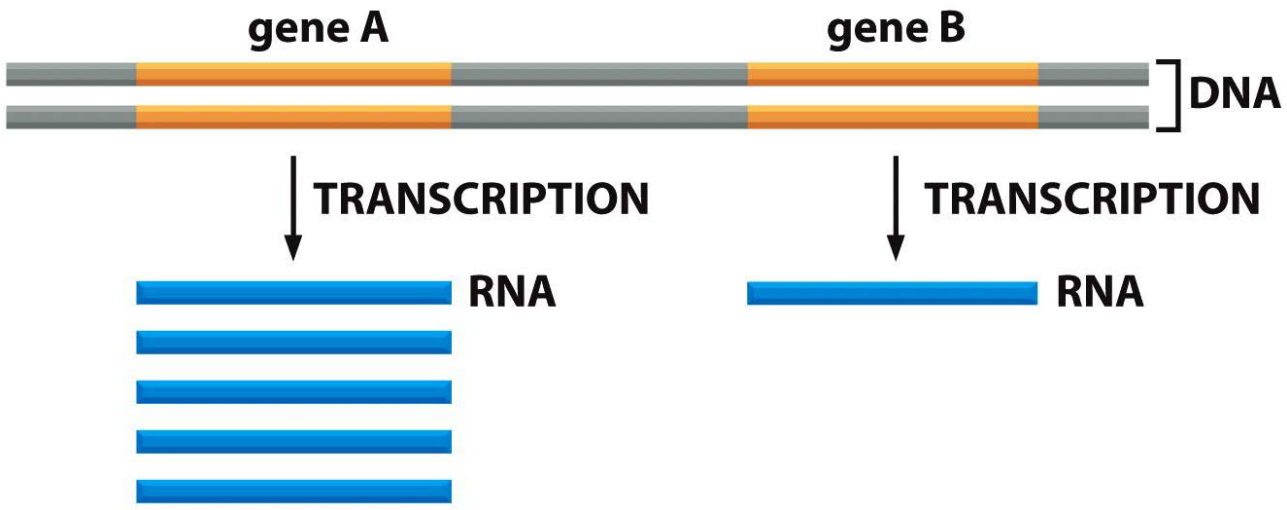


Figure 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

How?

Gene A is faster than gene B in transcription rates, so at DNA microarray you will see a lot more A than B.

Transcriptional Regulation

Gene expression in both prokaryotes and eukaryotes is regulated by:

- Gene Regulatory Proteins (transcription factors)

critical player determines the rate of transcription

Which bind specifically to:

- Regulatory regions of DNA (cis elements)

then influence the rate together (protein + a piece of DNA)

Gene regulatory proteins can turn genes:

-ON; Positive regulators; ● activators

-OFF; Negative regulators; ● repressors

(eg. *Trp* operon)

examples shown in prokaryotes because the studies started with them

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

He was right. Much of what we learned on gene expression came from studies on prokaryotes.

The Nobel Prize in Physiology or Medicine 1965
François Jacob, André Lwoff, Jacques Monod

The Nobel Prize in Physiology or Medicine 1965
François Jacob
André Lwoff
Jacques Monod


François Jacob


André Lwoff


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"*.

Bacterial Gene Regulation

Our model organism, won't have disease-causing strain

E. coli : notorious reputation for causing gas and & intestine diseases from water contamination

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

20min double in time

getting enough nutrients to carry on the rapid transcription rate

Prokaryotic feature: 5 genes for E.Coli

-Multiple genes can be transcribed into a single RNA molecule

- operon

(e.g. Tryp and Lac)

many genes lined up, transcribed into one RNA molecule

- there's no operon usually in eukaryotes;
- rare feature in eukaryotes;
- each gene transcribes to its own RNA molecule

