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Teaching Gene Regulation in the High School Classroom, AP Biology,

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High Impact Fellows Project Overview

Project Title, Course Name, Grade Level

Teaching Gene Regulation in the High School Classroom, AP Biology, Grades 9-12

Team Members

Student: *Leigh Smith*

High School Teacher: *Marie Fox* School: *Broome High School*

Wofford Faculty: *Dr. Stefanie Baker* Department: *Biology*

Brief Description of Project

This project sought to enhance high school students' understanding of gene regulation as taught in an Advanced Placement Biology course. We accomplished this by designing and implementing a lab module that included a pre-lab assessment, a hands-on classroom experiment, and a post-lab assessment in the form of a lab poster. Students developed lab skills while simultaneously learning about course content. Students' progress was evaluated through a multiple choice/essay test and their lab posters that they created on the lab experiment.

Standards:

From the South Carolina Common Core Standards for high school biology:

- B – 1: Scientific Inquiry

From the College Board AP Biology Curriculum Framework:

- Science Practice 1, 4, 5, 6, 7

List of Materials Required and Costs

Students in Mrs. Fox's AP Biology class travelled to Wofford College to perform the laboratory component. The lab protocol included in this packet is for the lab as performed at Wofford College. If you are unable to take your class to a nearby college or university, Carolina Biological and Ward's Science have kits that can be used to explore the regulation of gene regulation in bacteria.

Carolina Biological Kit Information

Carolina BioKits®: Introduction to Gene Regulation: The *lac* Operon (with prepaid coupon) Item # 171027 $89.95

High Impact Fellows Project Overview

Carolina BioKits®: Introduction to Gene Regulation: The *lac* Operon (with perishables) Item # 171027P $84.95

Ward's Science Kit Information

Regulation of the *lac* Operon Activity Item # 5115370 $141.43

Sequence for Lac Operon Lessons

Day One:

1. Introduce Gene Regulation by discussing Gene Regulation Power Point.
2. Have students complete guided reading on Gene Regulation (Chapter 18 guided reading)

Day Two:

1. Review and reinforce key concepts pertaining to Lac Operon.
2. Student should complete the lac operon tutorial.

Day Three:

1. Discuss any misconceptions students may have pertaining to the lac operon.
2. Student should complete Lac Operon Simulation.

Day Four:

1. Hand out Lac Operon Lab Part One and Part Two. Discuss the important features of the lab: Constant, Controls, Independent Variable, Dependent Variable.
2. Students should make predications based on their knowledge of the lac operon about what they think should happen in the lab.
3. Discuss Lac Operon lab Part II. Explain to students that their job is to create own lab to test the principals of the lac operon.
4. Hand out Lac Operon Lab Proposal. Discuss the key features that students must have in their lab proposal.

Day Five:

1. Allow students time to work on their proposal in their group. It is key for the teacher to discuss with each groups the importance of the correct constants, controls, and variables.

Day Six:

Lab at Wofford.

Show students a college level poster while at Wofford

Day Seven:

1. After students have complete lab discuss with them the how to display their findings in a poster format.

Lac Operon Tutorial:

Watch the following two tutorial and write a one page summary of each. Also complete and record your answers the quiz questions for each simulation. Due January 7th.

1. http://highered.mcgraw-hill.com/sites/9834092339/student_view0/chapter16/the_lac_operon.html

2. http://highered.mcgraw-hill.com/sites/0073403423/student_view0/chapter12/combination_of_switches__the_lac_operon.html

Name_____ **Period**_____

Chapter 18: Regulation of Gene Expression

Overview

The overview for Chapter 18 introduces the idea that while all cells of an organism have all genes in the genome, not all genes are expressed in every cell. What regulates gene expression? Gene expression in prokaryotic cells differs from that in eukaryotic cells. How do disruptions in gene regulation lead to cancer? This chapter gives you a look at how genes are expressed and modulated.

Concept 18.1 Bacteria often respond to environmental change by regulating transcription

1. All genes are not "on" all the time. Using the metabolic needs of *E. coli*, explain why not.

2. What are the two main ways of controlling metabolism in bacterial cells?

3. *Feedback inhibition* is a recurring mechanism throughout biological systems. In the case of *E. coli* regulating tryptophan synthesis, is it *positive* or *negative inhibition*? Explain your choice.

4. What is a *promoter*?

5. What is the *operator*? What does it do?

6. What is an *operon*?

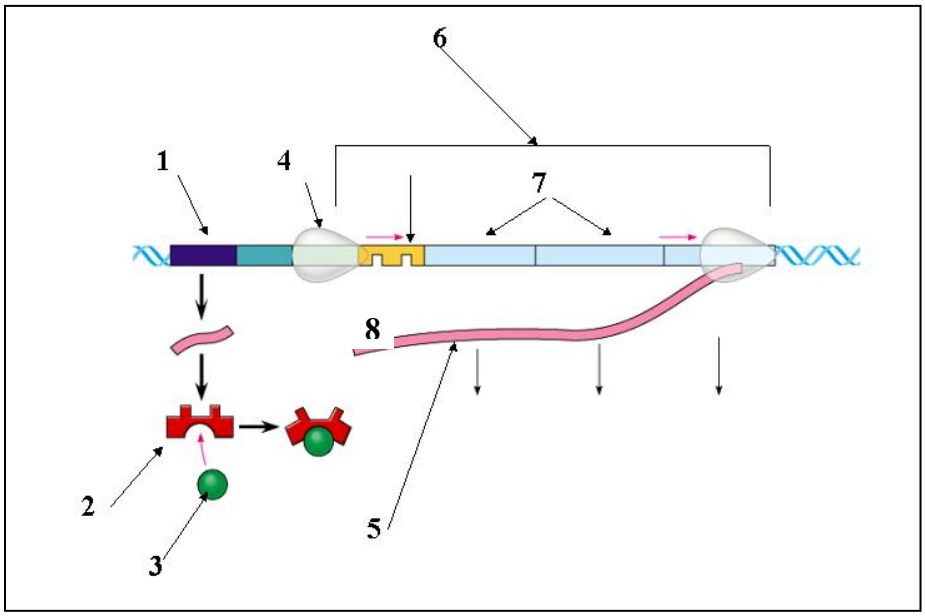
7. List the three components of an *operon*, and explain the role of each one.

8. How does a *repressor* protein work?

9. What are *regulatory genes*?

10. Distinguish between *inducible* and *repressible operons*, and describe one example of each type.

11. Label this sketch of the *lac operon* with the terms at right. Know the function of each structure.



Operon genes

Operon

RNA polymerase

mRNA

Repressor protein

Operator

Repressor

Regulatory gene

Inducer

12. Compare and contrast the *lac* operon and the *trp* operon. (Remember that *compare* means "to tell how they are similar," and *contrast* means "to tell how they are different.")

13. What happens when a repressor is bound to the operator?

14. What is *CAP*? How does *CAP* work?

15. Explain why CAP binding and stimulation of gene expression is *positive regulation*.

16. Describe the relationship between glucose supply, cAMP, and CAP.

17. How can both repressible and inducible operons be *negative regulators*?

Concept 18.2 Eukaryotic gene expression can be regulated at any stage

18. Even though all cells of an organism have the same genes, there is *differential gene expression*. What does this mean?

19. What percentage of the genes of a typical human cell is expressed at any given time?

20. What is the common control point of gene expression for all organisms?

21. Gene expression can be regulated by modifications of the chromatin. Distinguish between *heterochromatin* and *euchromatin* as to their structure and activity.

22. What occurs in *histone acetylation*? How does it affect gene expression?

23. What is *DNA methylation*? What role may it play in gene expression?

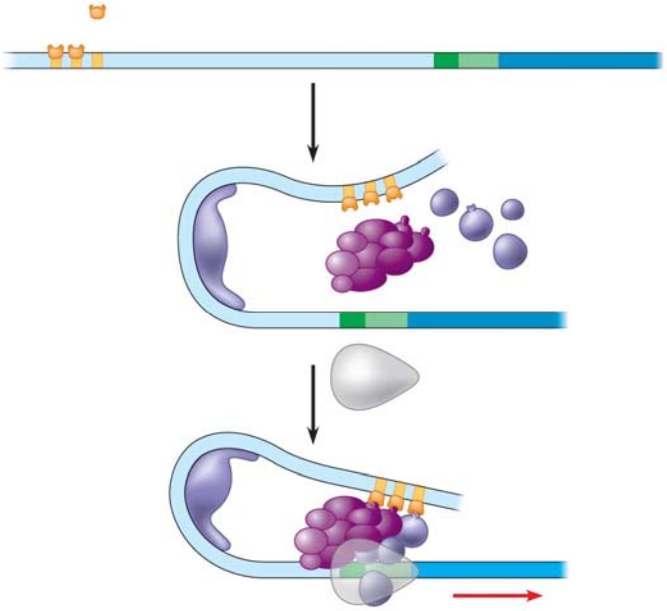
24. The inactive mammalian X chromosome is heavily methylated. What is the result of this methylation?

25. What is *genomic imprinting*, and how is it maintained? Give an example discussed earlier in human genetics.

26. Explain what is meant by *epigenetic inheritance*, and give an example of epigenetic changes discussed in the text or in class.

27. Use the sketch below to explain how enhancers and activators interact with transcription factors to affect gene expression. Label the following elements: *TATA box, promoter, gene, enhancer, activators, transcription factors, transcription initiation complex, RNA polymerase II,* and *DNA*. Then place your explanation to the right of the figure.

EXPLANATION



28. In prokaryotes, functionally related genes are usually clustered in a single operon. What has been found to be the case in eukaryotes?

29. Operons have not been found in eukaryotic cells, and the genes coding for the enzymes of a particular metabolic pathway are often scattered over different chromosomes. What is a plausible mechanism for the *coordination of gene expression*?

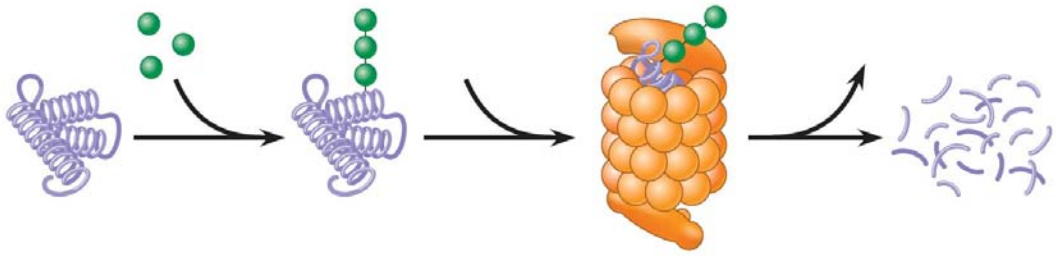
30. How can *alternative RNA splicing* result in different proteins derived from the same initial RNA transcript?

31. *Posttranscriptional control* includes regulation of *mRNA degradation*. Explain how this affects translation.

32. How can proteins be activated, processed, and degraded? Give an example or describe each process.

33. An article in *Scientific American* about *proteasomes* was entitled "Little Chamber of Horrors." Explain how proteins are targeted for degradation, and give a specific example of when this might occur.

34. How do these "little chambers of horrors" function? Annotate the sketch below to describe their action. Then explain their role in regulation of gene expression.

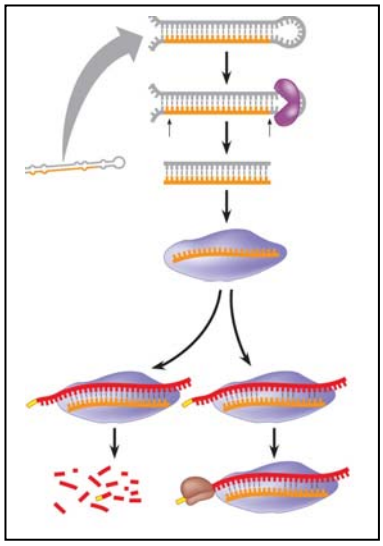


Concept 18.3 Noncoding RNAs play multiple roles in controlling gene expression

35. It is now known that much of the RNA that is transcribed is not translated into protein. these RNAs are called *noncoding RNAs*. Read carefully to discern a crucial role played by these RNAs. What is this role?

36. One of the *noncoding RNAs* that regulate gene expression is *microRNA*. On the sketch below, follow an RNA loop, called a "hairpin," from its creation. Explain the two modes of action of *microRNAs*.

    Be sure to label the location of hydrogen bonds and *Dicer*.



Concept 18.4 A program of differential gene expression leads to the different cell types in a multicellular organism

This concept deals with the regulation of gene expression in development. Animal development is also discussed in Chapter 47.

37. What three processes lead to the transformation of a zygote into the organism?

38. Explain what occurs in *cell differentiation* and *morphogenesis*.

39. Differential gene expression results from different activators in different cells. How do different sets of activators come to be present in two cells? Explain how each of these occurs:

    a. distribution of *cytoplasmic determinants*

    b. different *inductive signals*

40. What is meant by *determination*? Explain what this means within an embryonic cell.

41. What process ensures that all the tissues and organs of an organism are in their characteristic places? Where do the molecular cues that control this process arise?

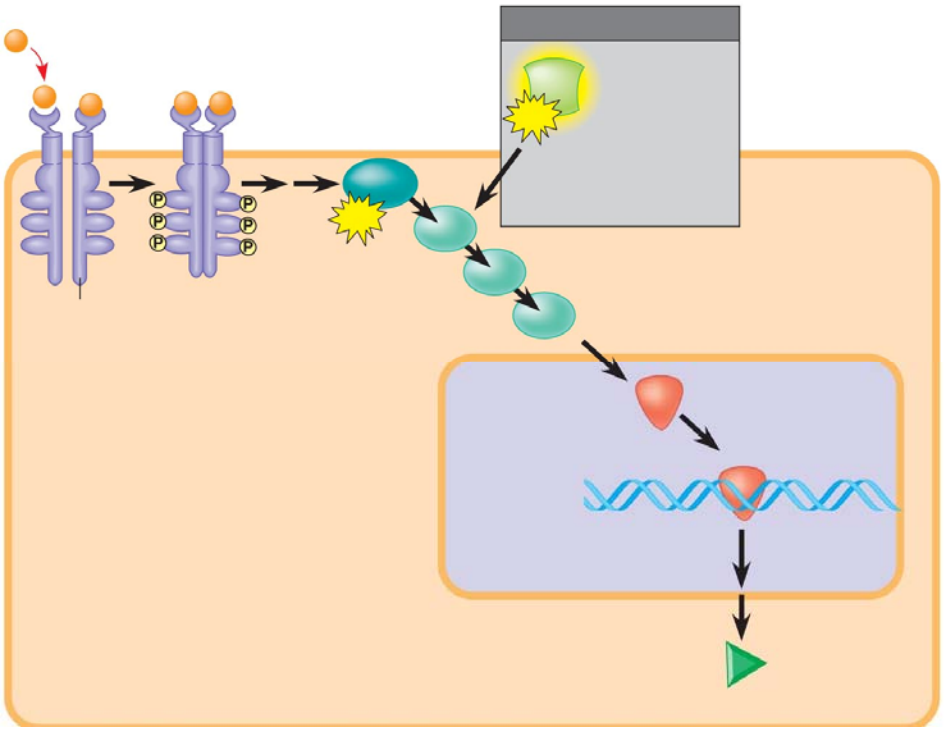
42. What is controlled by *homeotic genes*?

Concept 18.5 Cancer results from genetic changes that affect cell cycle control

43. What mechanism is involved in the beginning of tumor growth? Discuss *oncogenes* and proto-*oncogenes*.

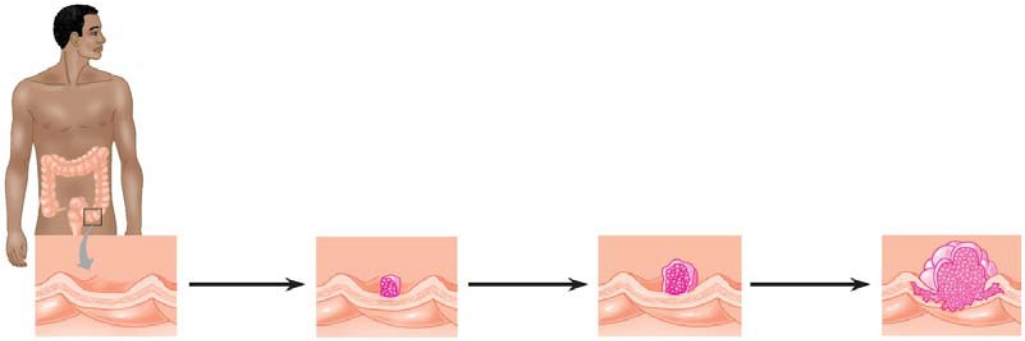
44. What are three mechanisms for converting a proto-oncogene to an oncogene?

45. There seem to be two categories of genes involved in cancer: *oncogenes*, which code for proteins to regulate cell growth, and should not be stuck "on," much like the accelerator in a car; and *tumor-suppressor genes*, which work like the brakes on a car and must function! Let's begin with a look at the *ras gene*, which codes for a G protein and is an *oncogene*. Label the sketch below to explain how a *ras* mutation leads to cancer.



46. *Tumor-suppressor genes* help prevent uncontrolled cell growth. One that is found mutated (and therefore nonfunctional) in more than 50% of human cancer is *p53*. So important is the *p53 gene* that it is sometimes called the "guardian angel of the genome." Describe the double whammy that results from mutation of *p53*.

47. Explain the *multistep model of cancer development* by using the specific example of colorectal cancer. The figure below may be labeled to help in your explanation.

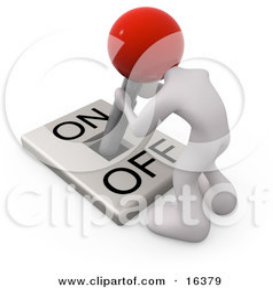


Testing Your Knowledge: Self-Quiz Answers
Now you should be ready to test your knowledge. Place your answers here:

1._____ 2._____ 3._____ 4._____ 5._____ 6._____ 7._____

8._____ 9._____ 10._____

GENE REGULATION CHAPTERS 18



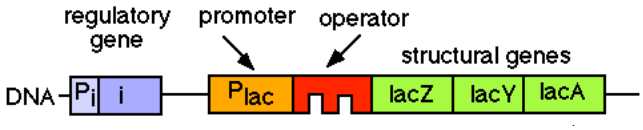
-Timing/coordination of specific events = necessary for normal development of organisms
- cell differentiation results from the expression of genes for tissue- specific proteins
- induction of transcription factors results in sequential gene expression. during development
- **Homeotic (HOX) genes** are involved in developmental patterns and sequences
- Embryonic induction in development results in the correct timing of events
-. **APOPTOSIS** (programmed cell death) plays a role in development/differentiation

- **MICRO RNA's**
- regulate genes/play role in development/ control of cellular functions
- Genetic mutations can result in abnormal development
- Environmental factors can influence gene expression
  EX: temperature and the availability of water determine seed germination in most plants.

PROKARYOTIC GENOME
• use substances/synthesize macromolecules just fast enough to meet needs
• If substance/enzyme needed, gene is transcribed.
• If substance/enzyme not needed, gene is turned off
• Allows for conservation of cell resources
• Controlling gene expression is one method of regulating metabolism

OPERON – Related genes grouped together with one promoter
• Allows for coordinated control of genes required for metabolism.
• One switch controls more than one gene
• Can be **inducible** or **repressible**.
• Not present in eukaryotes
Repressible and inducible enzymes = both examples of **NEGATIVE control** of a pathway
Activating the repressor proteins shuts off the pathway
POSITIVE control requires that an activator molecule switch on transcription

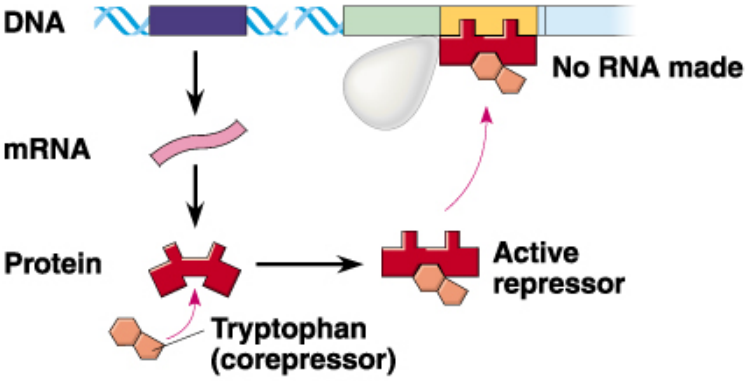
OPERATORS-
regions of DNA that control RNA access to promoter



REPRESSOR -
-regulatory protein binds to operator
- turns genes off (negative control mechanism)
- acts as a braking mechanism
- produced at a site away from the operon by regulatory gene.

Repressors alternate between active/inactive forms to control transcription.
• **Active form**- binds to operator/turns gene off
• **Inactive form**- conformation change prevents binding to operator
• Binding of **REPRESSOR** to **OPERATOR** prevents transcription -RNA polymerase not able to bind to promoter
~ **TURNS GENE OFF** -

Repressible Operons EX: trp operon	**Inducible Operons EX: lac operon**
Their genes are switched on until a specific metabolite activates the repressor.	Their genes are switched off until a specific metabolite inactivates the repressor.
They generally function in ANABOLIC pathways.	Function in CATABOLIC pathways
Pathway end product switches off its own production by repressing enzyme synthesis.	Enzyme synthesis is switched on by the nutrient the pathway uses



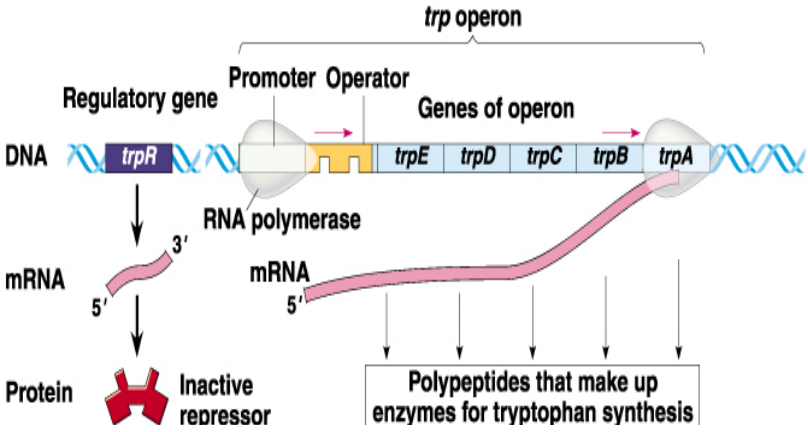
REPRESSIBLE: TRYPTOPHAN *trp* OPERON

Genes usually TURNED ON;
Repressor = INACTIVE;
Can be turned off
by activating repressor

Allows cell to use genes when tryptophan is needed and turn off genes when trp is plentiful

TRYPTOPHAN = corepressor
Presence of tryptophan activates repressor

If TRYPTOPHAN is present,
don't need to make more



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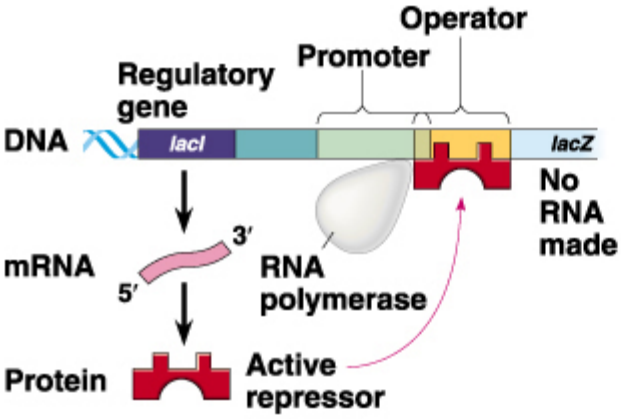
INDUCIBLE LACTOSE *lac* OPERON



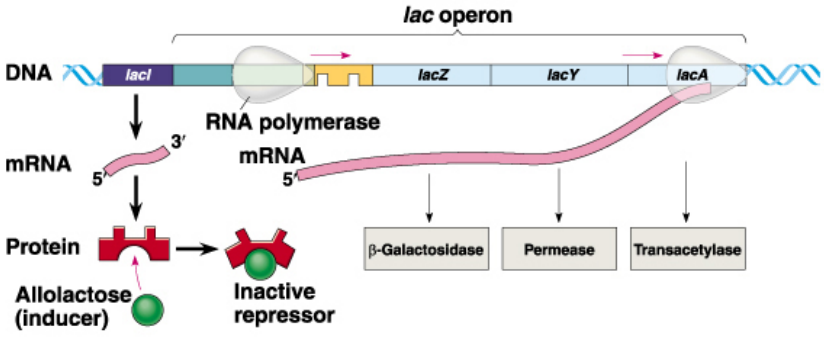
Genes usually TURNED OFF;
Repressor = ACTIVE; binds OPERATOR
Can be turned ON by deactivating repressor

Allows cell to turn on genes needed for lactose digestion when lactose is available

Keeps genes turned off unless needed

ALLOLACTOSE =inducer
presence inactivates repressor

Cell only turns gene on when needed

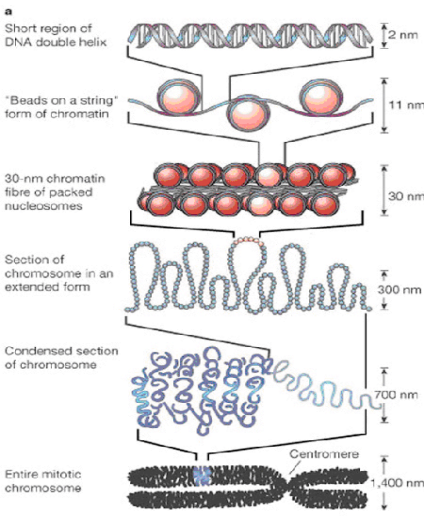


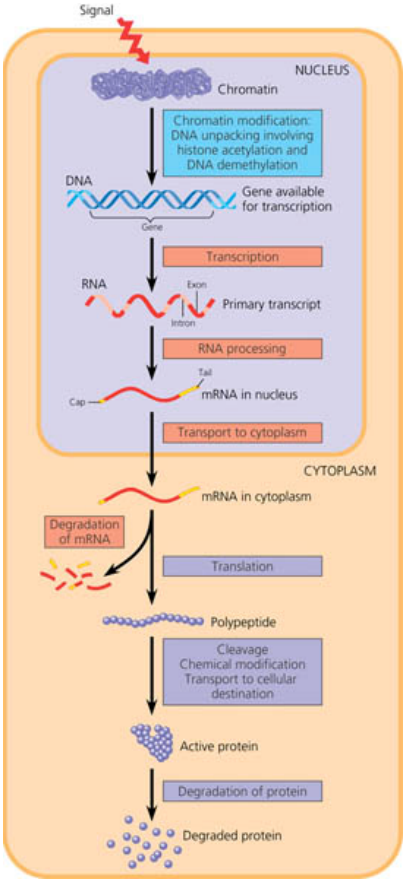
EUKARYOTIC GENOME REGULATION
-genes NOT grouped into operons

CHROMOSOME STRUCTURE

DNA PACKING/CHEMICAL MODIFICATION

HISTONES wrap DNA into beadlike bundles = **NUCLEOSOMES**
Tight wrapping around **HISTONES** turns genes off
Addition of **ACETYL GROUPS** to histones loosens wrapping
HETEROCHROMATIN-tightly packed
EUCHROMATIN-less tightly packed
DNA METHYLATION-adding ($-CH_3$) to cytosine blocks transcription
EX: Barr bodies, genomic imprinting (epigenetics)





TRANSCRIPTIONAL CONTROL

PROMOTER region at beginning of gene
binding of RNA polymerase/transcripton factors
controls speed of transcription

TATA BOX-helps position RNA Polymerase

ENHANCER sequences-upstream from gene
binding of proteins here speeds up transcription

POST TRANSCRIPTIONAL CONTROL

RNA PROCESSING
- Intron/exon editing
- Alternative RNA splicing
- 5′ CAP & Poly-A tail

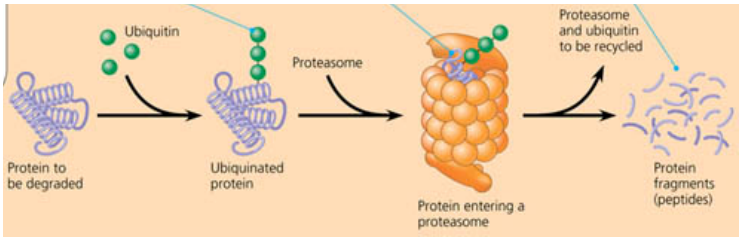
NUCLEAR TRANSPORT
-Control speed of exit out of nucleus

TRANSLATIONAL CONTROL

Regulatory proteins prevent ribosome binding to 5′ end of mRNA
Change rate of mRNA digestion
Change rate of aminoacyl-tRNA synthetase recharging tRNA's

POST-TRANSLATIONAL CONTROL

RNAinterference (RNAi) – short RNA's bind mRNA
MICRO RNA (miRNA) - block reading of message by ribosomes
SMALL INTERFERING (siRNA) RNA's tag message for degradation
CLEAVAGE-Cutting polypeptide chain to produce functional protein
EX: proinsulin (1 chain) → insulin (2 chains)
CHEMICAL MODIFICATION-
Add sugars, phosphates, etc
TRANSPORT TAGS-
Identify cellular destination
UBIQUITIN=protein tag
identifies proteins for degradation
digested by **PROTEASOMES**



LAC OPERON LAB SIMULATION

Biology Bench

http://www.phschool.com/science/biology_place/biocoach/lacoperon/intro.html

Introduction:

1. Summarize the purpose of this virtual lab (in paragraph form).

Concept 1:

1. Describe the importance of gene regulation in bacteria.

Concept 2:

1. What is an operon? What is produced?
2. Draw the LAC OPERON.

Concept 3:

1. What is an operator and a promoter?
2. Draw the lac operator and the promoter

Concept 4:

1. Describe the why the Lac regulatory protein is considered a repressor.

Concept 5:

1. That happened to the Lac repressor in the absence of lactose. Why does this occur?

Concept 6:

1. What happens to the lac genes when lactose is present?
2. What is allolactose? How does it behave with the repressor protein?

Concept 7:

1. Why is Allolactose called an inducer?
2. What happens to the lac repressor when Lactose is present? Absent?

Concept 8:

1. What happens when the enzymes encoded by the lac operon are produced?

Concept 9:

1. What is the preferred food source for E. coli?
2. What happens to the transcription of the lac genes when glucose is present?
3. What happens to the transcription of the lac genes when glucose is exhausted?

Concept 10:

1. What happens to transcription of the lac genes when both glucose and lactose are present?

Concept 11:

1. How does glucose affect the concentration of cyclic AMP?

Concept 12:

1. Use your previous knowledge to describe the function of cAMP (cyclic AMP).
2. What happens to cyclic AMP in the presence of lactose?
3. What happened to cAMP when glucose levels are low?

Self Quiz:

Complete the Self-Quiz and record your score_____.

GENE REGULATION: *LAC* OPERON LAB

(This laboratory procedure is a modification of:
Moss, Robert. 1999. A discovery laboratory investigating bacterial gene regulation. Pages 165 – 173, *in* Tested studies for laboratory teaching, Volume 20 (S. J. Karcher, Editor). Proceedings of the 20th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 399 pages.)

Bacteria have evolved for efficiency, and for adaptation. Environmental factors may alter a cell's physiology, by temporarily increasing or decreasing transcription of particular genes. The *lac* operon in *E. coli* is one of the simplest and best examples of such gene regulation. In this operon, lactose induces the synthesis of the enzymes involved in its breakdown, particularly β-galactosidase. This enzyme breaks lactose down to glucose + galactose.

$$\text{Lactose} \xrightarrow{\text{(β galactosidase)}} \text{Glucose + Galactose}$$

In the absence of lactose, these enzymes are not required, and thus are not synthesized, as it would be inefficient to do so. The primary regulator of the *lac* operon is a "negative control element" called the **lac repressor.** This protein is a negative regulator because when it binds to the regulatory region a gene, it down regulates expression of that gene.

When lactose is present in the cell, the sugar combines with and inactivates the repressor. Under these conditions, the genes of the lactose operon are expressed, and the enzyme required to utilize the lactose is synthesized. This process, whereby an environmental factor activates a gene, is called gene or transcription **induction**.

The repressor of the lactose operon is coded for by the *lacI gene*. The repressor binds to the operator area, O, and blocks the attachment of the RNA polymerase to the promoter site, P. The RNA polymerase must attach to the promoter site and move through the operator site if the lactose operon is to be transcribed. Therefore, when lactose is absent the repressor binds to the operator and transcription of the *lac* operon decreases. However, when lactose is present, the repressor does not bind to the operator and transcription of the *lac* operon increases (Figure 1).

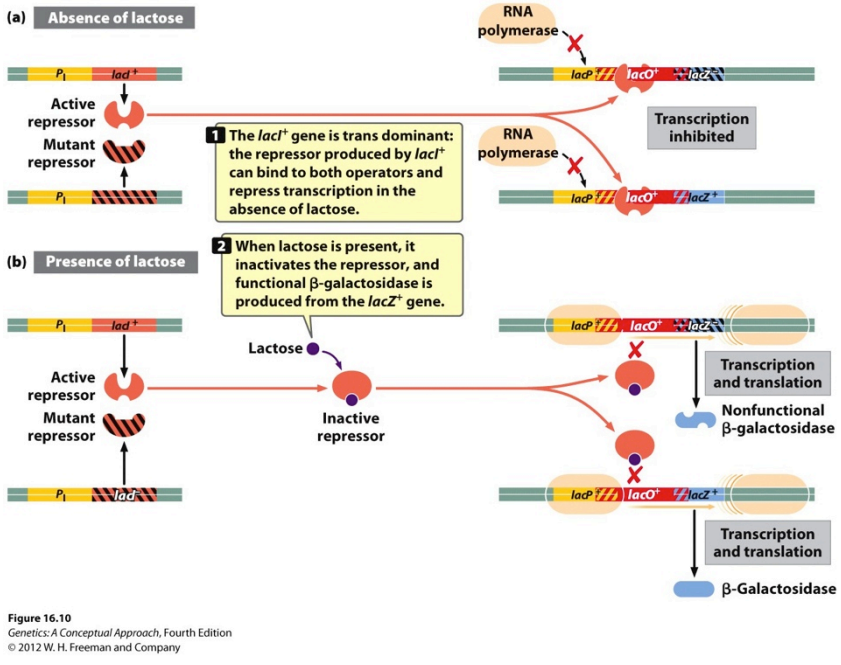


Figure 16.10
Genetics: A Conceptual Approach, Fourth Edition
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Figure 1: Regulation of the *lac* operon by lactose.

E. coli's preferred energy and carbon source is glucose because unlike lactose and other sugars that have to be "pre-processed," glucose can directly enter glycolysis to yield ATP. So, if both glucose and lactose are present in the environment, what will *E. coli* do? If you answered, "use the glucose first," you are correct. Since *E. coli* doesn't "need" the lactose to supply energy or carbon if glucose is present, *E. coli* suppresses the transcription of the *lac* operon by inactivating the **catabolite activator protein (CAP).** CAP is a transcription factor that binds to specific sites upstream of the *lac* promoter if the cyclic AMP (cAMP) is bound to it. Once the cAMP-CAP complex is bound to the appropriate regulatory sites, it recruits RNA polymerase to the scene resulting in increased transcription of the *lac* operon. The concentration of cAMP is regulated by glucose by an inverse relationship: when glucose concentration is high, cAMP concentration is low and vice versa.

We will measure the amount of enzyme produced by the gene under various conditions. In order to do so, we need to be able to measure one of the products of the enzyme's reaction as it is made. Unfortunately, we have no easy assay for glucose or galactose. However, β galactosidase will cleave other similar molecules, such as O-nitrophenyl galactopyranoside (ONPG). One of the cleavage products created when the enzyme operates on ONPG is bright yellow, and thus can easily be seen or measured.

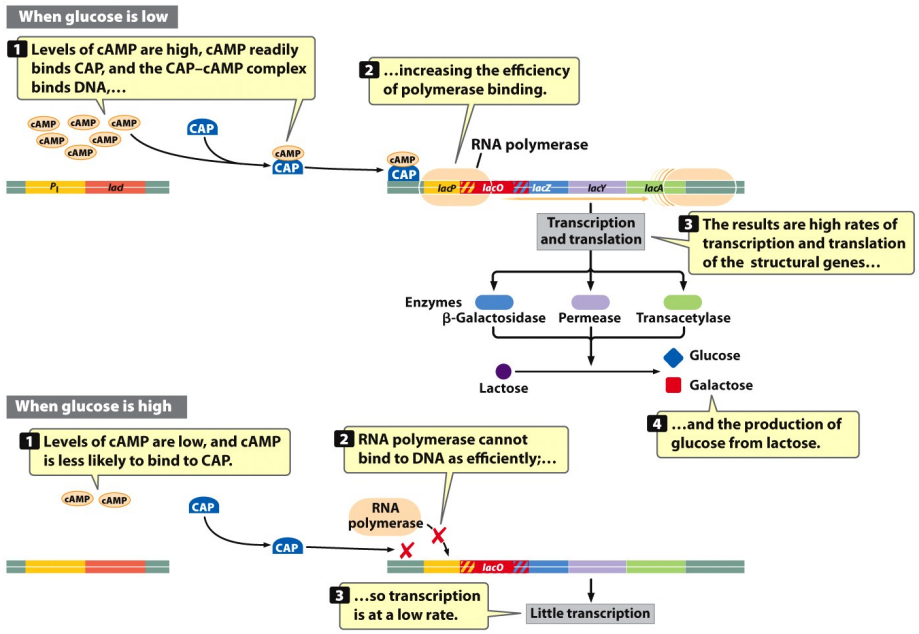

Figure 16.13
Genetics: A Conceptual Approach, Fourth Edition

Figure 2: Regulation of the *lac* operon by glucose.

WEEK 1 PROCEDURE

1) You should start the experiment at the beginning of the lab period, and we'll discuss the theory behind it during the first 75 min. incubation period.

2) You will be provided with cultures of each of three bacterial strains, labeled "A", "B", and "C". One of these will be "wild type" *E. coli*; one is a *lacZ*$^-$ strain, which lacks a functional β-galactosidase gene, and one is *lacI*$^-$, which doesn't have a gene for the lac repressor. Your job for the first week is to carry out an experiment that will identify each of the three strains. Make sure you write out a hypothesis or prediction of how each strain will react.

3) Set up 13 reactions according to the table below. *Measure as accurately as possible.* Use a separate pipette for each bacterial culture!! Make sure you swirl or mix the culture each time before removing cells. Dispose of pipette tips that come in contact with bacteria in biohazard waster container.

Tube	M9 + glycerol medium	Bacterial culture	Sugar
1	500 µl	800 µl of Strain A	100 µl of water (- control)
2	500 µl	800 µl of Strain A	100 µl of lactose
3	500 µl	800 µl of Strain A	100 µl of glucose
4	500 µl	800 µl of Strain A	100 µl of lactose analog TMG
5	500 µl	800 µl of Strain B	100 µl of water (- control)
6	500 µl	800 µl of Strain B	100 µl of lactose
7	500 µl	800 µl of Strain B	100 µl of glucose
8	500 µl	800 µl of Strain B	100 µl of lactose analog TMG
9	500 µl	800 µl of Strain C	100 µl of water (- control)
10	500 µl	800 µl of Strain C	100 µl of lactose
11	500 µl	800 µl of Strain C	100 µl of glucose
12	500 µl	800 µl of Strain C	100 µl of lactose analog TMG
13	500 µl	800 µl of Strain A	100 µl of water

4) Incubate the cultures at 37˚C in a shaking water bath for 75 min.

5) Place tubes on ice and add one drop of sarkosyl to each tube.

6) Add 0.7 ml 0.5% ONPG to each tube, **except tube 13**. **Add 0.7 ml of water to tube 13.** Briefly vortex each tube at low speed to mix, MAKING SURE THE TUBE IS NOT POINTED TOWARD ANYONE, AND MAKING SURE THE SPEED IS LOW ENOUGH SO THAT THERE IS NO SPILLAGE FROM THE TUBES.

   To ensure all reactions proceed for exactly the same length of time, stagger addition of ONPG by 30 seconds.

7) Incubate for at least 15 minutes at 37 - 45˚C (the higher the temperature within this range, the quicker the enzyme works), until a rich yellow color develops in some of the tubes. Make sure to incubate all the tubes for exactly the same length of time.

8) When the first tube has incubated exactly the length of time you want, add 2 ml of ice cold 0.1 M $NaHCO_3$ to stop the reaction, briefly mix contents, and put tube 1 on ice. Thirty seconds later, repeat the stopping procedure with tube 2. Continue stopping the reactions in this manner until you have added $NaHCO_3$ to all 13 tubes. Leave the tubes on ice as you're

waiting to use the spectrophotometer. Record your observations about the relative color of each tube "by eye" before taking readings.

9) Determine the absorbance at 420 nm (A_{420}) for each of the samples using tube 13 to blank the spectrophotometer. Be sure to wipe the condensation off of each tube before putting it into the machine.

   NOTE: If the reading for any of your tubes is higher than 1.5, you must make a dilution to get an accurate A_{420} reading [I suggest a 10 fold dilution; 0.5 ml of sample plus 4.5 ml of cold water] and re-take your reading. To find out how concentrated your sample was BEFORE the dilution, multiply the reading by the "dilution factor." For example, your reading for the undiluted sample was 2.6. Since that is greater than 1.5, find the A_{420} for a 10-fold dilution of your sample. The A_{420} for the diluted sample is 0.3. The accurate A_{420} for your undiluted sample = 0.3 x 10 = 3.0.

QUESTIONS (I will not be grading these questions, but they are fair game for the lab final exam)

1) What is the role of each of the following molecules in this lab: lactose, glucose, ONPG, sarkosyl?

2) Explain what happens, ***on the molecular level [what's happening at the operon]***, when bacteria are growing in a medium with lactose? with glucose? with both?

3) Of what advantage to a cell is negative control [with repressor] of the *lac* operon?

4) Of what advantage to a cell is CAP?

5) If we had a mutant strain that had a deletion of the *lac* operator, what would the phenotype be?

WEEK 2

This week, you will perform the experiment you designed to examine some aspect of the regulation of the production of β-galactosidase.

In addition to the procedures you used last week, you might find the procedures below to be helpful when designing your own experiment this week.

1) To ensure all reactions proceed for exactly the same length of time, stagger addition of ONPG by 30 seconds. When the first tube has incubated exactly the length of time you want, add 2 ml of ice cold 0.1 M $NaHCO_3$ to stop the reaction. Then leave the tubes on ice as you're waiting to use the spectrophotometer. Record your observations about the relative color of each tube "by eye" before taking readings.

2) Place the tubes into a spectrophotometer to measure the color density. The wavelength should be set to 420 nm for ONPG, which is yellow. Then, you must calibrate the machine. Place your blank into the machine. Press the "0 Absorbance" button to set the machine to zero. Now you're ready to go. Place each tube in, and read the ABSORBANCE number. Absorbance is directly proportional to the concentration of the colored molecule. You might want to RE-CALIBRATE the machine, using the SAME blank, after every reading. Don't forget to wipe the condensation and fingerprints off each tube before putting it into the machine!