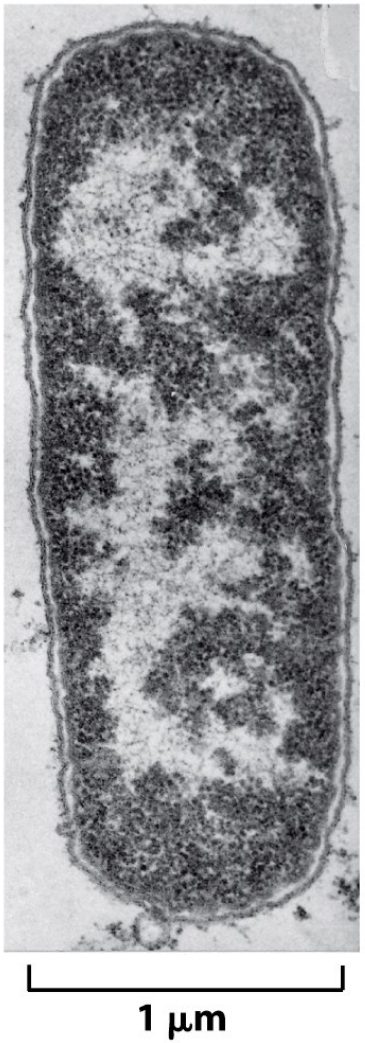


Figure 1-18b Molecular Biology of the Cell 5/e (© Garland Science 2008)

(the operon that has genes to make enzymes produce Tryp)

Example 1: The Tryptophan Operon

Turn on transcription using simple mechanism (Tryp, W)

It's important for the bacteria to have all the amino acids it needs to synthesize that 20 genes in the cell. That's why regulation on Tryp synthesis is important.

Tryptophan operon

- Five genes

if tryptophan is low, the e.coli will turn on tryptophan operon. If trp is high, e.coli will turn off trp operon

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

Bacteria only wants to turn on Tryp synthesis to make its own when there's not enough Tryp in the environment, saving energy and resources

expression of five genes regulated
and initiated by one promoter start site when sigma factor binds

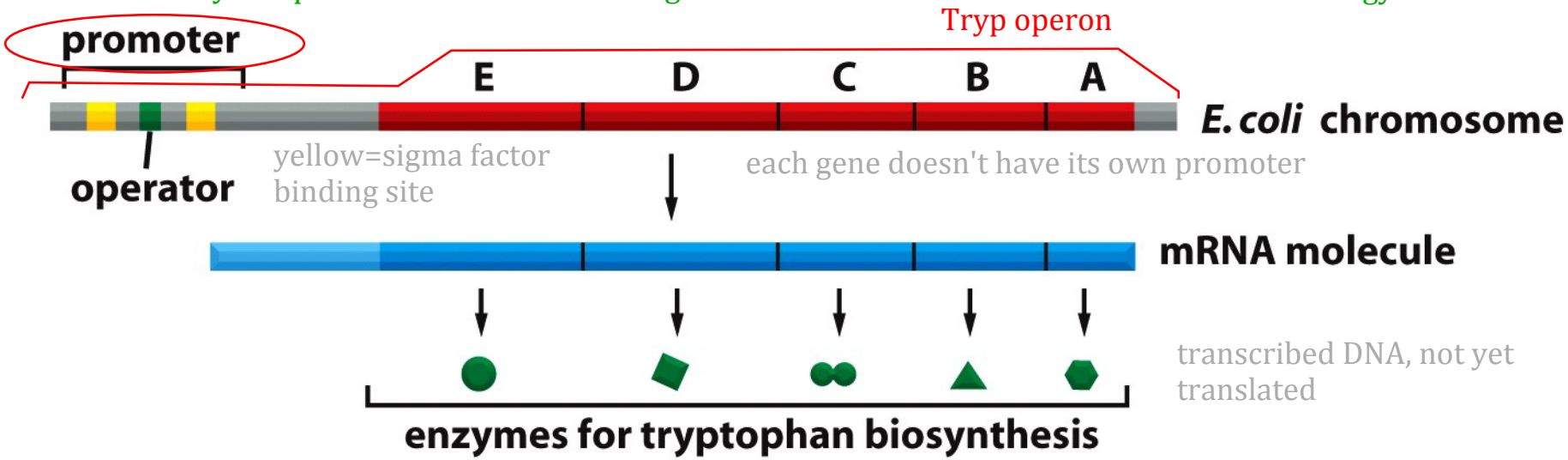


Figure 7-34 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The Tryptophan Operon