3) If the reading for any of your tubes is higher than 1.5, you must make a dilution to get an accurate A_{420} reading [I suggest a 5- or 10-fold dilution]. To make a 5-fold dilution, mix 1 ml of sample with 4.0 ml of cold water. After making the dilution, re-take your reading. To find out how concentrated your sample was BEFORE the dilution, multiply the reading by the "dilution factor." For example, your reading for the undiluted sample was 2.6. Since that is greater than 1.5, find the A_{420} for a 5-fold dilution of your sample. The A_{420} for the diluted sample is 0.6. The accurate A_{420} for your undiluted sample = 0.6 x 5 = 2.5.

In addition to the reagents included in the first week, the following will be made available:

- Glucose, maltose, fructose, sucrose and 'TMG' sugars [Note: If you wish to try combinations of glucose and lactose in the same sample, the glucose must be added 20 minutes prior to the lactose]

- You may design experiments requiring other reagents or equipment; every effort will be made to accommodate your needs as long as you give us enough advance notice.

- Some possible questions you might want to address [you aren't limited to these]:
  - How specific is the Lac repressor for lactose?
  - How does glucose effect the gene induction?
  - What concentration of lactose is required to induce?
  - How long does it take for the cell to respond to new lactose in its environment? [Make sure you take time points out to 75'. Incubate with ONPG for 30', which is longer than usual.]

NOTES:

- ***MAKE SURE you have both positive and negative controls in your experiment.*** A control should only differ from the experimental group by one variable.

- Once your second incubation is over, STOP THE REACTION AND PLACE ALL OF YOUR TUBES ON ICE right up until you take your readings (see additional procedures above). You don't want the reactions to continue while you are waiting for a spectrophotometer for instance.

- If you're working with wild type bacteria, they might make a very small amount of enzyme under the conditions you choose. So to "boost sensitivity", your ability to detect smaller amounts of enzyme, I suggest you increase the length of the second incubation to 30'.

Laboratory Preparation

Week 1:

Materials:

Shaking incubator or water bath set at 37˚C
Vortex mixers
Sterile test tubes for culturing bacteria
Sterile flasks for culturing bacteria
Nutrient agar plates, Carolina Biological #821862
E. coli, strain K12 (Wild type strain), Carolina Biological Item #124500 or ATCC #23716
E. coli lacZ$^-$ mutant, Carolina Biological Item # 211561 or ATCC #23735
E. coli lacI$^-$ mutant, ATCC #15224
Thiomethyl galactoside (TMG), Sigma #M8146
O-Nitrophenyl-β-D-galactoside (ONPG), Sigma #N1127
Sarkosyl, Sigma #L9150
M9 minimal salts, Fisher #DF048517
Yeast extract, Fisher #DF2109-29
Glycerol, Fisher #BP2291
Glucose, Carolina Biological #857450
Lactose, Carolina Biological #871750
Sodium bicarbonate ($NaHCO_3$), Fisher #BP328-500
Spectrophotometer
Cuvettes for spectrophotometer
P200 pipettors and tips
P1000 pipettors and tips
Sterile water

Solutions:

3% w/v glucose solution (made fresh and stored in refrigerator to discourage bacterial growth)
3% w/v lactose solution (made fresh and stored in refrigerator to discourage bacterial growth)
1% w/v TMG
0.5% w/v ONPG prepared in 50 mM sodium phosphate buffer, pH 7.0
1% w/v sarkosyl in dropper bottles
M9 + glycerol medium
Per liter:
950 ml water
4 g glycerol
10 g M9 minimal salts
0.2 yeast extract
Stir to dissolve and check that the pH is 7.0 – 8.0. Adjust with NaOH or HCl if necessary.
Autoclave to sterilize.
0.1*M* $NaHCO_3$

Instructions for bacterial cultures:

Store cultures from supply companies in refrigerator. If cultures are on slants or plates, transfer cultures to new medium within two weeks of receipt. Streak for single colonies on nutrient agar plates and incubate plates at 37˚C overnight. The following day, inoculate 1 – 2 ml of M9 +

glycerol medium with a single colony from nutrient agar plate. Incubate culture at 37˚C for 24 – 48 h with shaking. Two days prior to lab, inoculate 100 ml of M9 + glycerol medium with the 5 ml culture and incubate at 37˚C with shaking. Label each of the bacterial strains with a letter only, such as A, B, or C. To preserve cultures for future use, prepare 15% glycerol stock culture and store at -80˚C.

Additional materials for Week 2:

Maltose, Carolina Biological #873750
Sucrose, Carolina Biological #892860
Fructose, Carolina Biological #872340

Additional solutions for Week 2:

3% w/v maltose
3% w/v sucrose
3% w/v fructose

Lac Operon Proposal

Rough Draft is due: Jan 16th

Final Draft is due Jan 21st.

Proposal: Use this outline to help you write your Lac Operon Lab Proposal. Use the Lac Operon Lab I as guide to help you create your own unique lab. The results and conclusion section with be a PREDICTION of what you think will happen in your lab. You also need to describe how you plan on recording and analyzing your results. The more detail you include in the proposal the better because you will be using your proposal on Jan 24th at Wofford to complete your lab.

Good luck and please schedule a time with me for extra help if you so desire.

Guidelines for writing general proposals and lab reports:

All labs must be written or typed and be submitted to the teacher. Always use third person (no personal pronouns) when writing all parts of a lab report. A cover sheet with the lab title, your name, date, and period should be stapled to the front of the lab report. Place the lab report in the front pocket of the Notebook. When typing your report put lab headings and lab questions in bold type (underline when writing). Use Times New Roman font 12 point only and only 1" margins.

Title
The title should indicate clearly & concisely the subject and scope of the report.

Introduction
The introduction should give background information about the experiment. It should also state the purpose of the investigation. (at least 3 references)

Hypothesis
The hypothesis should be a single statement telling the exact thing you are trying to prove in your experiment. Never write this statement using "first person".

Methods and material
This section includes one or more paragraphs explaining the step-by-step procedures used. The description should be so thorough that someone else could use your listed materials and procedures to conduct the same experiment & get the same results. **Include the independent, dependent variable, constants and controls.**

Results
All data should be collected and organized in a logical order. Results should be illustrated as charts, tables, graphs, &/or diagrams. All graphs should include a title, the independent variable on the horizontal axis, and the dependent variable on the vertical axis. All charts and graphs should be explained with a brief description. *All lab questions and answers should be included also with this section.* (Bold the questions & italicize the answers)

Discussion and Conclusion
In this section, you should interpret the collected data and relate it to the restated hypothesis. Explain the significance of the results. If additional research and experimentation is needed, explain in this section. References should be used in this section as well. Research should be used to support your data. You should also discuss further experiments that could be performed.

BIO 214 RESEARCH POSTER RUBRIC

Explanation of grading of performance	*Inadequate: Performance not acceptable (F, earns 0-59 points)*	*Below average: Performance not up to minimum standards but shows marginal grasp of concept (D, earns 60-69 points)*	*Average to above average: Performance met all minimum standards (C, earns 70-79 points).*	*Good to very good: Performance representative of good to noteworthy achievement (B, earns 80-89 points).*	*Particularly strong to exemplary: Performance demonstrated that this team moved beyond expectations and came up with original ideas that provided unique insight (A, earns 90 – 100 points).*
Introduction (15 points)	Intro is not present, is incoherent, or is unrelated to experiment.	Intro disorganized, or lacks reference to relevant primary literature, or missing purpose/hypothesis.	Background info too broad or too narrow, or weak/missing purpose or hypothesis, or lacks sophistication and/or may contain fallacies of logic.	The intro smoothly pulls reader into topic. It is well organized, flows from general to specific, and makes clear the purpose of experiment.	Intro is uniquely well written and is crafted in such a way (e.g., relevant examples from 1° lit) as to educate the poster audience in a noteworthy and effective way.
Appropriate use of primary literature; including Lit Cited section (10 points)	No 1° literature cited or it was plagiarized and/or citations missing or inadequate.	Attempts at paraphrasing border on plagiarism, or cited literature seems random and/or irrelevant to topic, and/or literature cited section inadequate and improperly formatted.	Use of reference literature perfunctory without clear context, and/or attribution inappropriate or misplaced, and/or literature cited section is inadequate.	Background information used in context (esp. in intro and discussion). Citations are appropriate and correctly located within text, with literature cited in appropriate format.	Effective use of 1° literature, which is cited appropriately. Paraphrasing of other works represents noteworthy grasp of referenced articles applied to poster's context.
Methods (10 points)	Methods absent or blatantly inaccurate.	Reader would have a tough time knowing what happened in the experiment.	Methods gave general view of experiment but were incomplete or imprecise.	Clearly states how experiment was conducted, and how data were collected and analyzed.	Methods are sophisticated, clear, and concise, giving particularly good insight (perhaps with visual aids) into how the study was performed and how the data were analyzed.
Results (15)	No results, no figures, or what is presented is grossly inaccurate.	Results are misleading or un-interpretable for reader due to mistakes or omissions (e.g., no figure captions).	Graphs/tables don't conform to minimum standards (e.g., contain raw data), or captions are incomplete, axes are misleading, or supporting text is inaccurate.	Figures & tables provide useful information for discussion. Captions are complete and accurate and supporting text is informative.	Results demonstrate effort beyond the norm, e.g., evidence that authors worked extra hard to create visually appealing, clear, and concise figures and supporting text.

This rubric is a slightly modified version of the Wofford College BIO 150 Research Poster Rubric. Funding supporting Bio 150 came from NSF grant 0836851. For additional information about the above document, please contact Dr. Ellen Goldey (goldeyes@wofford.edu).

BIO 214 RESEARCH POSTER RUBRIC

Discussion (15 points)	Discussion is not present, is incoherent, or is unrelated to experiment.	Discussion makes little attempt to address the purpose of the experiment or relate results to prior work and/or misuse of terms suggests ignorance of key concepts.	Discussion attempts to address the purpose of the experiment and relate results to other works, but misuse of scientific terms and/or lack of coherence with other sections confuses reader.	Authors relate their finding to their hypothesis/purpose, and/or situated their finding in the context of what was previously known about the topic, and/or given broader meaning for this work, but not all three	Communicates original synthesis of evidence in a way that is complex and free of logical fallacies. Future study recommendations are *specific* and reasonably follow from this study. Findings are discussed in broader context.
Overall unity of poster across sections (10)	Sections vary widely in quality and accuracy, resulting in a confusing hodgepodge.	Poster lacks unity and coherence (sections appear to have been developed separately then thrown together).	Acceptable coherence across some sections, but some sections (hint: often the intro and discussion) still lack any relationship to each other.	Demonstrates generally coherent and unified writing across sections providing a unified whole.	Sections are well integrated and interdependent (e.g., topics of intro are resolved in discussion) with smooth transitions. Discussion reflects back on other sections to provide novel, even exciting, insights.
Title & Acknowledgements (5 points)	Both are missing	Either is missing or misleading	Both are present, but title is not descriptive of experiment.	Both present, title is descriptive and accurate.	Both present, title is descriptive yet brief (perhaps even creative).
Sentence structure, grammar, punctuation, spelling (10 points)	Sentence structure seriously flawed, and/or numerous grammar and punctuation problems, and/or many spelling errors.	Several problems with sentence structure, spelling, grammar and punctuation make poster unprofessional.	Errors in grammar, punctuation, and/or spelling detract from quality. Vocabulary immature or misused. Text would benefit from additional editing for brevity.	A few errors in grammar, punctuation, or spelling do not detract too much from overall poster quality. Sentence structure is generally good, but may still contain excess words.	Poster is free of errors in grammar, punctuation, or spelling, and sentences are well structured. Poster demonstrates authors' careful editing. Vocabulary is notably sophisticated.
Creativity (10)	Poster is visually unappealing; use of template not apparent, and/or extraneous clutter completely hides the poster's purpose.	Poster fails to meet minimal visual standards. Sections are not intact or obviously unbalanced in length relative to suggested template.	Poster meets minimal requirements (e.g., template used "as is"), but problems (such as overcrowding, reducing text size to squeeze in words, and lack of appropriate white space) makes it less appealing.	Authors have followed guidelines and added visual interest while preserving white space.	Authors have added creative (e.g., color, images, humor) touches that enhance their poster's effectiveness ***without distracting*** from the poster's purpose. Be careful here!

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lac Operon Questions

1) Northern blotting is used to detect RNA that is transcribed from a particular gene or operon. If a specific RNA is present, then a band will appear on the Northern blot; the darker the band, This technique can also be somewhat quantitative; the darker the band, the more RNA that is present. Bacteria containing a normal *lac* operon were grown under different types of conditions, and then the mRNA was isolated from cells, and subjected to Northern blot analysis.

   Which is more effective at shutting down the *lac* operon, the binding of the *lac* repressor or the removal of CAP? Explain your answer based on the results in the Northern blot.

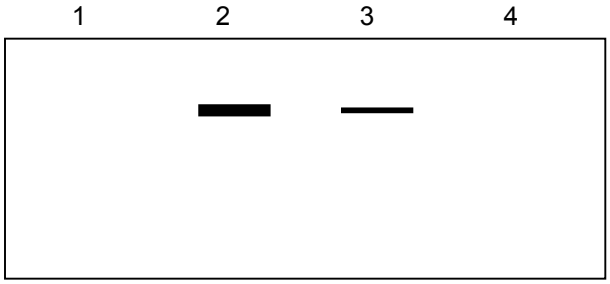

   Figure 1. Northern blot analysis of *lac* operon. Lane 1: RNA from cells grown in medium containing only glucose; lane 2: RNA from cells grown in medium containing only lactose; lane 3: RNA from cells grown in medium containing glucose and lactose; lane 4: RNA from cells grown in medium containing no glucose or lactose.

2) One of your friends who attends a different high school is writing his lab report about the *lac* operon. He called you to ask for help when writing his discussion because he doesn't have clue about how the *lac* operon works. He explains that his lab group performed an experiment very similar to what we did in the *lac* operon 1 lab. His results didn't turn out as expected because none of the tubes turned yellow after the second incubation. Which of the following observations could account for his observations? (select all answers that apply.) Explain your answers.
   A) His lab group forgot to add sarkosyl.
   B) His lab group forgot to add ONPG.
   C) His lab group did not add lactose to any of the tubes.
   D) His lab group did not add glucose to any of the tubes.

3) Jacob, Monod, and Pardee used various mutants to help determine how the *lac* operon is regulated. Here are the descriptions of some of the mutants they studied.
   - *lacI*^{S} mutant: This mutant produces a super repressor that is not inactivated by allolactose.
   - *lacO*^{C} mutant: This mutant has an altered operator sequence so that the repressor protein is no longer able to bind.
   - A – sign indicates that the gene or regulatory element is inactive. For example, *lacI*$^{-}$ = no repressor protein is produced.

   These mutants are used in an experiment similar to the *lac* operon lab I that we performed this morning. One set of the various strains of *E. coli* were mixed with M9 + glycerol medium and TMG and then incubated at 37˚C for 75 minutes. A second set of the various strains of *E. coli* were mixed with M9 + glycerol only. Sarkosyl was added followed by the addition of ONPG. Using a scale of – (no color) to +++ (intense yellow color) predict the level of yellow color for each of the following strains:

   A) *lacY*$^{-}$
   B) *lacP*$^{-}$
   C) *lacI*^{S}
   D) *lacO*^{C}

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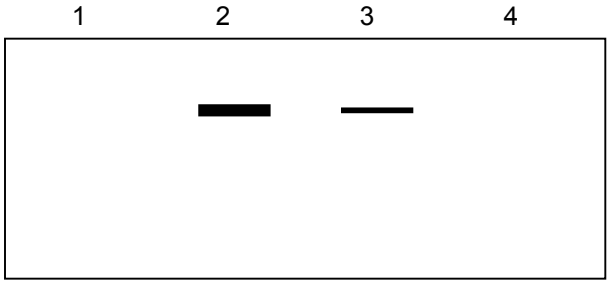

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   Binding of repressor is more effective at decreasing transcription of *lac* operon then removal of CAP. Due the absence of lactose, the repressor was active in samples for lanes 1 and 4 resulting in no detectable *lac* mRNA. The sample in lane 3 has low cAMP due the presence of glucose and the repressor is inactive due to the presence of lactose resulting in the production of a small amount of *lac* mRNA.

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   C) His lab group did not add lactose to any of the tubes.
   D) His lab group did not add glucose to any of the tubes.

   A) His lab group forgot to add sarkosyl therefore the cells remained intact resulting in β-galactosidase being inside cells and ONPG substrate outside cells. If enzyme can't interact with substrate, then no yellow product can be produced.

   B) His lab group forgot to add ONPG so there was no substrate for the enzyme to act upon.

   C) His lab group did not add lactose to any of the tubes so transcription of the operon was not induced. Therefore, no β-galactosidase was available to cleave ONPG.

   D) Cannot explain lack of yellow color because leaving out glucose would not have a negative effect on transcription of the operon.

4) Jacob, Monod, and Pardee used various mutants to help determine how the *lac* operon is regulated. Here are the descriptions of some of the mutants they studied.
   - *lacI*^{s} mutant: This mutant produces a super repressor that is not inactivated by allolactose.

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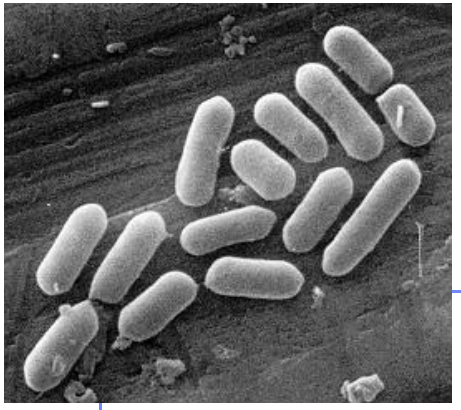
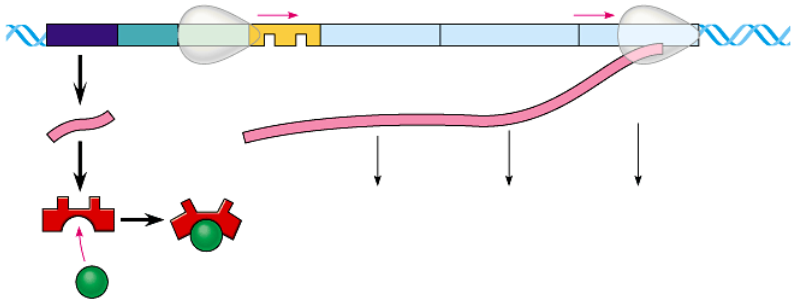
These mutants are used in an experiment similar to the *lac* operon lab that we performed this morning. One set of the various strains of *E. coli* was mixed with M9 + glycerol medium and TMG and then incubated at 37˚C for 75 minutes. A second set of the various strains of *E. coli* was mixed with M9 + glycerol only. Sarkosyl was added followed by the addition of ONPG. Using a scale of – (no color) to +++ (intense yellow color) predict the level of yellow color for each of the following strains:

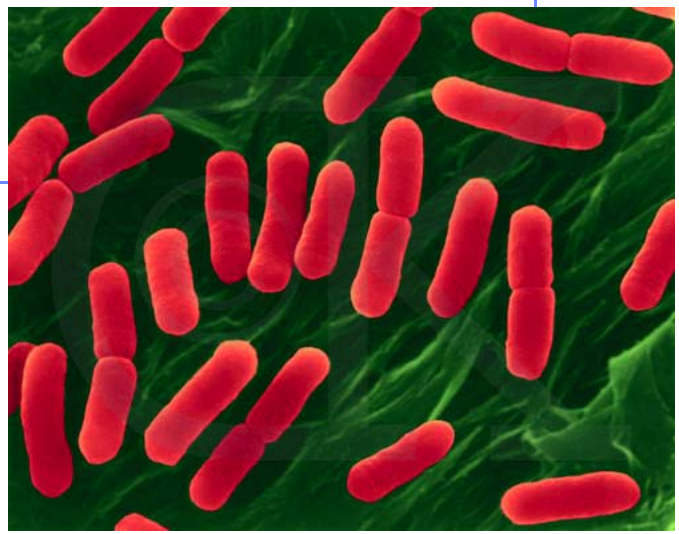
A) *lacY*$^{-}$
B) *lacP*$^{-}$
C) *lacI*^{S}
D) *lacO*^{C}

Strain	+ TMG	-TMG
lacY^{-}	- because TMG can't enter cell to induce transcription of operon	-
lacP^{-}	- because binding site RNA polymerase was removed.	-
lacI^{S}	- because TMG can't bind to and inactive repressor	-
lacO^{C}	+++	+++ because repressor will not bind to mutated operator region.