Tryptophan (*Trp*) operon promoter

Two proteins can bind to the same starting site

Two protein-bound states:

1) Bound by RNA polymerase

- Trp gene expression ON

2) Bound by a tryptophan repressor protein

negatively regulate Tryp gene expression

- Trp gene expression OFF

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

- operator

there's a competition going on between RNA polymerase and tryp repressor protein: if tryp repressor binds, it blocks RNA polymerase from binding

negative number = before first nucleotide that gets transcribe (untranscribed)

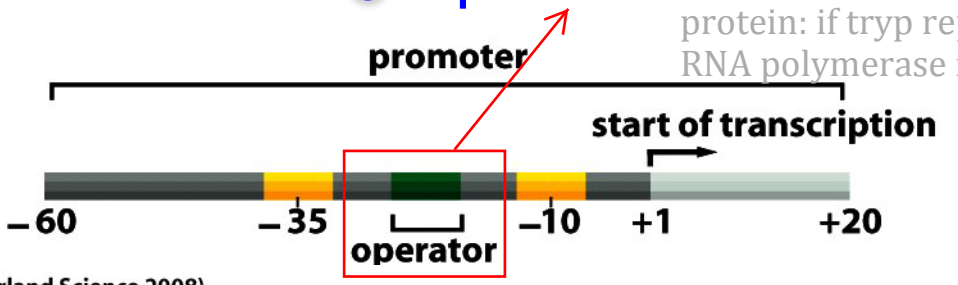


Figure 7-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The Tryptophan Operon

Tryptophan (*Trp*) operon promoter

Tryptophan repressor binding blocks promoter access

- RNA polymerase cannot bind when Tryp repressor ON
- Negatively regulates *Trp* expression

BUT, Tryptophan repressor DNA-binding activity is regulated: there are 2 molecules of free Tryp -> 2 free Tryp bind Tryp repressor protein -> Tryp repressor binds promoter DNA sequence -> Tryp repressor is ON -> RNA polymerase is OFF

- Must bind two molecules of Tryptophan to bind DNA

When there are free Tryp floating around, bacteria won't make its own.

Repressor and operator provide a simple switch to control tryptophan biosynthesis according to the availability of free tryptophan mechanism of Trp repressor in the cell is reflected by the Trp amount in the environment so the cell doesn't have to make its own when there's a lot to use from the environment

The Tryptophan Operon

e. coli - 5 genes

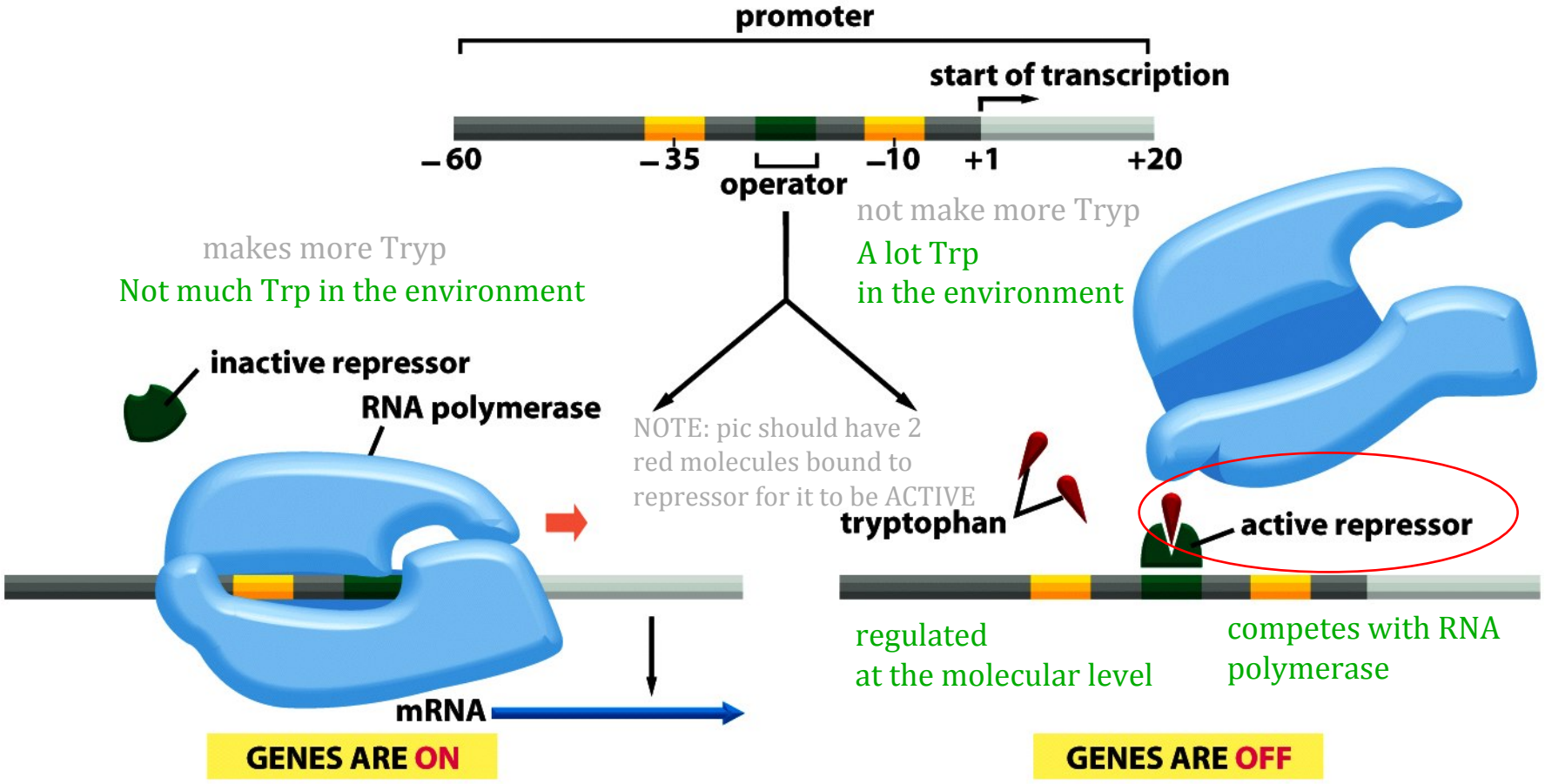


Figure 7-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- LOW Tryptophan Levels
- HIGH Tryptophan Levels

Example 1: The Tryptophan Operon

works as a dimer

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

a monomer
2nd protein structure
alpha-turn-alpha

Helix-Turn-Helix

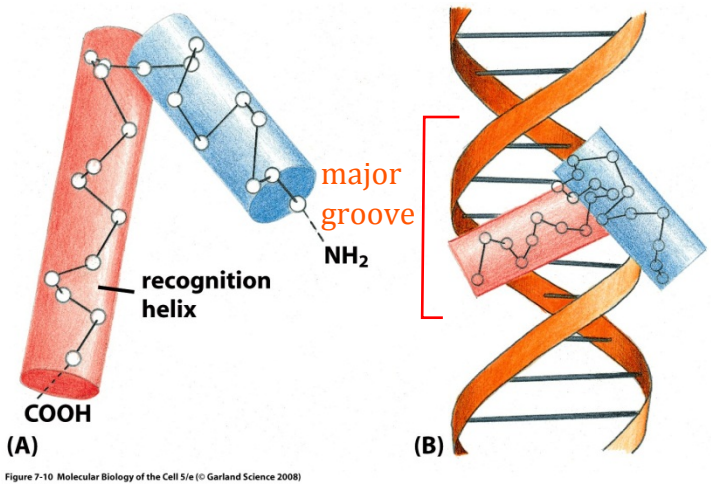


Figure 7-10 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Tryptophan Repressor