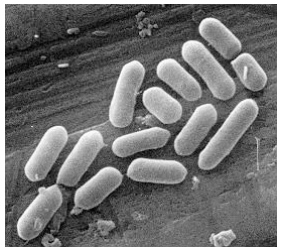




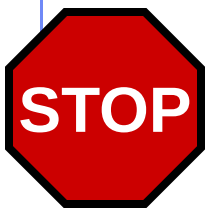
GENE REGULATION



Bacterial metabolism



- Bacteria need to respond quickly to changes in their environment
  - if they have enough of a product, need to stop production

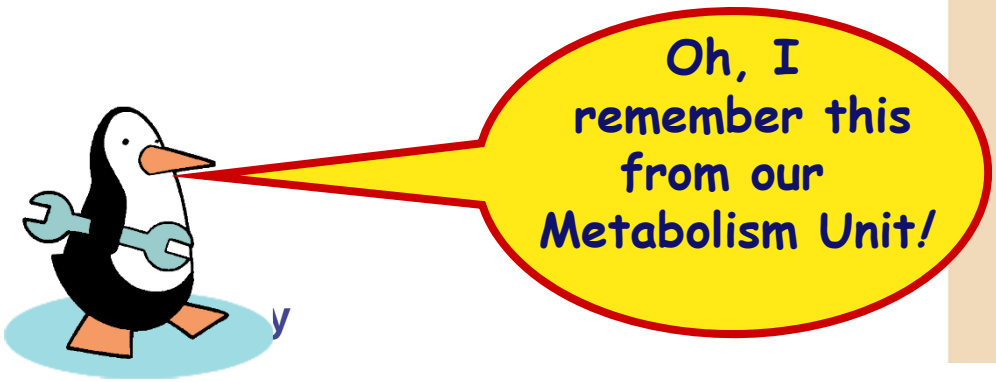


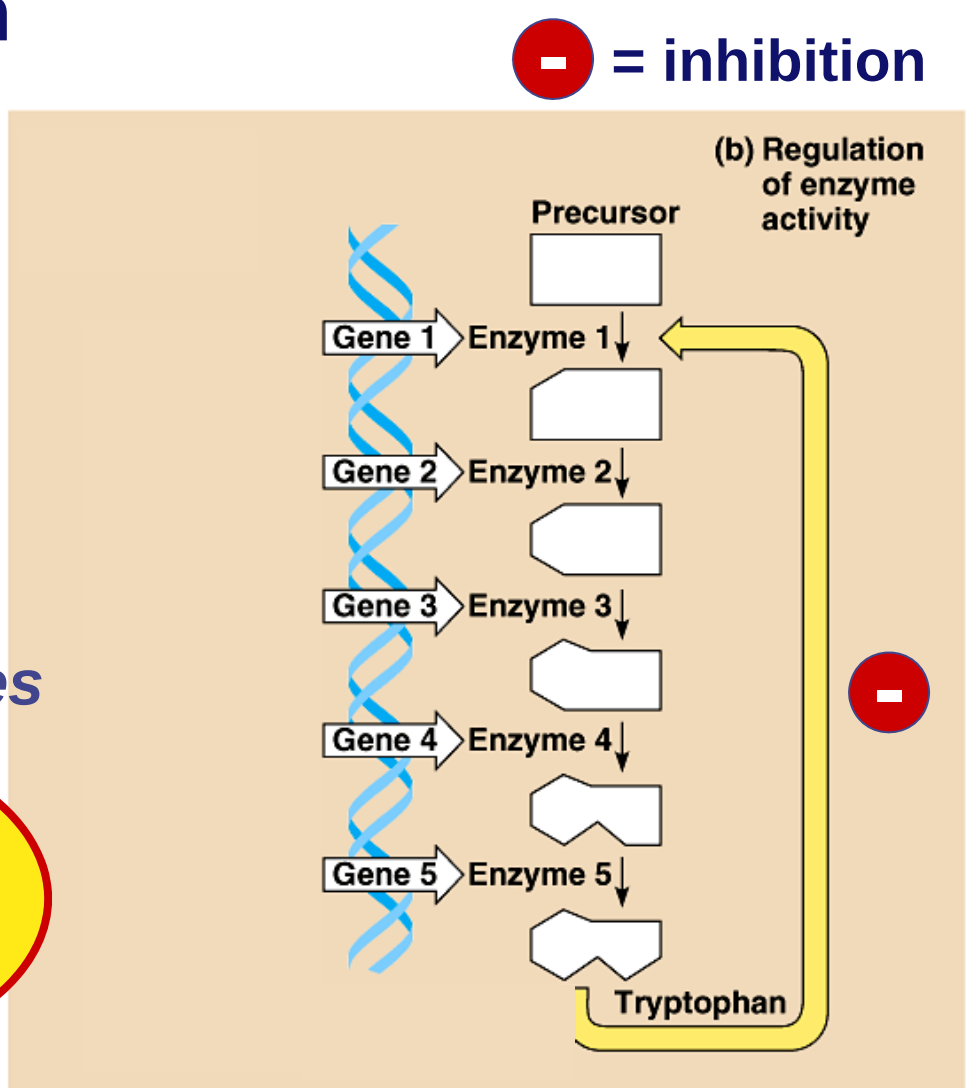
    - why? waste of energy to produce more
    - how? stop production of enzymes for synthesis
  - if they find new food/energy source, need to utilize it quickly

    - why? metabolism, growth, reproduction
    - how? start production of enzymes for digestion

Remember Regulating Metabolism?

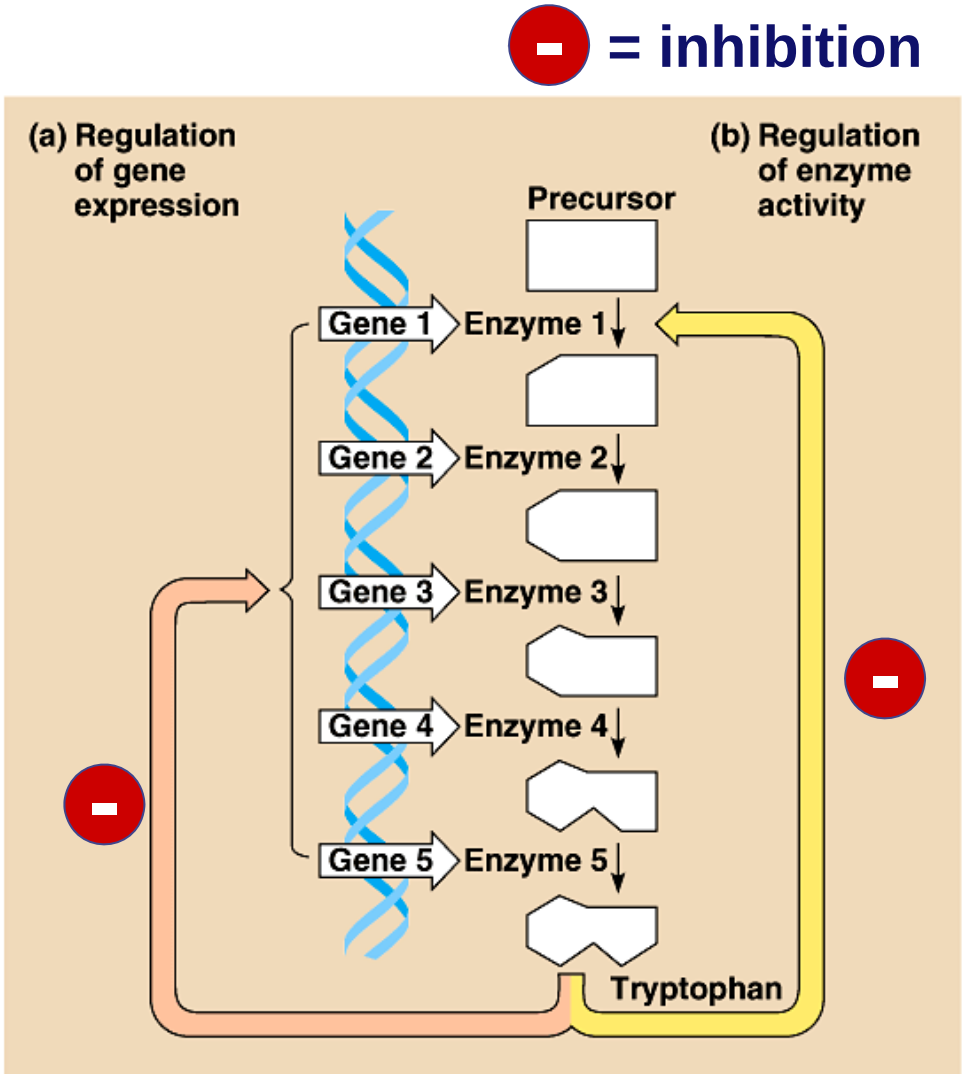
- **Feedback inhibition**
  - product acts as an allosteric inhibitor of 1st enzyme in tryptophan pathway
  - *but this is wasteful production of enzymes*



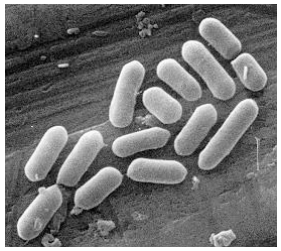


Different way to Regulate Metabolism

- **Gene regulation**
  - instead of blocking enzyme function, block transcription of genes for all enzymes in tryptophan pathway
    - saves energy by not wasting it on unnecessary protein synthesis



Gene regulation in bacteria



- Cells vary amount of specific enzymes by regulating gene transcription
  - turn genes on or turn genes off
    - turn genes OFF example

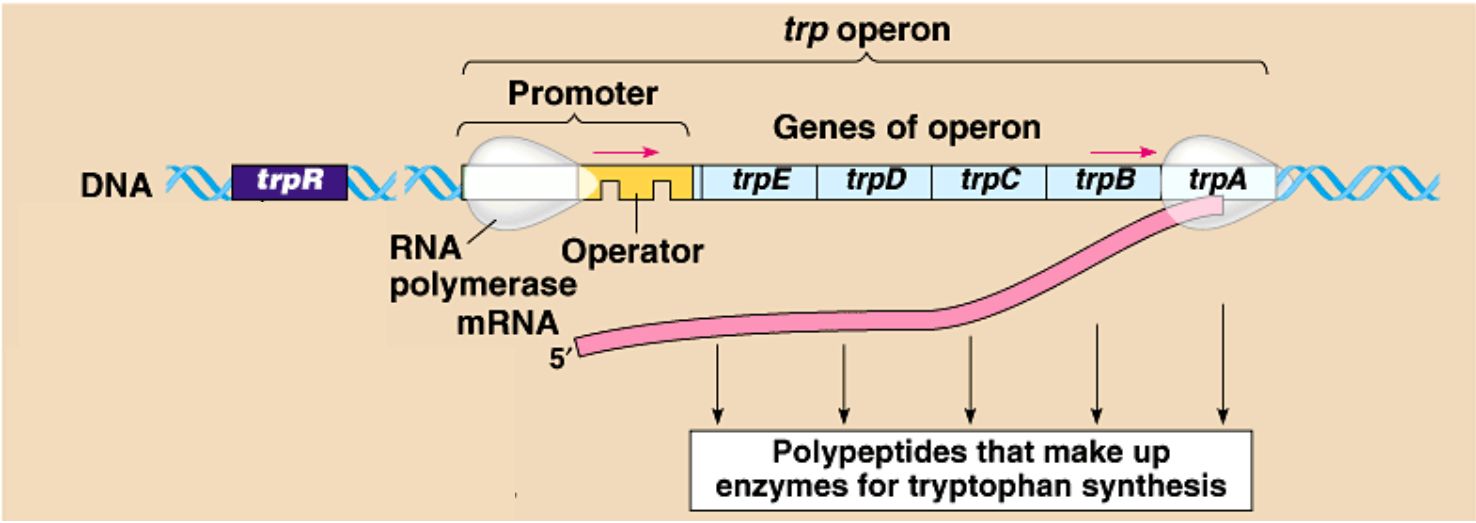
if bacterium has enough tryptophan then it doesn't need to make enzymes used to build tryptophan

    - turn genes ON example

if bacterium encounters new sugar (energy source), like lactose, then it needs to start making enzymes used to digest lactose

Bacteria group genes together

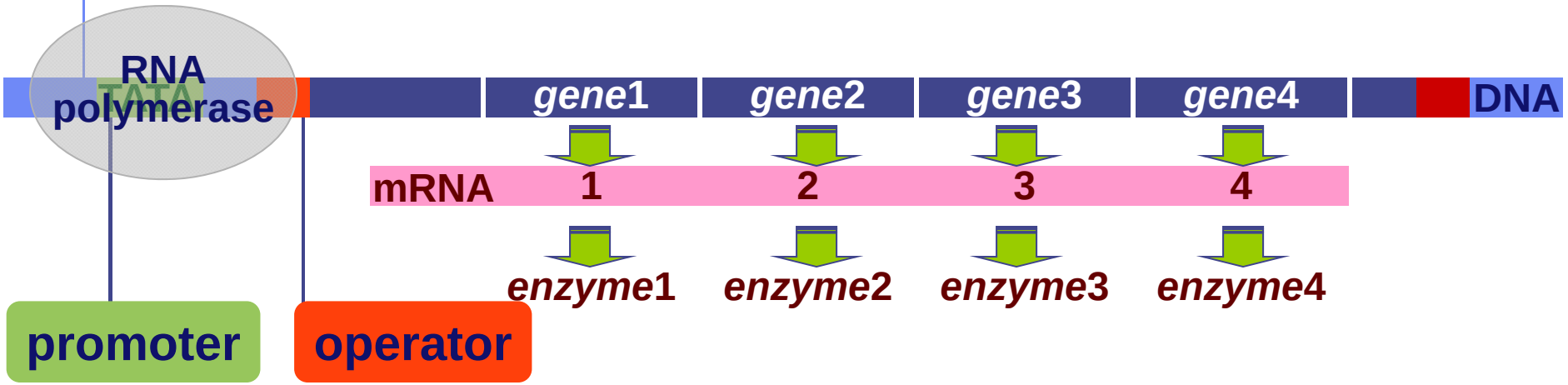
- **Operon**
  - genes grouped together with related functions
    - example: all enzymes in a metabolic pathway
  - promoter = RNA polymerase binding site
    - single promoter controls transcription of all genes in operon
    - transcribed as one unit & a single mRNA is made
  - operator = DNA binding site of repressor protein



Operon model

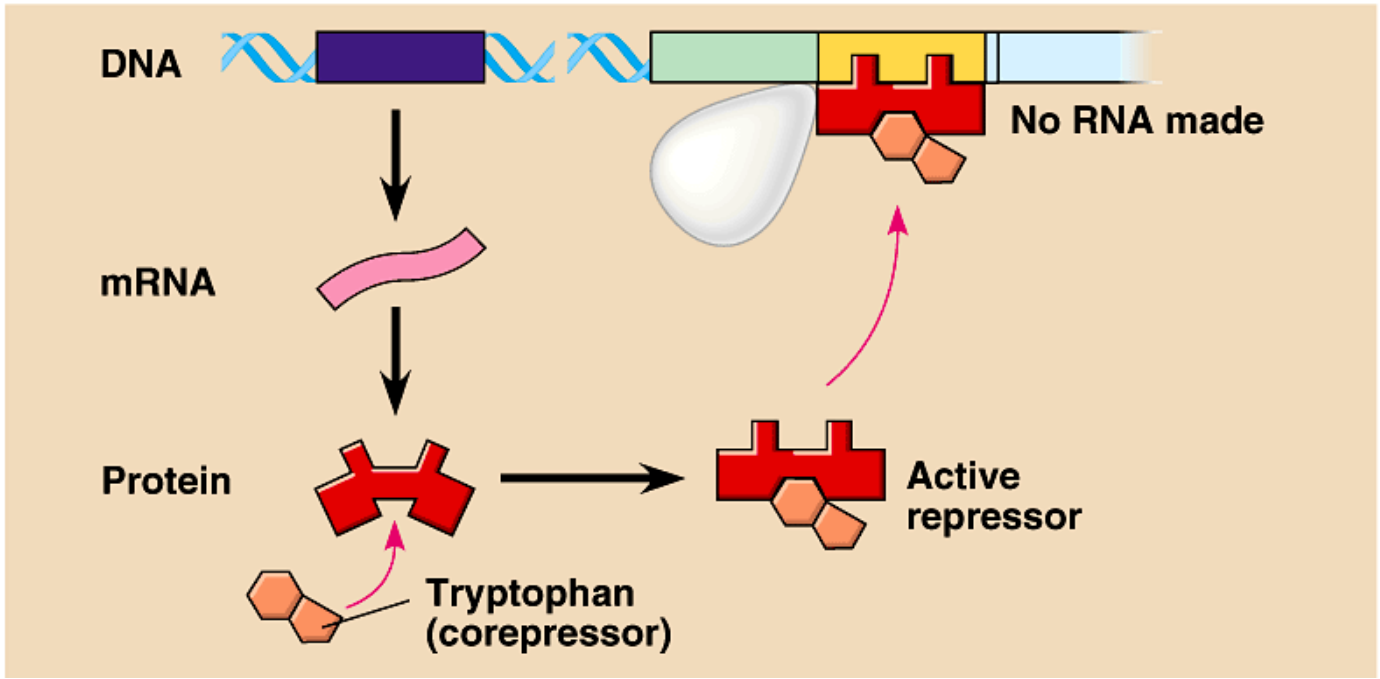
When gene is turned ON:

Polymerase binds promoter
Gene is transcribed



So how can these genes be turned off?

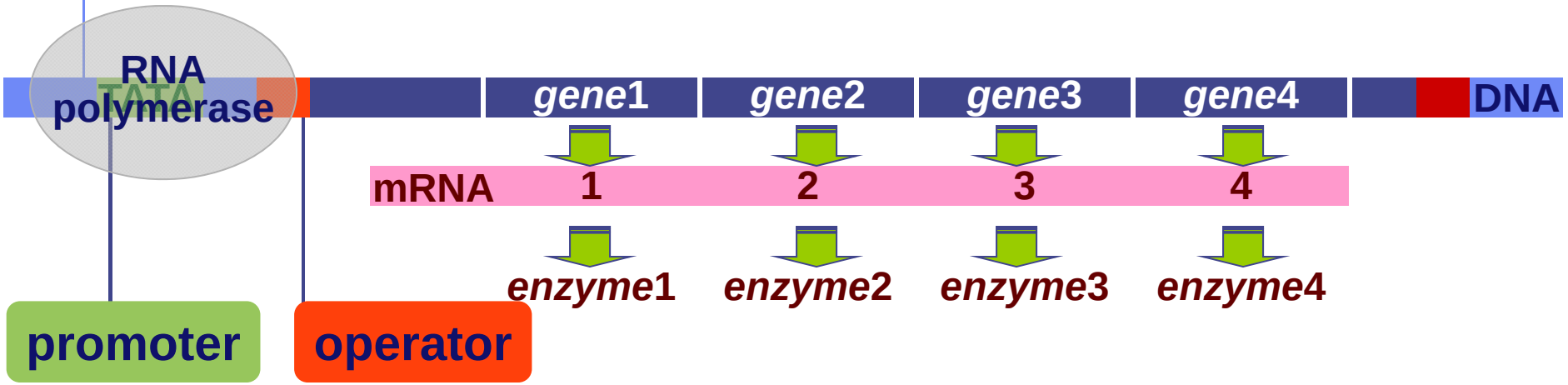
- **Repressor protein**
  - binds to DNA at operator site
  - blocking RNA polymerase
  - blocks transcription



Operon model

Operon:
operator, promoter & genes they control

serve as a model for gene regulation

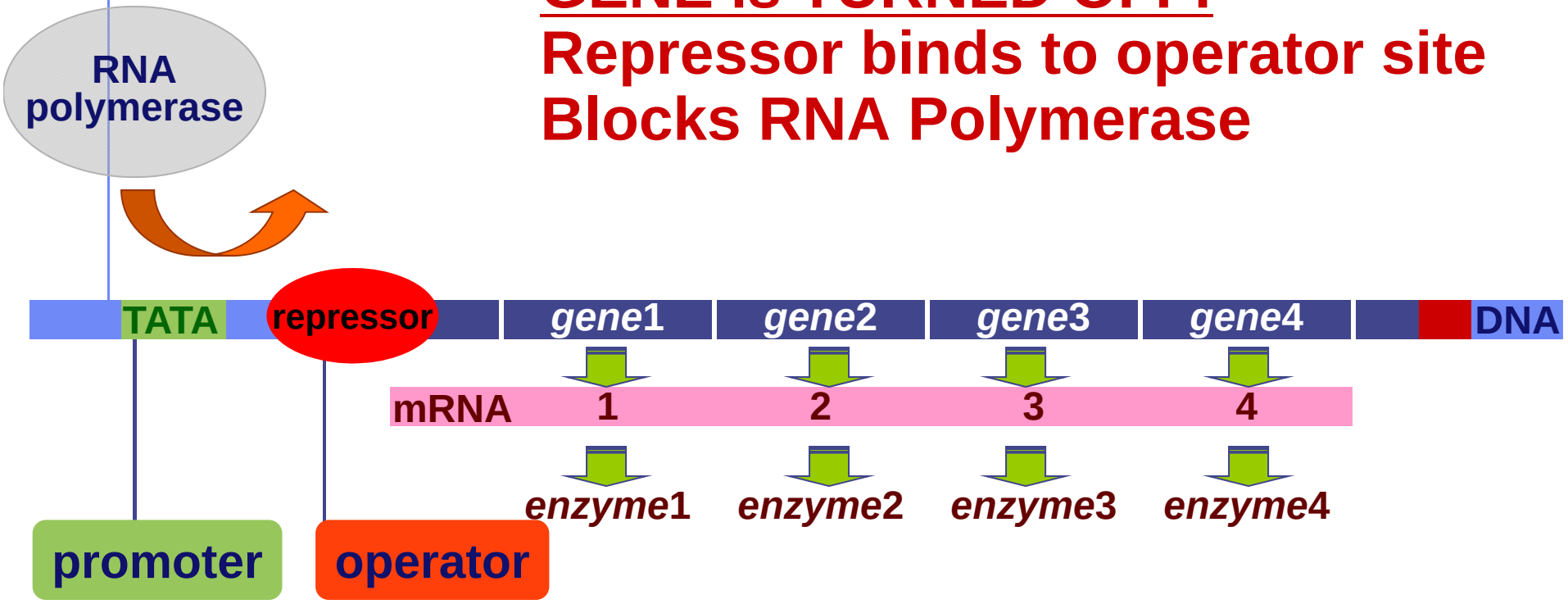


Operon model

GENE is TURNED OFF:

Repressor binds to operator site

Blocks RNA Polymerase



repressor = repressor protein

- **REPRESSIBLE OPERONS are ON**
  Can be turned off
    EX: *trp* operon
      makes enzymes used in tryptophan synthesis

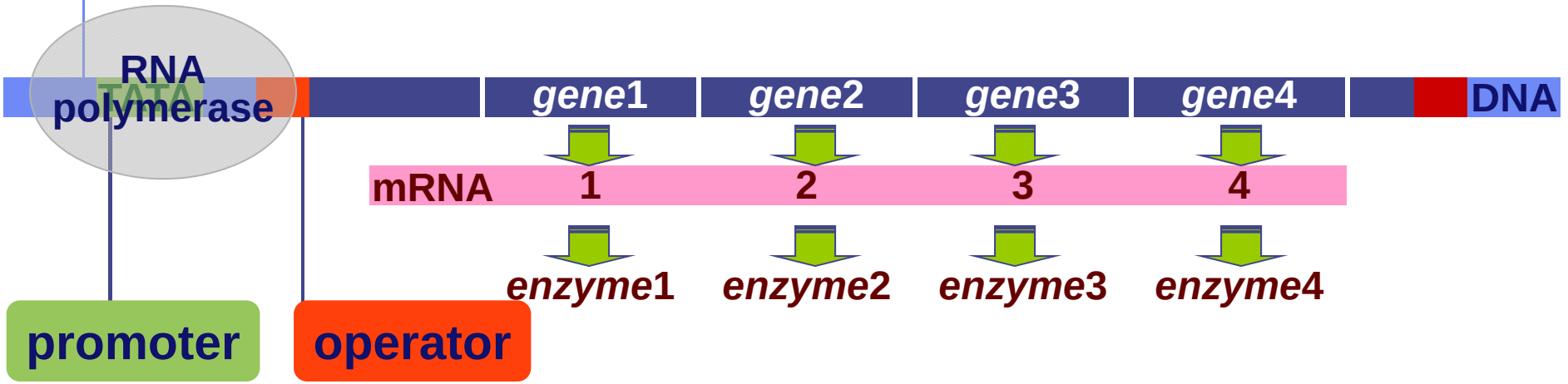
- **INDUCIBLE OPERONS are OFF**
  Can be turned on
    EX: lac operon
      makes enzymes used in lactose digestion

Repressible operon: tryptophan

Gene is on when tryptophan is needed

Repressor protein exists as an inactive form

Cell makes enzymes for tryptophan synthesis



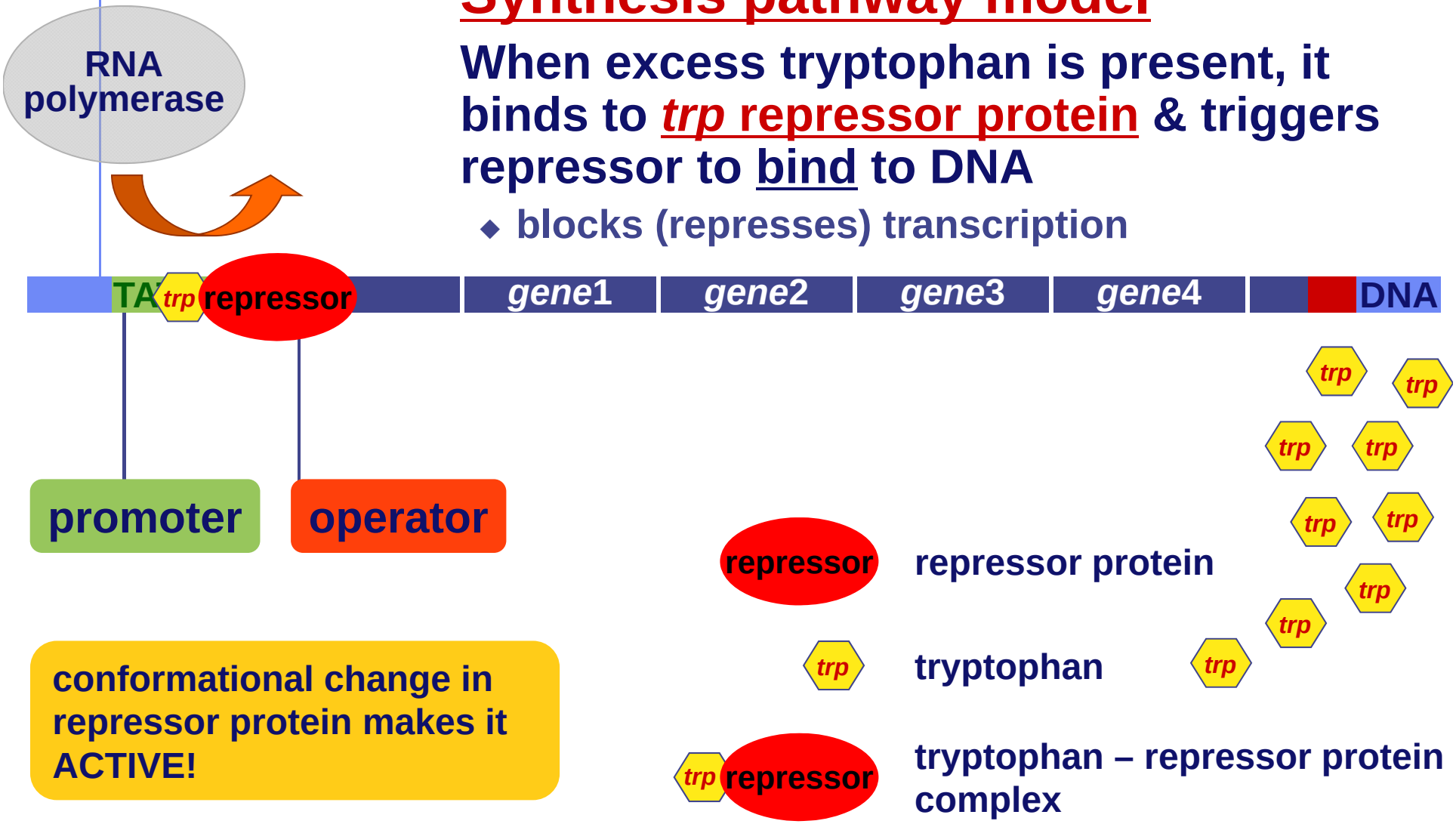
repressor — Inactive repressor protein

Repressible operon: tryptophan

Synthesis pathway model

When excess tryptophan is present, it binds to *trp* repressor protein & triggers repressor to bind to DNA

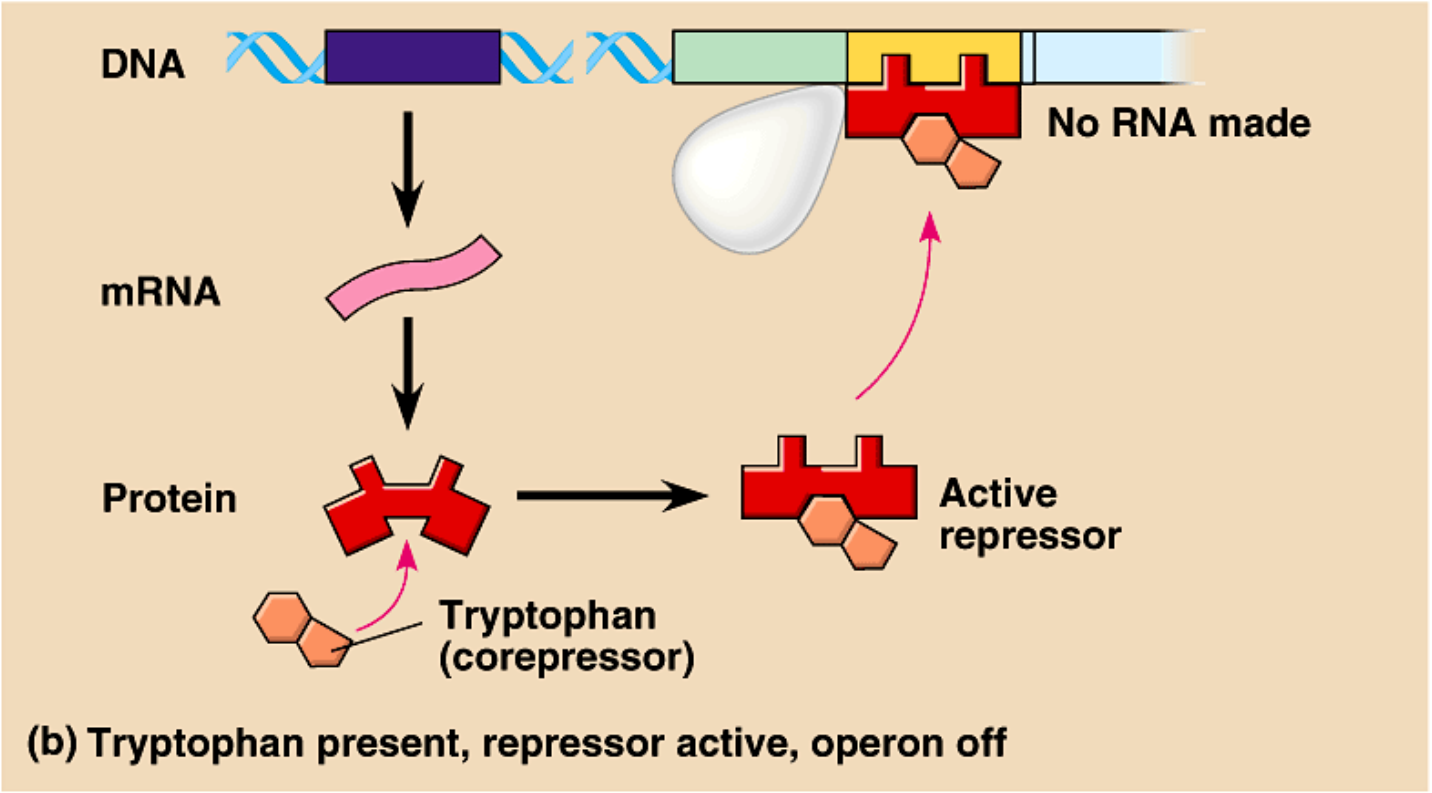
- **blocks (represses) transcription**



Tryptophan operon

When tryptophan is present

Don't need to make tryptophan-building enzymes



Tryptophan is allosteric regulator of repressor protein

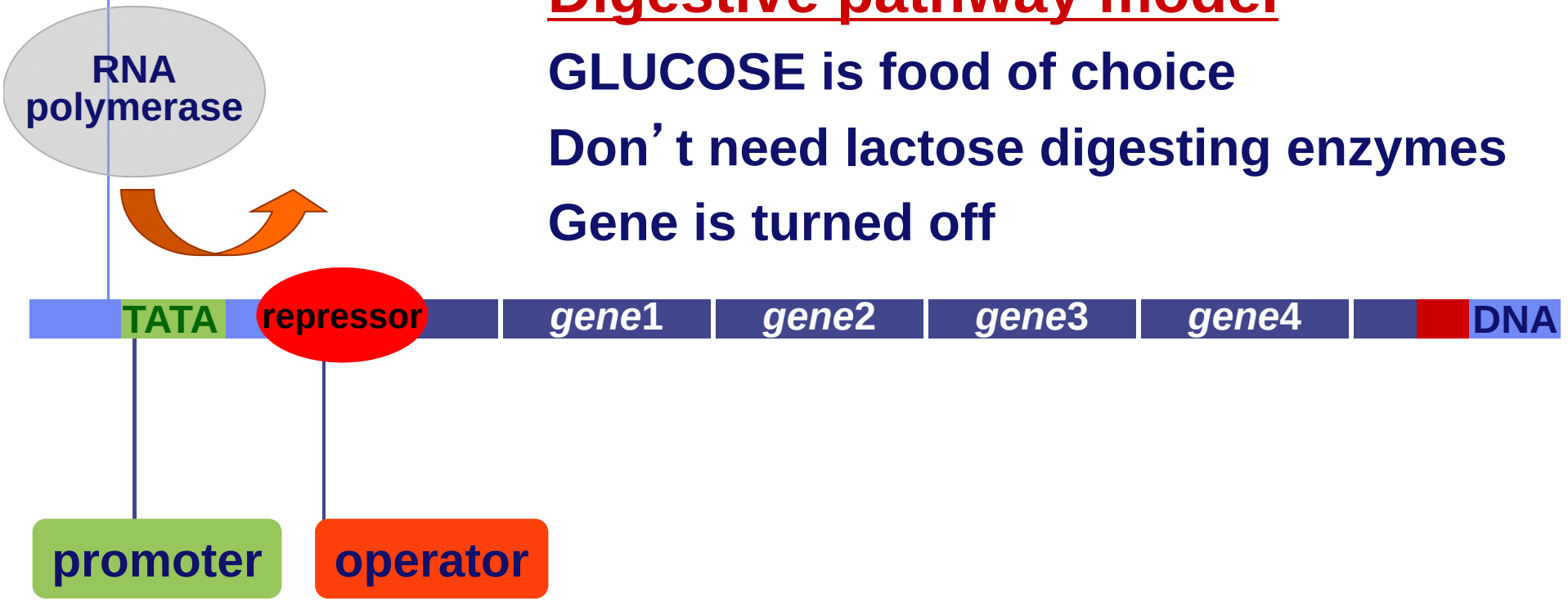
Inducible operon: lactose

Digestive pathway model

GLUCOSE is food of choice

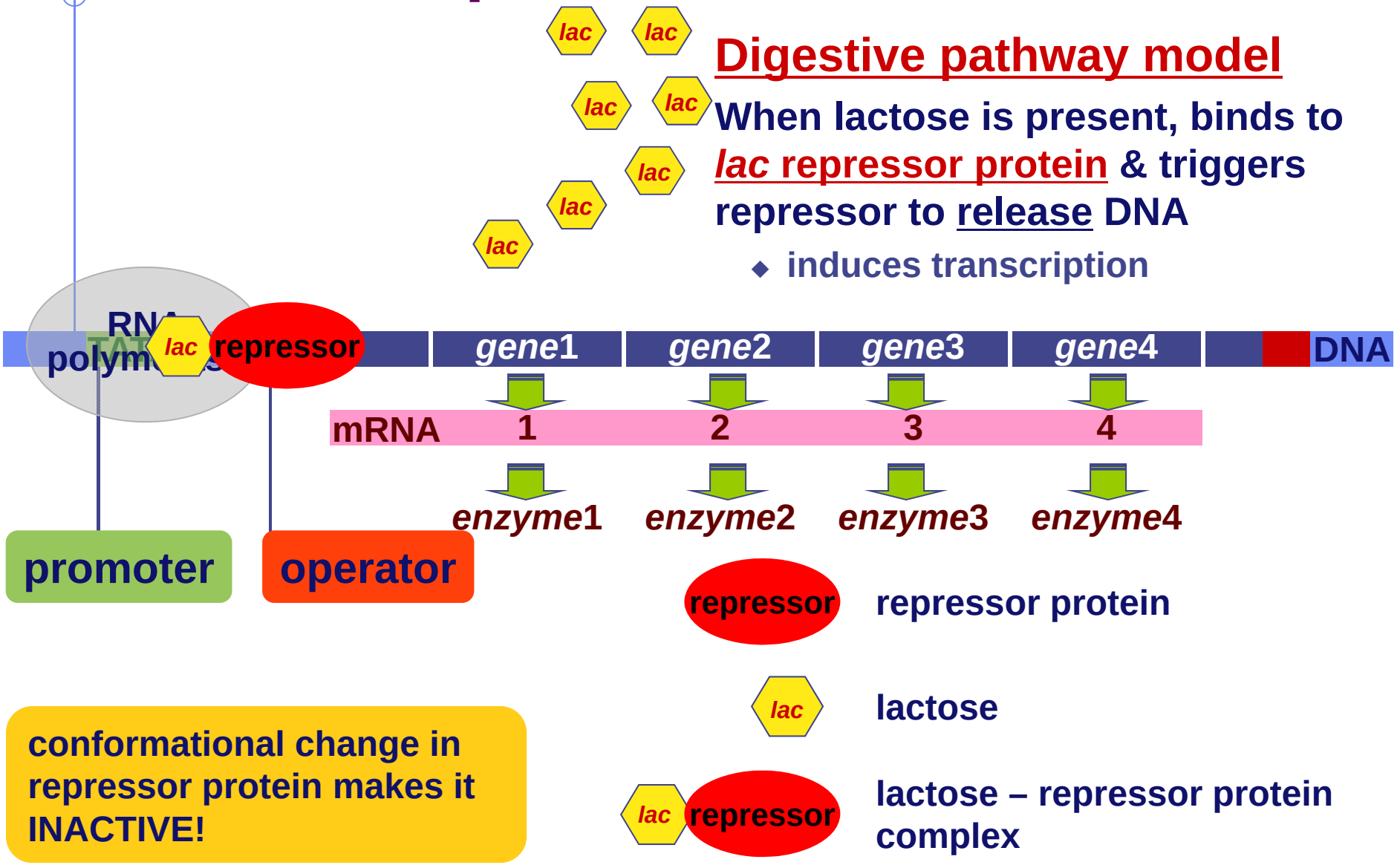
Don't need lactose digesting enzymes

Gene is turned off



repressor ACTIVE repressor protein

Inducible operon: lactose



Digestive pathway model

When lactose is present, binds to *lac* repressor protein & triggers repressor to release DNA

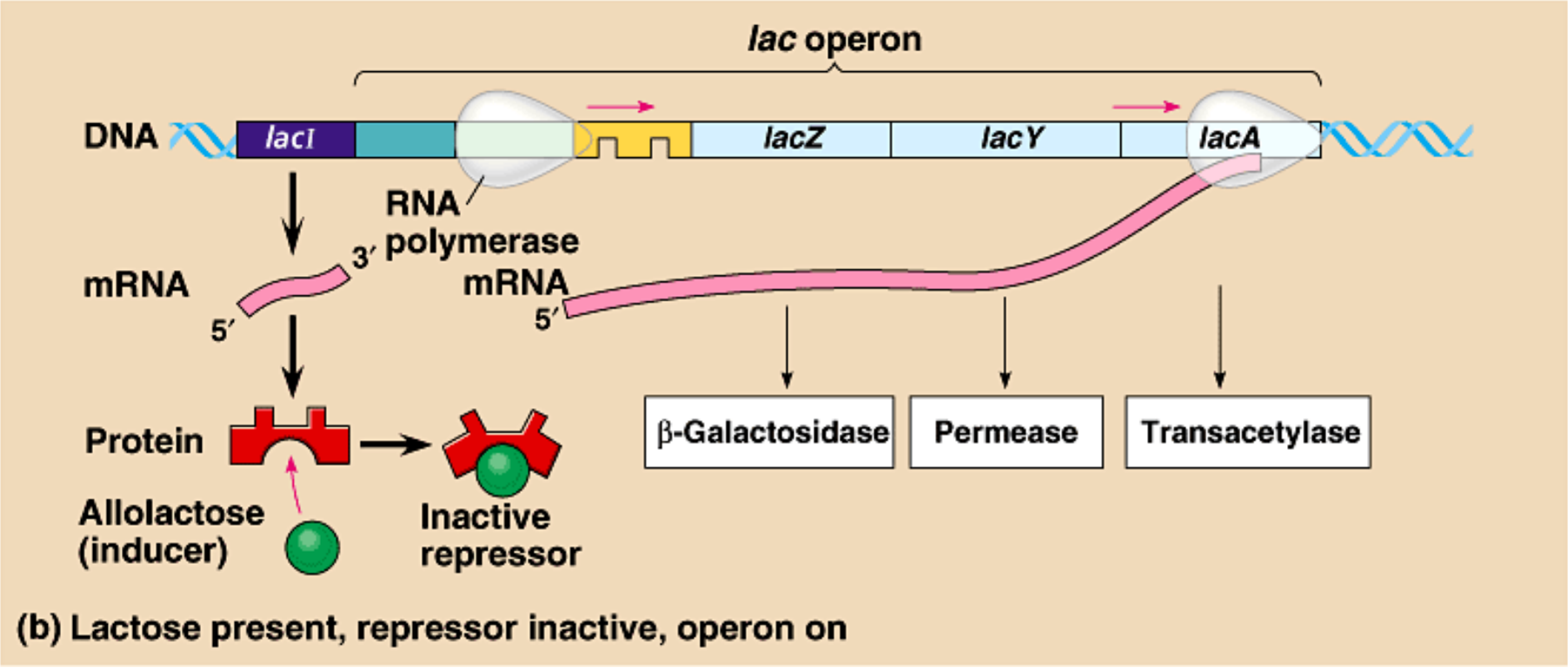
- induces transcription

conformational change in repressor protein makes it INACTIVE!

Lactose operon

What happens when lactose is present?