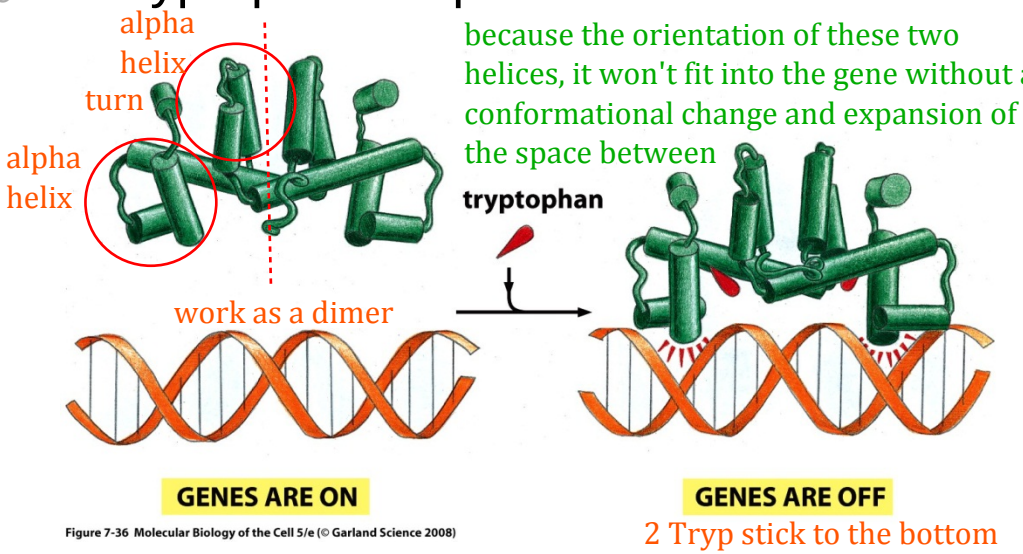


Figure 7-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

because the orientation of these two helices, it won't fit into the gene without a conformational change and expansion of the space between

2 Tryp stick to the bottom to make it flatter to fit

Binds in ● major groove of DNA double helix

represses expression of Tryp operon

Tryptophan binding induces

- ● conformational change
- ● fits in major groove

(the operon that makes transporters get some lactose and break it down)

Example 2: The *Lac* Operon

E. coli Lac operon:

(lactose: to break down lactose to make glucose available for bacteria to use)

plasma membrane surrounding the cell is not freely permeable for most molecules, e.g. glucose needs an active transporter like GLUT2 or GLUT4

- three genes required for transport of lactose into the cell and for its catabolism
  - use glucose from environment as preferable source
  - make lactose when there's not enough glucose around from outside
- enables use of lactose in the absence of glucose
- dual regulation: both positive and negative control

1) Activator: ● Catabolite Activator Protein (CAP)
   Promotes *Lac* expression ● Low glucose/ High glucose