Need to make lactose-digesting enzymes

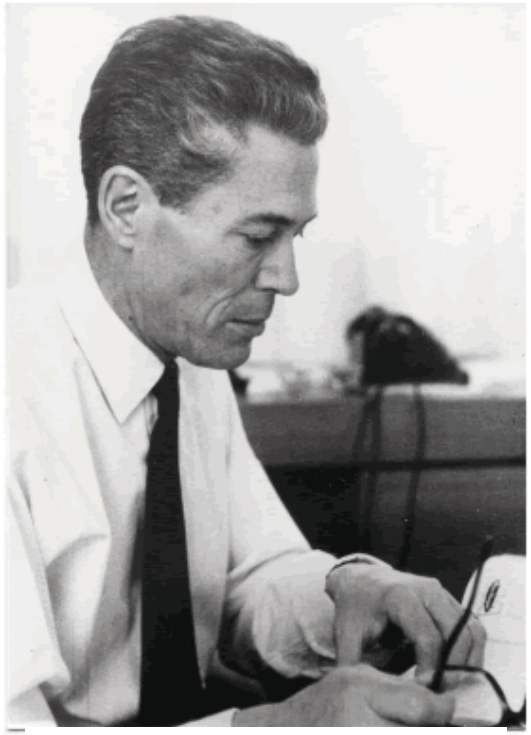


Lactose is allosteric regulator of repressor protein

Jacob & Monod: *lac* Operon

1961 | 1965

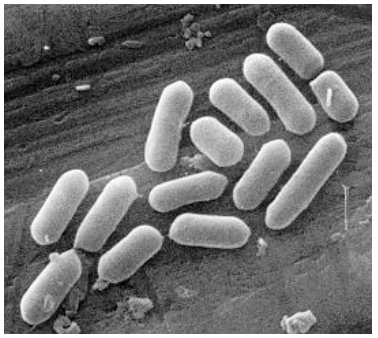
- Francois Jacob & Jacques Monod
  - first to describe operon system
  - coined the phrase “operon”



Jacques Monod

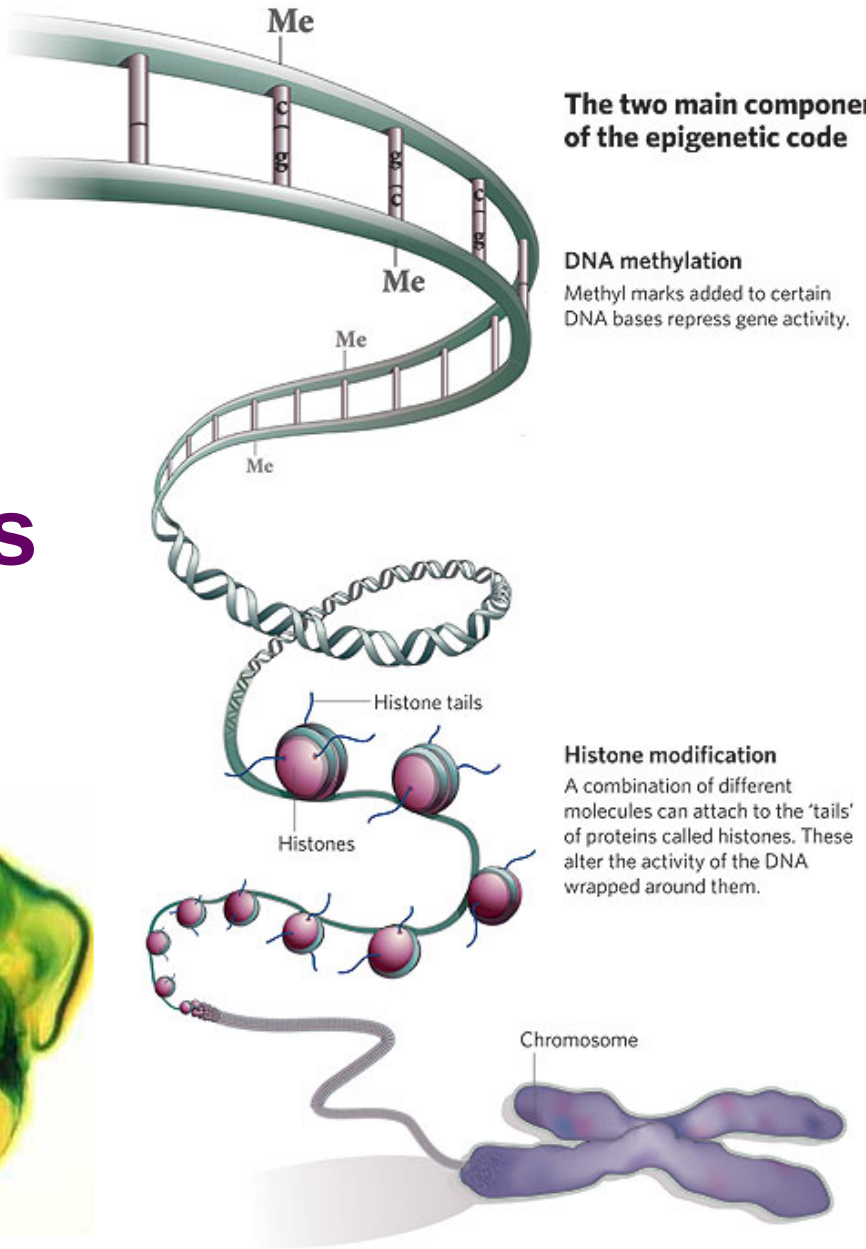
Francois Jacob

Operon summary



- **Repressible operon**
  - usually functions in **anabolic** pathways
    - **synthesizing** end products
  - when end product is present in excess, cell allocates resources to other uses
- **Inducible operon**
  - usually functions in **catabolic** pathways,
    - **digesting** nutrients to simpler molecules
  - produce enzymes only when nutrient is available
    - cell avoids making proteins that have nothing to do, cell allocates resources to other uses

Control of Eukaryotic Genes



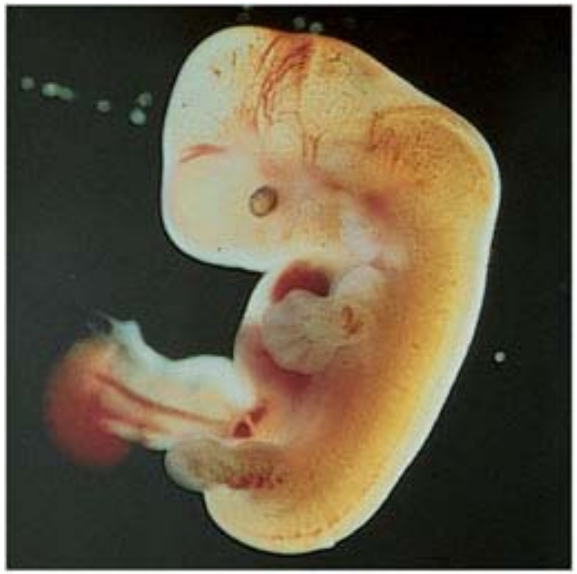
The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

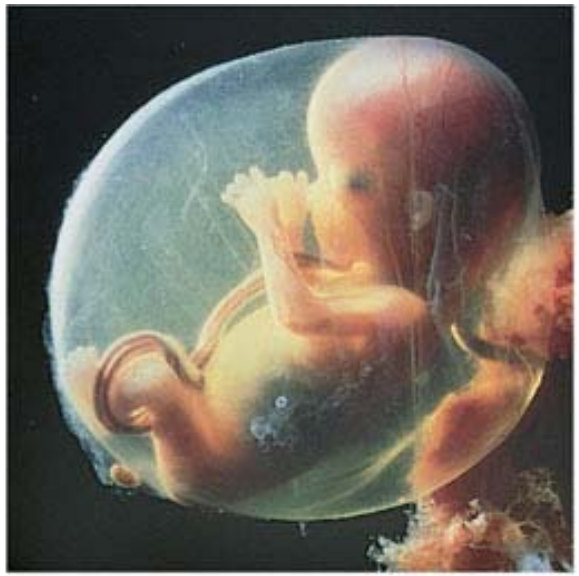
Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

The BIG Questions…

- How are genes turned on & off in eukaryotes?
- How do cells with the same genes differentiate to perform completely different, specialized functions?



(a) 5 weeks.

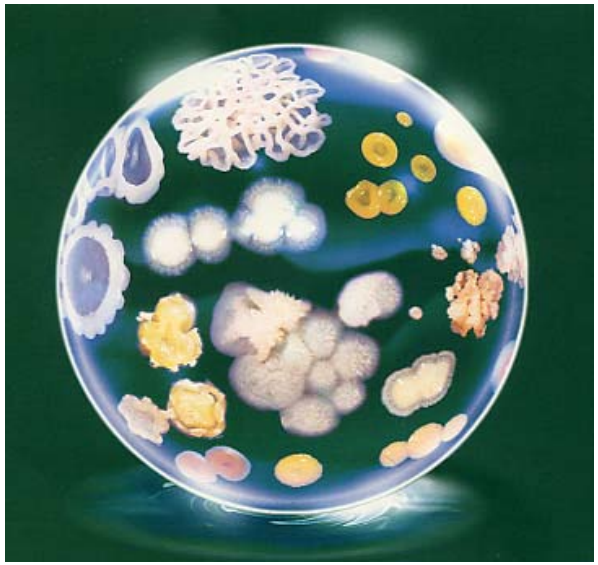


(b) 14 weeks.

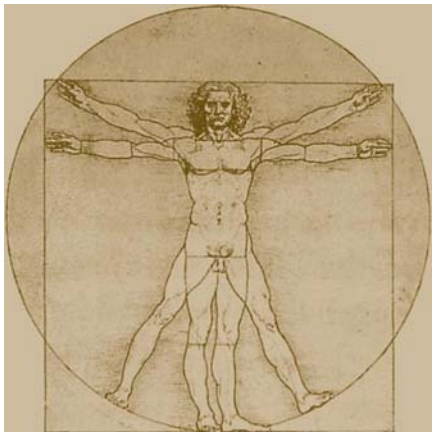
(c) 20 weeks.

Evolution of gene regulation

- Prokaryotes
  - single-celled
  - evolved to grow & divide rapidly
  - must respond quickly to changes in external environment
    - exploit transient resources
- Gene regulation
  - turn genes on & off rapidly
    - flexibility & reversibility
  - adjust levels of enzymes for synthesis & digestion



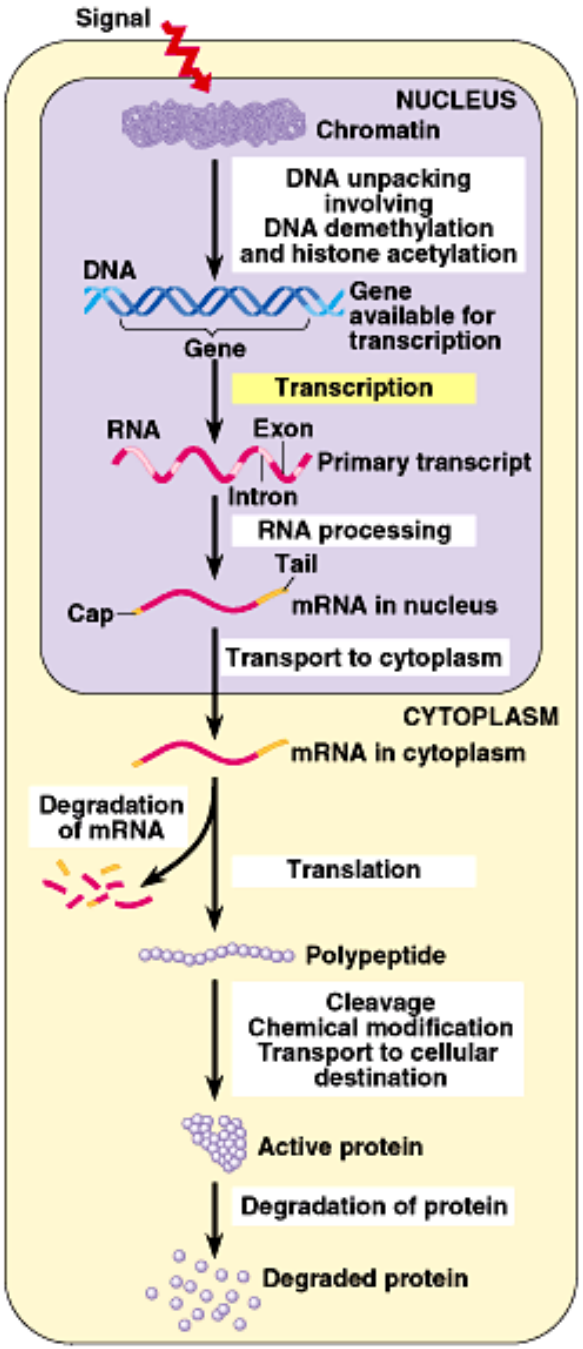
Evolution of gene regulation



- Eukaryotes
  - multicellular
  - evolved to maintain constant internal conditions while facing changing external conditions
    - homeostasis
  - regulate body as a whole
    - growth & development
      - long term processes
    - specialization
      - turn on & off large number of genes
    - must coordinate the body as a whole rather than serve the needs of individual cells

Points of control

- **The control of gene expression can occur at any step in the pathway from gene to functional protein**
  1. packing/unpacking DNA
  2. transcription
  3. mRNA processing
  4. mRNA transport
  5. translation
  6. protein processing
  7. protein degradation

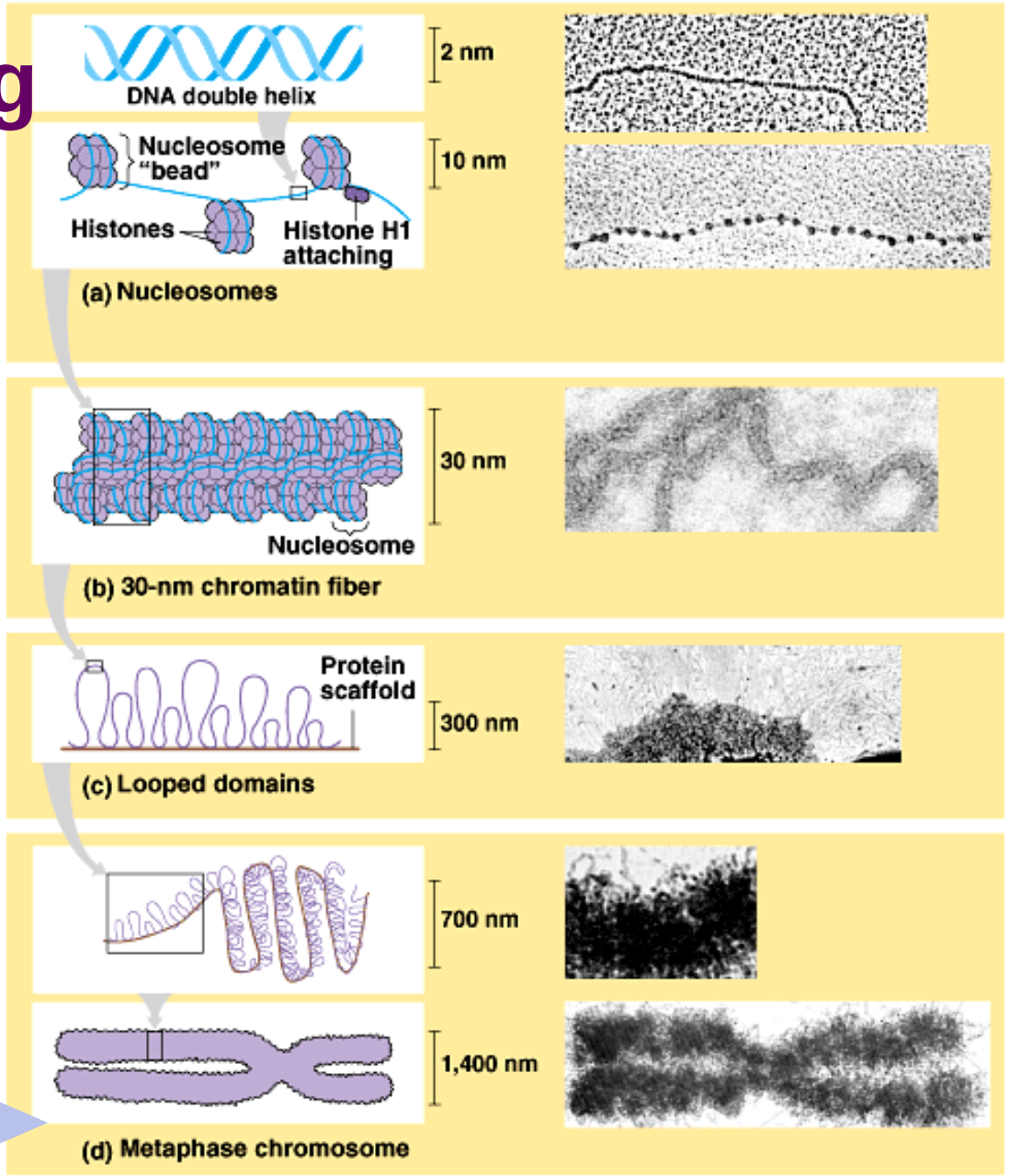


1. DNA packing

How do you fit all that DNA into nucleus?

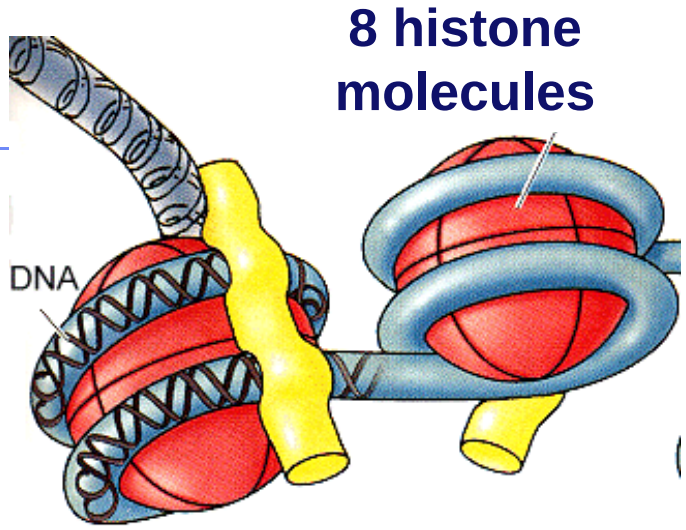
- DNA coiling & folding
  - double helix
  - nucleosomes
  - chromatin fiber
  - looped domains
  - chromosome

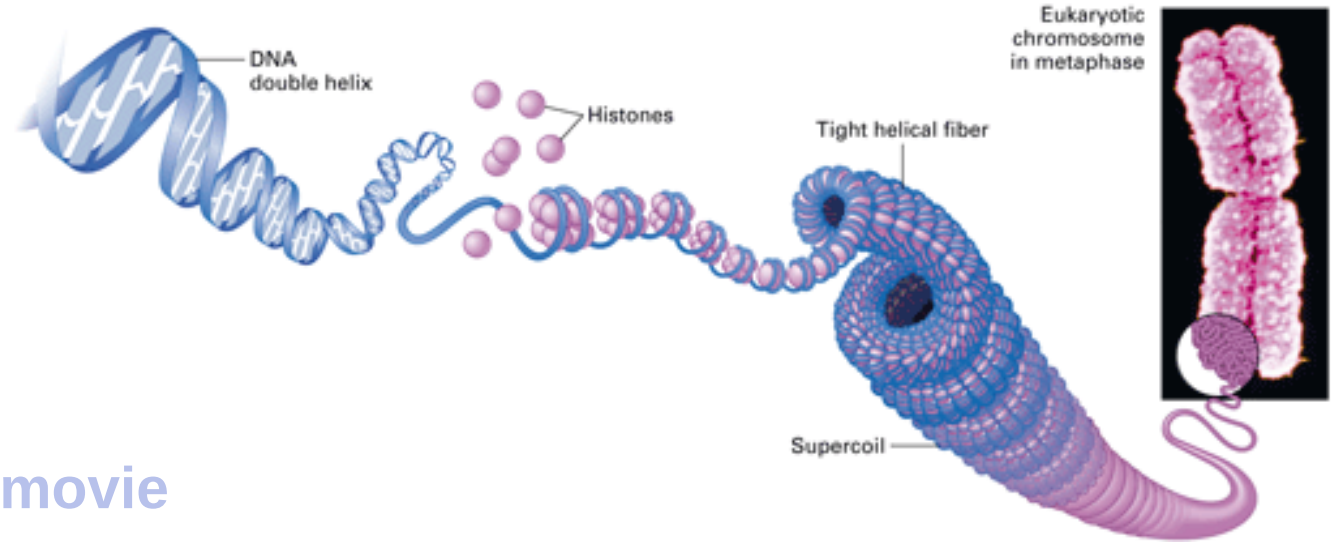
from DNA double helix to condensed chromosome



Nucleosomes

- "Beads on a string"
  - 1st level of DNA packing
  - histone proteins
    - 8 protein molecules
    - positively charged amino acids
    - bind tightly to negatively charged DNA