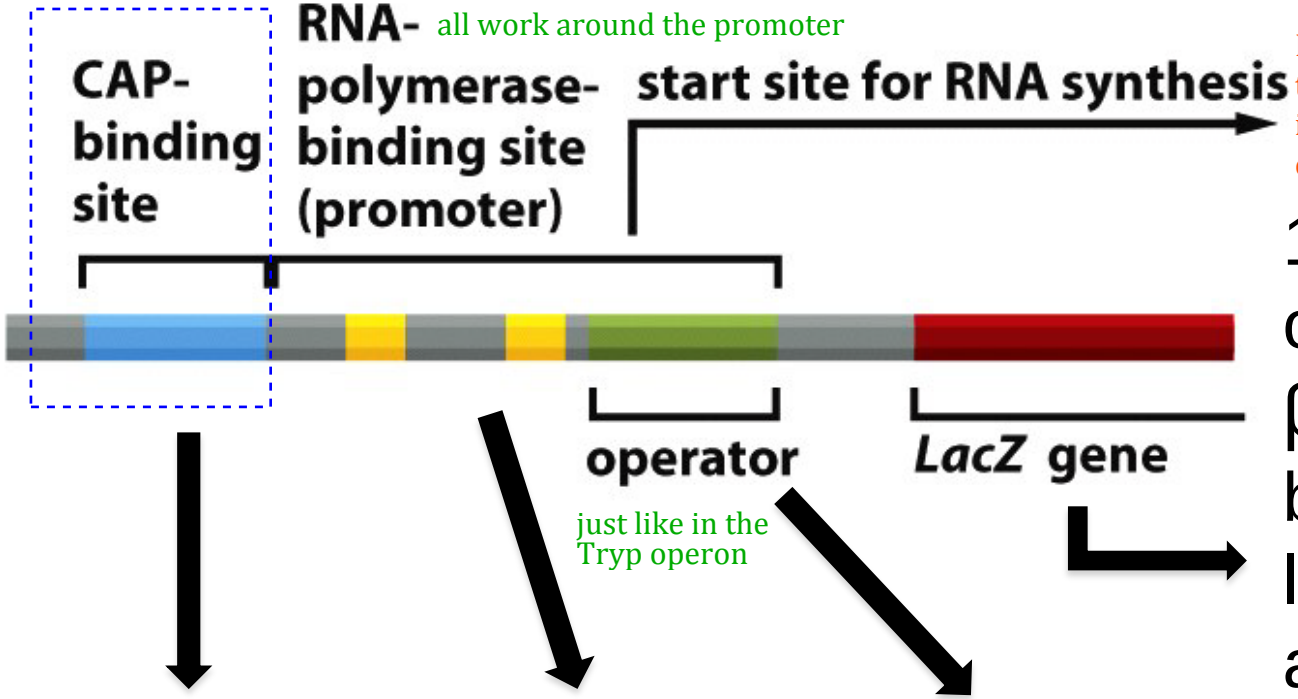
2) Repressor: ● Lac Repressor Protein
   Inhibits *Lac* expression ● Low Lactose

1. E. coli's first choice is to use glucose available already;
2. When there is low glucose AND high lactose, then it will use lactose (both of these conditions must be true, because you need to have at least some lactose there to break down)
3. To use lactose, it will transcribe the lac operon (turn on), make its own glucose available

The *Lac* Operon

Operon's definition: "all genes regulated by one promoter"

unique,
where activator binds



Lac Z: first of three genes encoded in the operon; other two genes are involved in uptaking and modification of lactose.

1st gene of *Lac* operon; encodes an enzyme β-galactosidase; breaks down lactose to glucose and galactose

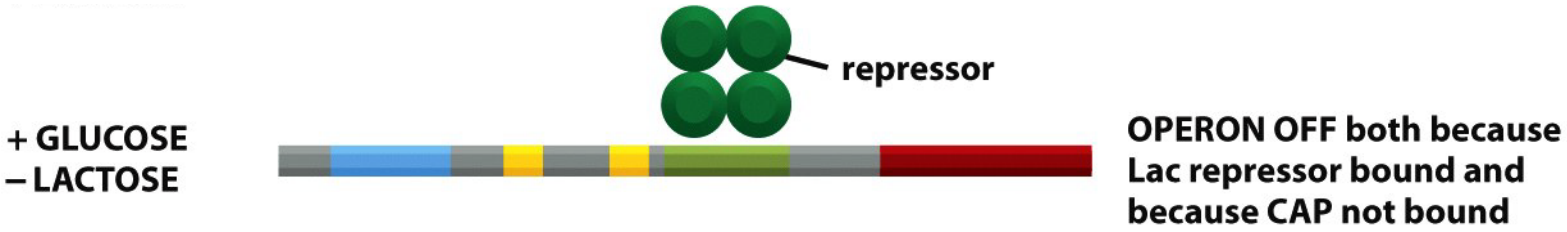
Where CAP binds

Where RNA polymerase binds

Where *Lac* repressor binds

so, what if there's low lactose? There's is always a leaky expression of the lac operon and it's never completely off. Then why bothering to express B-galactosidase to break down this non-existent lactose?

The *Lac* Operon



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

Because there is no lactose for B-galactosidase to break down into glucose and galactose

Lac operon gene expression is OFF (but never completely)

Increased lactose removes repressor from operator- How?

get ready to break down lactose

The *Lac* Operon

actually produced by β-galactosidase, only looks a bit different than lactose

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

(enzyme encoded in Lac Z of Lac operon)

Product of the 1st gene is what's responsible for turning on Lac operon, i.e. "inducer." There's a little bit of allolactose floating around in the cell so it can always switch back and forth

Allolactose binds to Lac repressor:

- Conformational change

lactose low=allolactose low
lactose high=allolactose high

- Decreases DNA-binding activity

Lac repressor falls off from the operator

- Release from the operator

If Lac repressor is not sitting on the operator, can RNA polymerase just come in and start transcribing? It's not that simple. RNA polymerase has low affinity to this promoter by itself, so CAP works as an activator to increase affinity of RNA polymerase, to help it bind to DNA sequence of 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 (most common DNA-binding motif)
- CAP contains a helix-turn-helix DNA binding domain

just like one of the monomers of a Tryp repressor protein's dimer

+ GLUCOSE
+ LACTOSE

OPERON OFF
because CAP not bound

so RNA polymerase got no one helping it to bind to promoter

- How is CAP binding regulated?

Recall: E. Coli only wants to turn on the operon when glucose is low in the environment.

(next page: the decrease of environmental glucose level increases CAP binding activity)

The *Lac* Operon

- CAP DNA-binding activity is activated by
  - low glucose - How?

both eukaryotes and prokaryotes use cAMP as signaling regulator

- Decreasing glucose levels increase the levels of a signaling molecule called
  - cyclic AMP (cAMP)

- cAMP binds CAP protein:

Glucose low=cAMP high
glucose high=cAMP low

(no need to know the specifics of this biochemical reaction for now)

  - Conformational change
  - Increases DNA-binding activity
  - Binds to CAP-binding site

- CAP recruits RNA polymerase to the *Lac* promoter

they are all located nearby of each other

The *Lac* Operon

The only time when this Lac operon is on: low glucose & high lactose

low glucose = high cAMP = cAMP increases CAP-binding = CAP binds DNA site = CAP as an activator recruits RNA polymerase = RNA polymerase can bind to promoter = Lac Operon is ON