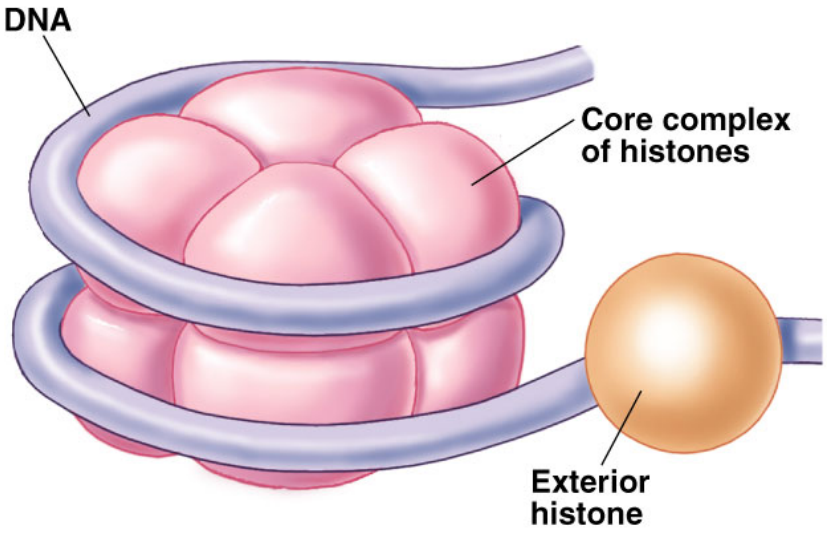


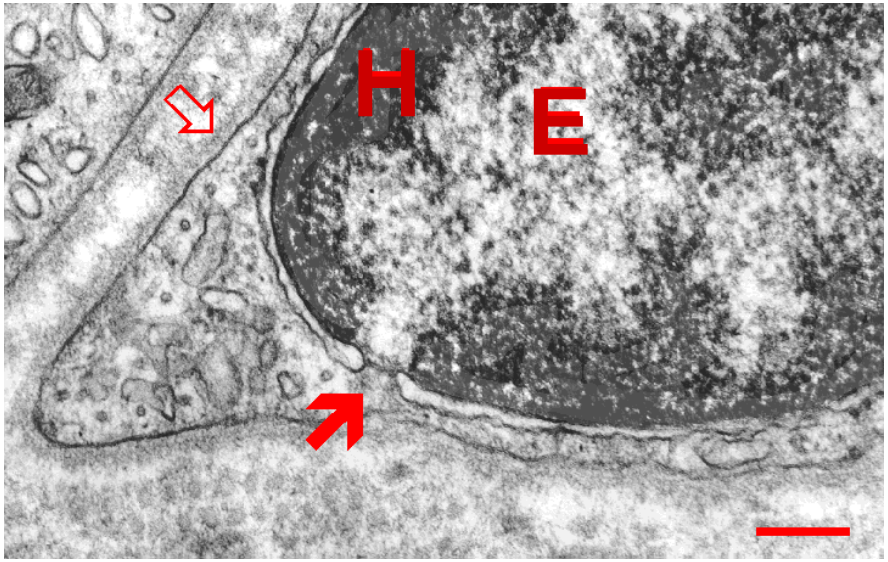
DNA packing movie

DNA packing as gene control

- Degree of packing of DNA regulates transcription
  - tightly wrapped around histones
    - no transcription
    - genes turned off

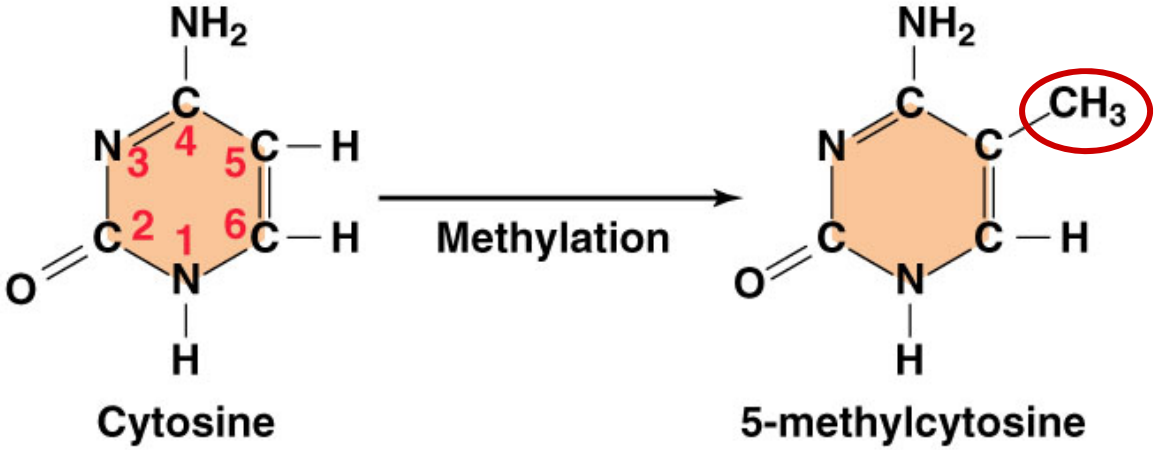
- heterochromatin
  darker DNA (H) = tightly packed
- euchromatin
  lighter DNA (E) = loosely packed





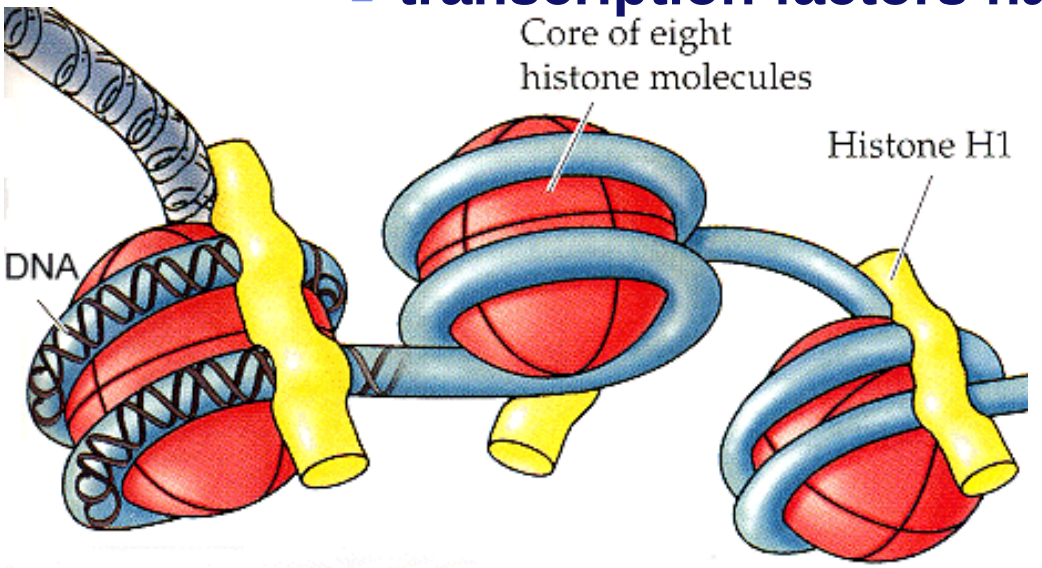
DNA methylation

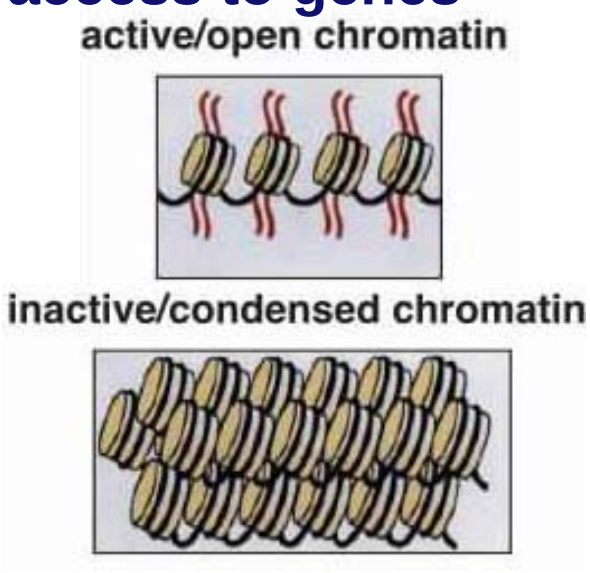
- **Methylation of DNA blocks transcription factors**
  - no transcription
    - → **genes turned off**
  - attachment of methyl groups ($-CH_3$) to cytosine
    - C = cytosine
  - nearly permanent inactivation of genes
    - ex. inactivated mammalian X chromosome = Barr body



Histone acetylation

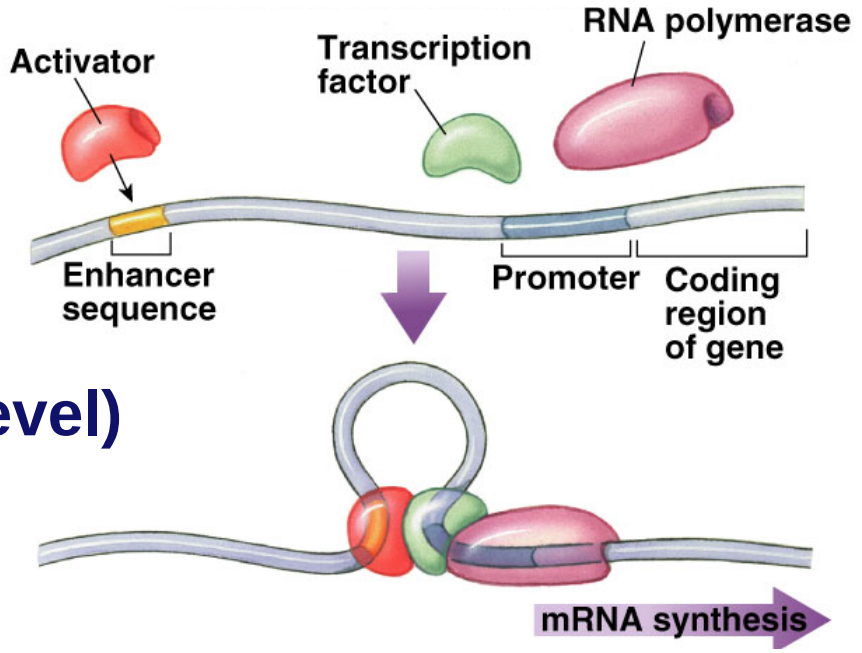
- **Acetylation of histones unwinds DNA**
  - **loosely wrapped around histones**
    - **enables transcription**
    - **genes turned on**
  - **attachment of acetyl groups (–$COCH_3$) to histones**
    - **conformational change in histone proteins**
    - **transcription factors have easier access to genes**





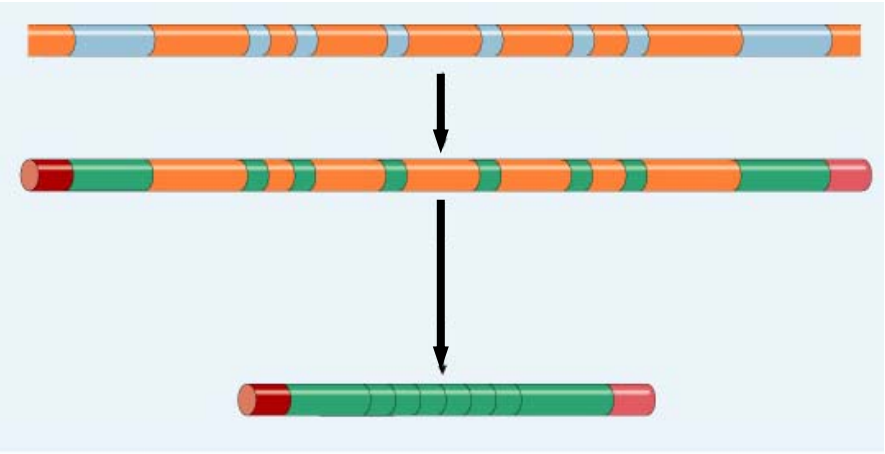
2. Transcription initiation

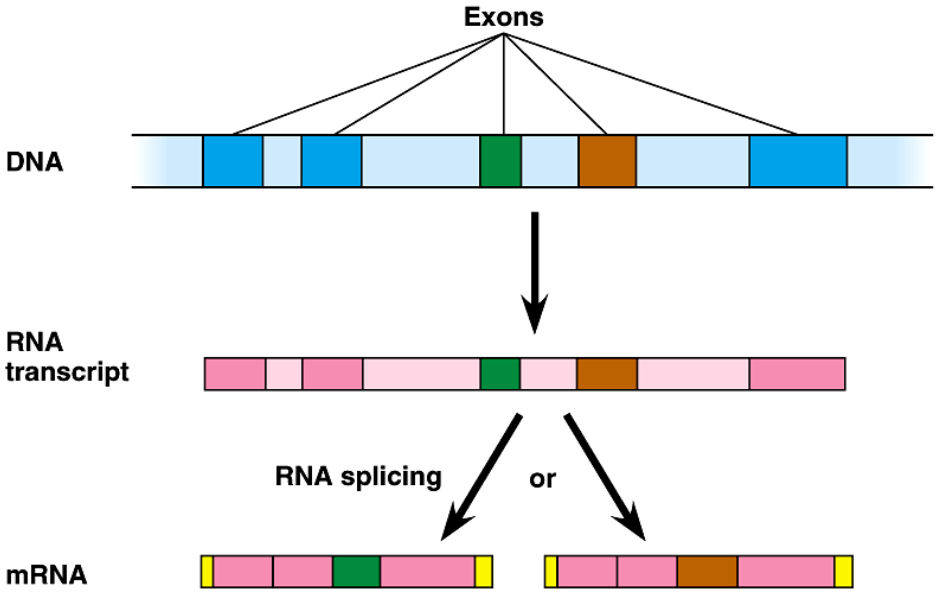
- Control regions on DNA
  - promoter
    - nearby control sequence on DNA
    - binding of RNA polymerase & transcription factors
    - "base" rate of transcription
  - enhancer
    - distant control sequences on DNA
    - binding of activator proteins
    - "enhanced" rate (high level) of transcription



3. Post-transcriptional control

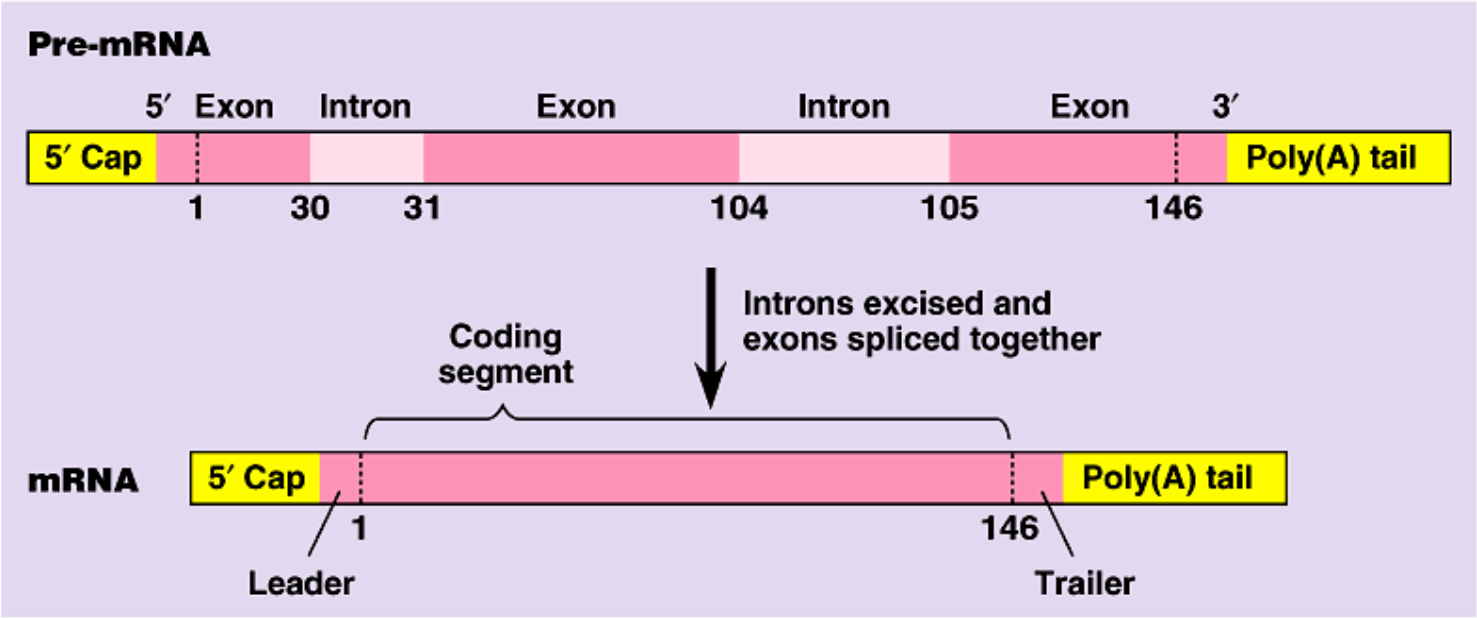
- **Alternative RNA splicing**
  - **variable processing of exons creates a family of proteins**





4. Regulation of mRNA degradation

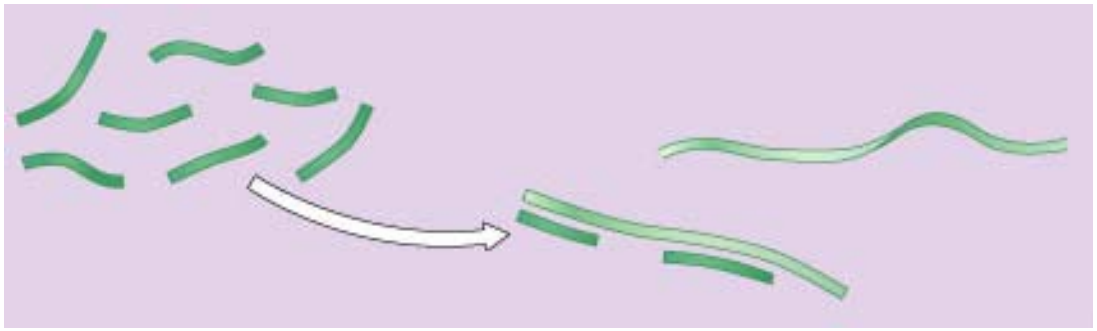
- **Life span of mRNA determines amount of protein synthesis**
  - **mRNA can last from hours to weeks**



RNA interference

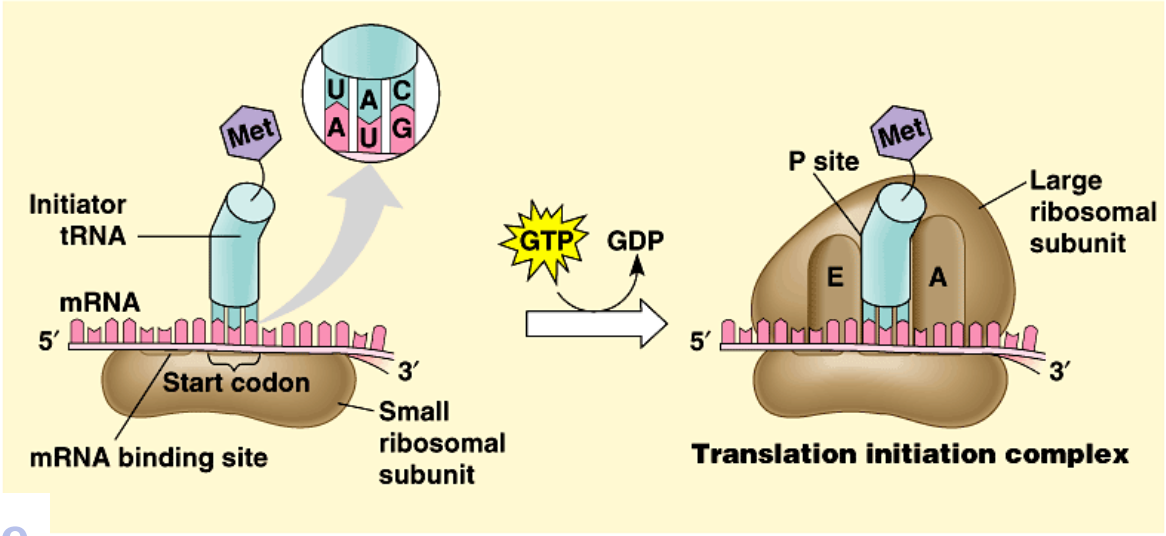
- **Small interfering RNAs (siRNA)**
  - short segments of RNA (21-28 bases)
    - bind to mRNA
    - create sections of double-stranded mRNA
    - "death" tag for mRNA
      - triggers degradation of mRNA

siRNA



5. Control of translation

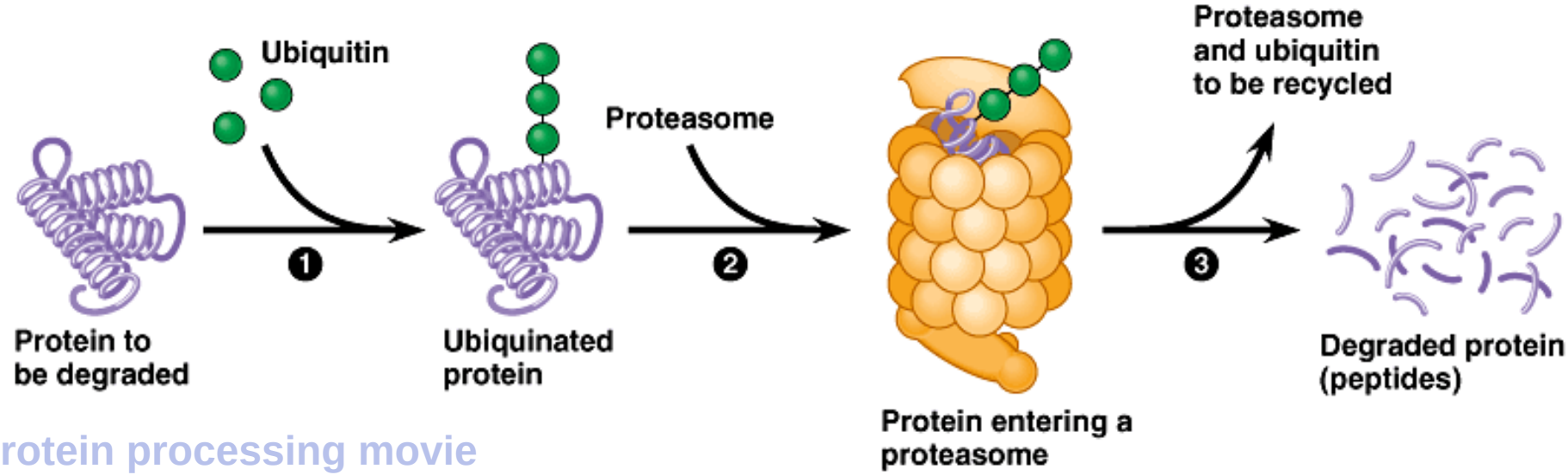
- **Block initiation of translation stage**
  - **regulatory proteins attach to 5' end of mRNA**
    - **prevent attachment of ribosomal subunits & initiator tRNA**
    - **block translation of mRNA to protein**



Control of translation movie

6-7. Protein processing & degradation

- **Protein processing**
  - folding, cleaving, adding sugar groups, targeting for transport
- **Protein degradation**
  - **ubiquitin** tagging
  - **proteasome** degradation

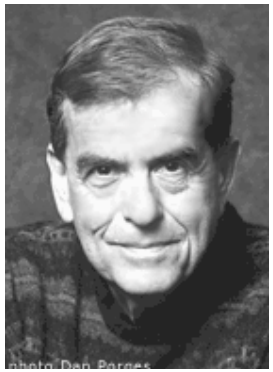


Protein processing movie

1980s | 2004

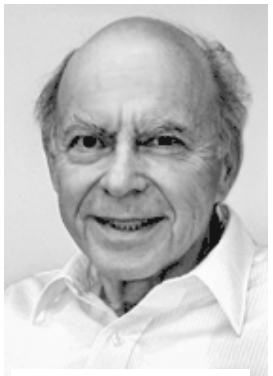
Ubiquitin

- "Death tag"
  - mark unwanted proteins with a label
  - 76 amino acid polypeptide, ubiquitin
  - labeled proteins are broken down rapidly in "waste disposers"
    - proteasomes



Aaron Ciechanover
Israel

Avram Hershko
Israel



Irwin Rose
UC Riverside

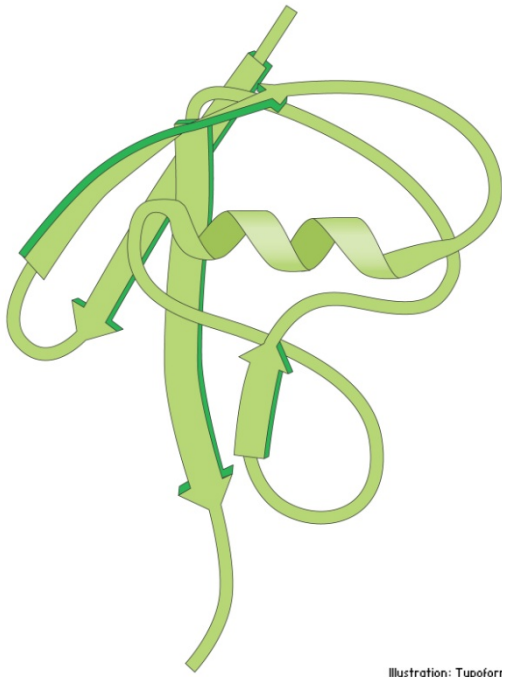
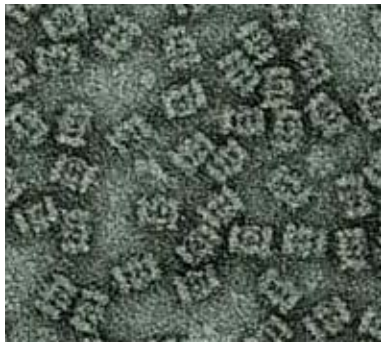
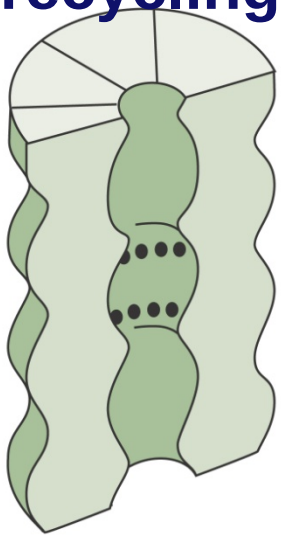


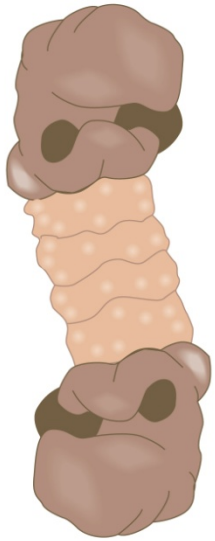
Illustration: Tupoforr

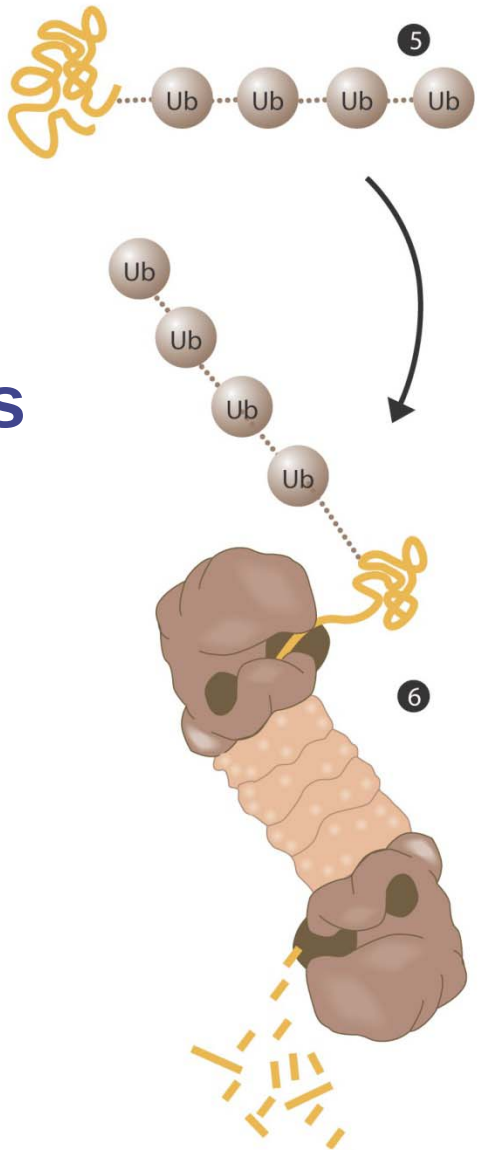
Proteasome

- **Protein-degrading "machine"**
  - **cell's waste disposer**
  - **breaks down any proteins into 7-9 amino acid fragments**
    - **cellular recycling**



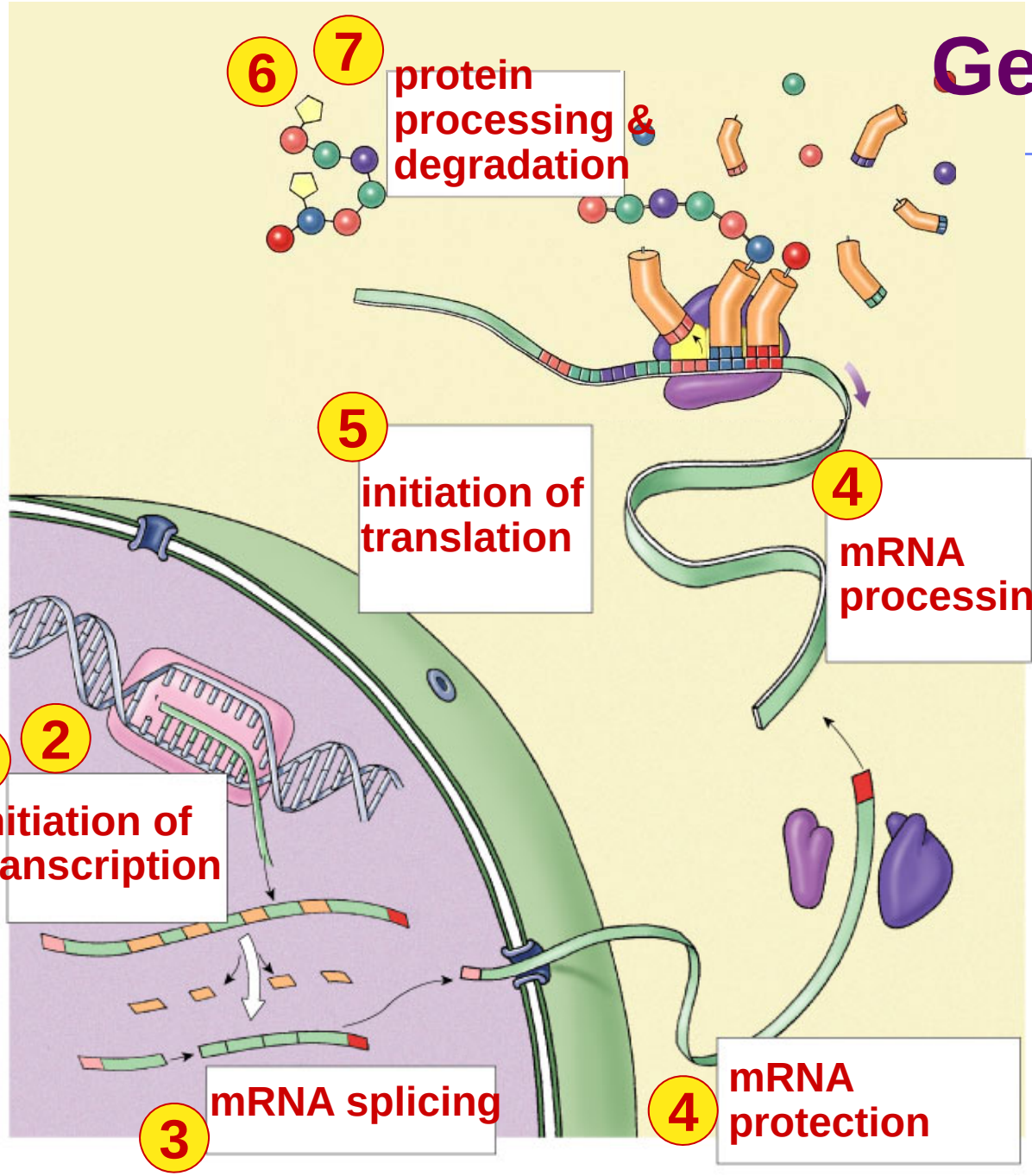






play Nobel animation

Gene Regulation



1 & 2. transcription
- DNA packing
- transcription factors

3 & 4. post-transcription
- mRNA processing
- splicing
- 5’ cap & poly-A tail
- breakdown by siRNA

5. translation
- block start of translation

6 & 7. post-translation
- protein processing
- protein degradation

Title (should be descriptive of the work)

Your Names Here

Wofford College, South Carolina

Introduction

This section should provide background information (appropriately cited) from the reference literature about your topic. Typically, an introduction moves from general to specific, and it ends with your hypothesis (sometimes stated as the experiment's purpose). The background information should inform the reader about the bigger biological issues under investigation, and how this experiment will add something new to (or clarify) what is already known about the topic. To avoid plagiarizing the work of other authors, summarize relevant findings in your OWN words and then cite the source of the idea. For citation format, you may number the citations, or you may use the author and year system (e.g., Smith, et al. 2009). A bibliographic reference for all cited works must appear in the literature cited section.

Methods

This section includes information on how the experiment was performed. It should be brief but descriptive (a list of supplies is not appropriate). Consider using a flow chart or picture if you think it would help the reader understand what was done. Remember that the reader should be able to get the gist of the experimental methods here (a research poster doesn't give as much detail as a full article, but it should provide enough detail that a naive reader will understand what you did). If applicable, be sure to name/describe the statistical test that you used to analyze your data.

Results

This section should be dominated by results that appear in graphical (usually) or table form. Each graph should have a descriptive caption that begins with Figure 1, Figure 2, etc. The caption should include information that allows your reader to quickly interpret the findings depicted in the figure without having to read the rest of the poster. Statistics should be displayed appropriately. For example, sample means should show the variability, such as standard error bars, in the data. Your results **SHOULD NOT** include raw data.

Other hints for preparing your poster:

1.WORK AS A TEAM – not like workers on assembly lines of different car companies. Such workers end up with all the parts of a car, but none of them fit together! So, all team members should work on every section together, rather than Joe does the Intro, Jenny does the Methods, etc. There is a grade for posters that lack flow and coherence, and you don't want that grade!

2.If you want to change the background, logo, etc., on this template, go to "slide master" under the edit tab.

3.If you have room, consider adding relevant photos to your poster – they capture the eye and draw people in.

4.This template is set up to print on Ledger size paper (11' X 17"), which your professor will print for you in color (send him/her the electronic file). If you want to print an 8.5" X 11" size copy, be sure to go to page setup and change the paper size.

5.Brevity is key to success– write out a draft of your text and then remove as many words as possible. Note: You are looking at too many words on this poster template!

6.Get many people to critique your poster. Remember that peer-review is a hallmark of good scholarship.

Discussion

The body of a discussion tends to go from the specific to the general. Start with your team's **interpretation** of the results but do not restate them (i.e., don't just describe your figures again). What is the biological significance of your findings (i.e., did you achieve your stated purpose and/or do the results support your hypothesis)? Consider your results in relationship to what others have done – how do your findings clarify or contribute new information in light of previous studies (and cite them)? What additional scientific questions arise from your experiment, and what follow-up experiments might address those questions?

At the end, link the work you've done here to a larger context. This is the "So what? Who cares" part of your poster.

Literature Cited

Use the appropriate format for your citations (you can use the format from the references/literature cited section of one of your primary sources as a guide here).

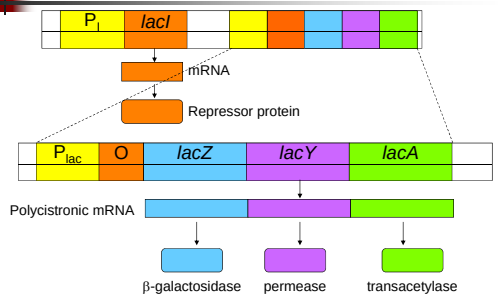
It is *not* OK to list a bunch of web sites. Your literature cited section must have at least two primary sources (published articles from refereed journals that you have read) and one other resource (review, textbook, etc…) related to your topic.

Acknowledgements

Use this section to thank those who helped you or to list with whom your group collaborated (if you collaborated with another group).

E. coli Lactose Operon

Review of *lac* Operon Parts



Control of *lac* Operon: Absence of Lactose

- *LacI repressor* protein binds to operator preventing RNA pol from binding to promoter.
- So, if no RNA is produced, then no permease should be made. How does lactose get into cell to induce transcription of the operon?

Control of *lac* Operon: Absence of Lactose

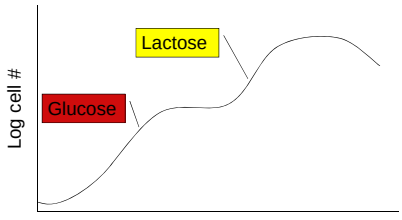
- Expression is *"leaky."*
  - Even in absence of lactose a tiny amount of transcription takes place. Why important?

Choices for Lunch

Pizza

Creamed Spinach

Biauxic Growth of *E. coli*



Catabolite Repression

- Presence of one catabolite (sugar) inhibits metabolism of other sugars.
- Glucose prevents induction of the *lac* operon.
  - **Catabolite activator protein (CAP)** is required for maximal expression of *lac* operon.
  - CAP is inactive in when glucose is present.

Catabolite Repression Occurs in the Presence of Glucose

↑Glucose ↓cAMP ↓transcription

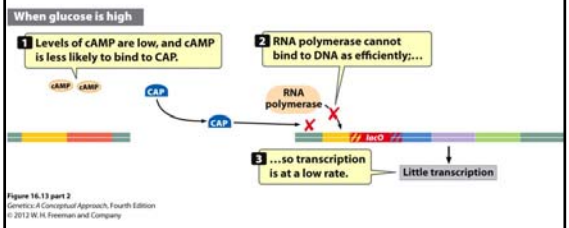


Figure 16.13 part 2
Genetics: A Conceptual Approach, Fourth Edition
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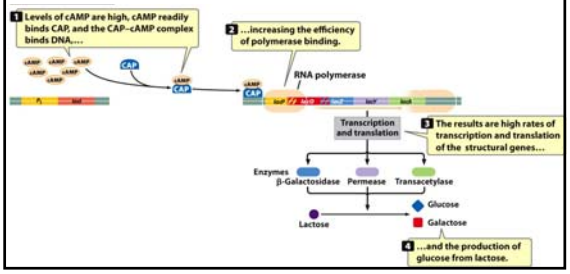
No Catabolite Repression When [Glucose] is Low

↓Glucose ↑cAMP ↑transcription



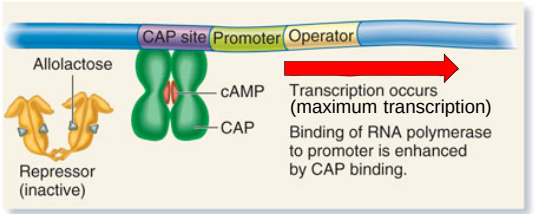
Role Play - Glucose / + Lactose & + Glucose / + Lactose

- Actors
  - RNA polymerase
  - Lac Repressor
  - Polycistronic mRNA (3 actors)
  - Ribosomes
  - β-galactosidase
  - Permease
  - CAP

Activity

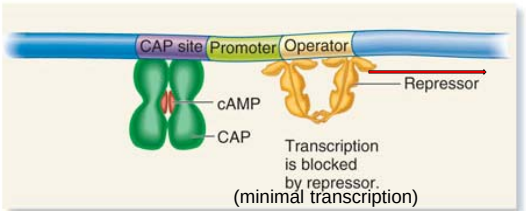
- Describe the conditions shown in the following scenarios concerning the *lac* operon.
  - Is lactose present or absent?
  - Is [glucose] high or low?
- Explain to class how you arrived at your answer.

Scenario 1
+ Lactose /↓ [Glucose] (↑ cAMP)



Scenario 2

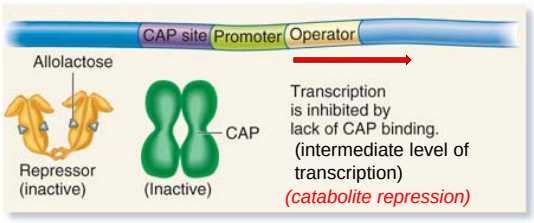
- Lactose /↓ [Glucose] (↑ cAMP)



(minimal transcription)

Scenario 3

+ Lactose / ↑ [Glucose] (↓ cAMP)

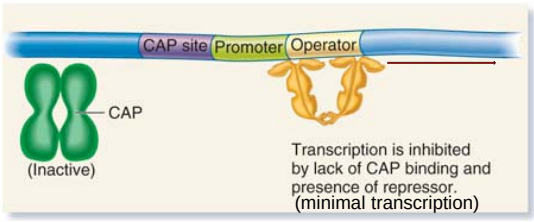


(intermediate level of transcription)

(catabolite repression)

Scenario 4

- Lactose /↑ [Glucose] (↓ cAMP)

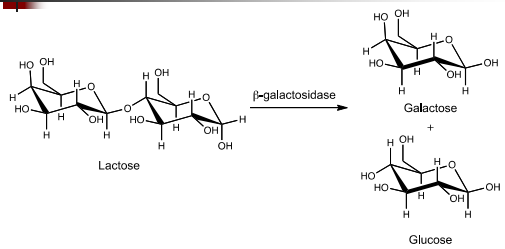


(minimal transcription)

First Task

- Three strains A, B, C.
- Figure out which strain is wildtype, which is *lacZ*$^-$ and which is *lacI*$^-$.

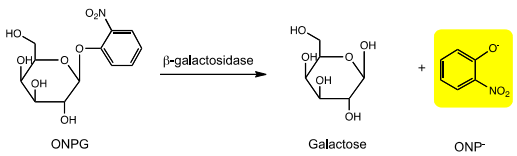
Normal β-galactosidase Activity



Problem: It's difficult to detect formation of glucose & galactose

Solution to Problem

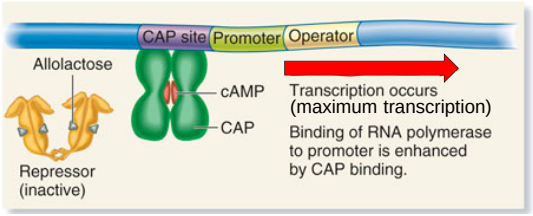
Use a different substrate for β-galactosidase



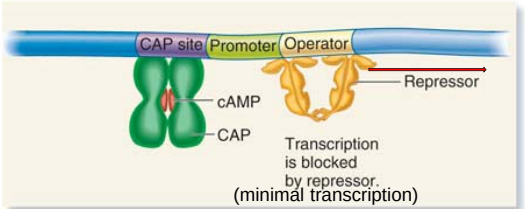
Procedure

- 4 scenarios
  - Water (negative control)
  - Glucose
  - Lactose
  - TMG (a lactose analog)
- Predict which strains will result in yellow product in each of the scenarios listed above.

Scenario 1

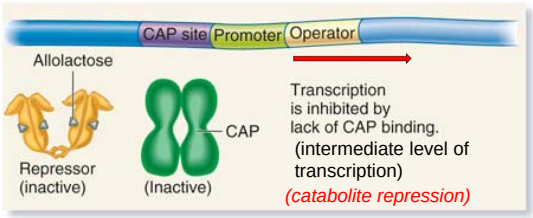


Scenario 2



(minimal transcription)

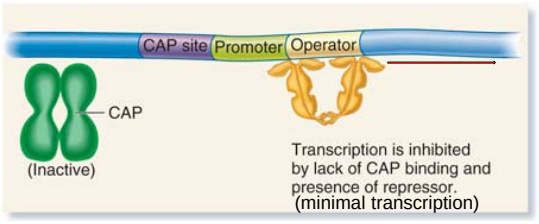
Scenario 3



(intermediate level of transcription)

(catabolite repression)

Scenario 4


CAP site
Promoter
Operator
CAP
(Inactive)
Transcription is inhibited by lack of CAP binding and presence of repressor.
(minimal transcription)