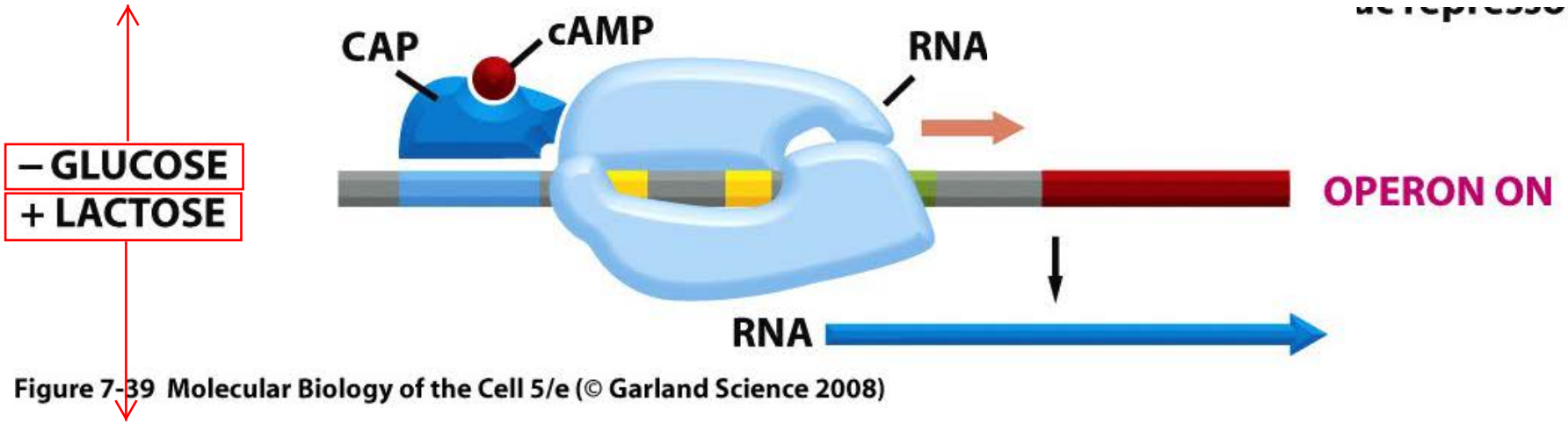


Figure 7-39 Molecular Biology of the Cell 5/e (© Garland Science 2008)

lactose high= β-galactosidase makes allolactose high = allolactose binds to the Lac repressor = Lac repressor cannot bind DNA operator site = RNA polymerase can bind promoter site = Lac Operon is ON

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

Reason:
Glucose level low -> Lac repressor will bind operator
Lactose is required to remove Lac repressor from operator in order for to bind promotor to turn on Lac operon

glucose low=cAMP hgh=cAMP binds cap=CAP binds DNA=RNA polymerase can bind
Lactose low=allolactose low=allolactose does NOT bind to repressor=repressor binds to DNA=RNA polymerase cannot bind

The *Lac* Operon

Question 2: Why is important that expression of the *Lac* operon is a always slightly on?

A) *E. coli* needs β-galactosidase encoded by the Lac operon to utilize glucose.

B) *E. coli* needs β-galactosidase encoded by the Lac operon to respond to lactose.

because it converts glucose to allo-lactose .....LacZ to convert o ...

C) RNA polymerase requires β-galactosidase encoded by the Lac operon to bind DNA.

D) The *Lac* can be completely shut down without consequence.

Prof refers to slide 61: if glucose is high and lactose low then repressor binds to DNA to shut lac operon down. However, you need a bit of B-galactosidase even if lactose is low, just in case lactose increases so that you can quickly convert it to allolactase which can bind to repressor and turn on expression to get rid of the lactose

The *Lac* Operon

Question 3: Which of the following gene regulatory proteins has decreased DNA binding activity when bound to a small molecule?

A)Trp repressor

B)Lac repressor helix-turn-helix

C) Catabolite activator protein

when lactose is around and high, it gets converted to allolactose (which is also now high) and binds to lac repressor, which undergoes conformational change, making it release its bind to DNA (decreases DNA binding so that lac operon is on and it can express for B-galactosidase that will break down lactose into glucose

	CAP (RNA polymerase activator)	RNA polymerase	*Lac* repressor	Lac Z (encodes β-galactosidase)	*Lac* Operon
Glucose + cAMP - Lactose + Allolactose +	not bound	not bound	not bound	on	OFF (not completely)
Glucose - cAMP + Lactose - Allolactose -	bound	not bound	bound	off	OFF (not completely)
Glucose + cAMP - Lactose - Allolactose -	not bound	not bound	bound	off	OFF (not completely)
Glucose - cAMP + Lactose + Allolactose +	bound	bound	not bound	on	**ON**

***β-galactosidase produces allolactose from lactose, so the two's levels are synched**
high level of cAMP indicates not enough glucose