

C2005/F2401 '08 -- Lecture 15 -- Last Edited: 10/29/08 03:13 PM

© Copyright 2008 Deborah Mowshowitz and Lawrence Chasin Department of Biological Sciences Columbia University New York, NY.

Handouts: 15A -- Induction vs Repression; Repression vs Feedback Inhibition & 15 B -- [Operons](#)

I. Intro to Regulation in Prokaryotes (See handout 15A)

A. Why regulation of enzyme synthesis is reasonable and/or necessary -- consider some typical enzymes -- glycolytic enzymes, beta-galactosidase (needed to breakdown and metabolize lactose = dimer of glucose and galactose), and trptophan synthetase (needed to synthesize trp). (See Becker 23-1 & 23-2.) When are these enzymes needed?

1. *Glycolytic enzymes* -- always needed

2. *Beta-galactosidase* -- only needed if lactose **present** (and needs to be broken down); enzyme level should be low until lactose added to medium.

3. *TS (trp synthetase)* -- only needed if trp low or **absent** (then trp must be synthesized in order to make proteins) -- enzyme level should be high until trp added to medium.

4. *Why not make all enzymes all the time (even if not needed)?*

Enzyme synthesis uses a lot of energy.

B. The Phenomena -- Are enzymes (like those above) actually made only when they are needed? Graphs on handout 15A show what happens to level of appropriate enzyme if you add, or take away, the appropriate small molecule, namely lactose (lac) or tryptophan (trp).

1. *Example of Induction* -- Lactose (small molecule) = inducer = signal to turn **on** synthesis of appropriate enzyme ; synthesis of beta-galactosidase (enzyme) is called inducible; phenomenon is known as induction. See also Sadava 13.16 (13.13)

2. *Example of Repression* -- tryptophan (small molecule) = co-repressor = signal to turn **off** synthesis of appropriate enzyme ; synthesis of trp synthetase (enzyme) is called repressible; phenomenon is known as repression.

3. *Constitutive synthesis* -- Synthesis of some proteins, such as enzymes of glycolysis, is called constitutive = synthesis of enzymes is "on" at all times.

C. Summary of Terminology -- see table in middle of Handout 15A

Regulation is covered in problem set 12. To review the material in parts A-C, see Problem 12-1, parts A & B.

D. Comparison of Repression to feedback. Why do you need both types of regulation? Factors to consider:

- Speed (inhibition is faster)
- Which enzymes are affected (first in pathway in f.b. inhibition vs all enzymes of pathway in repression)
- What is changed -- enzyme activity (inhibition) vs synthesis of enzymes or **gene** activity (repression)

Overall, have coarse control (repression/induction) vs fine control (inhibition/activation). See chart and picture on bottom half of handout 15A. See also Sadava 13.17 (13.14). Note: Enzyme activation and induction can be compared in a similar way -- Activation increases enzyme activity while induction turns on enzyme synthesis

Today's lecture will focus on induction; we will go over the mechanism of repression in detail next time. Wait to do the problems on repression and/or repression vs. feedback until next time.

II. Mechanism of Prokaryote Regulation (See handout 15B) -- Operons

Note: This mechanism was largely figured out by analyzing mutants. How it was done is fascinating, but complex, so we will explain the mechanism first, and then you can try your hand at predicting the effects of mutations. See E below for more details.

A. How is co-ordinate control achieved? Upper Left Panel on handout -- idea of cluster or operon. (See Sadava 13.18 (13.16) or Becker fig. 23-3.)

- 1. Genes regulated together are linked** -- genes to be controlled co-ordinately (turned on and off together) are next to each other on the DNA.
- 2. Polycistronic mRNA.** The linked genes are transcribed as a unit to give one single mRNA. One mRNA is made per operon (not one mRNA per gene), because all the genes in a cluster share a single promoter. An mRNA able to code for several peptides (mRNA that comes from several genes) is called polycistronic

mRNA. (cistron = another term for gene).

3. *Transcriptional Control* -- Regulation is at level of transcription. The level of translation is controlled by regulating the synthesis of mRNA. This is the usual method for regulation of protein synthesis in prokaryotes.

Since mRNA has a short half life in prokaryotes, regulating mRNA synthesis controls the steady state level of mRNA. Translation per se (and degradation of mRNA) are not regulated here. (In some prok. cases and many euk. cases, these are regulated too.)

4. *Definition of an operon* = group of linked structural (enzyme coding) genes that share common regulatory sites and that are transcribed as a single unit.

(Note: The linked regulatory sites are considered part of the operon; the gene for the repressor protein is sometimes considered part of the operon and sometimes not. Whether the repressor gene is considered part of the operon or not is usually clear from context; the role of repressor is discussed further below.)

5. *Punctuation*. Reminder: DNA replication, transcription, and translation, have different stop and start signals. DNA replication starts at origins, transcription starts at promoters, and translation begins at start codons (AUG). Origins vs. Promoters was covered before. What about promoters vs. start codons?

a. mRNA has UTR's. It has leaders (untranslated region on 5' end before first AUG or 5' UTR) & trailers (untranslated 3' end or 3' UTR).

b. Numbers: Number of transcription starts (Promoters) for a message is one; number of translation starts may be many (one per peptide) in prokaryotes.

c. Translation of a polycistronic mRNA starts at multiple start codons. A ribosome assembles at the first AUG and starts translation. After each peptide is completed, the ribosome may continue down the mRNA to the next start codon and start a new peptide chain. Alternatively, the ribosome may detach (and disassociate into subunits) when it comes to a stop codon. In that case a new ribosome forms at the next start codon and starts translation of the next peptide.

B. How transcription of cluster is turned off -- Upper Right Panel of 15B -- Role of Repressor & Operator -- operon that is "off" (See Becker fig. 23-4, top panel or Sadava fig. 13.19 (13.17) top

panel.)

1. Role of operator (O) = DNA site to act as part of on/off switch -- binds repressor (regulator) protein when repressor is in appropriate or active form (rectangle on handout).

2. Role of repressor = other half of on/off switch (with O). Repressor is a protein that binds to operator and prevents RNA polymerase from binding to DNA and transcribing the operon. (Purves fig. 13.15 in 7th ed).

a. There is a different repressor protein for each operon. Repressor binds to specific sequence of DNA found in its respective operator.

b. Synthesis of repressor protein is constitutive -- gene is always on. (State of repressor protein varies, not the amount; see below.)

c. Terminology. The terms 'repressor' and 'repressor protein' are used interchangeably. The term 'repressor' is used in both induction & repression because the job of the protein is to turn the operon off. However some prefer to use the term 'regulator protein' instead of 'repressor protein' when referring to induction.

Question: Does the gene for repressor protein have a promoter? an operator?

C. How induction (and repression occur) -- Role of Effectors

1. Repressor protein is allosteric (has two forms) -- one that sticks to the operator and blocks transcription (rectangle on handout) and one that doesn't (round on handout). See Becker Fig. 23-5.

2. Repressor binds effector (inducer or co-repressor). Each repressor/regulator protein is unique in that it binds the proper co-repressor or inducer (see below) as well as the proper operator.

3. Effector determines which form the repressor is in. The amount of repressor protein present doesn't change (see above); the form repressor is in **does** change. The small molecule effector (inducer or co-repressor) shifts the

balance between the two forms thus shifting the equilibrium between free and bound repressor and turning the operon "on" or "off."

4. How does repressor get on or off the DNA? The picture on the handout implies that the repressor is either "on" or "off" the operator. There is actually an equilibrium between free and bound "sticky" repressor -- "rectangular" molecules are spontaneously coming on and off. The effector shifts that equilibrium, by binding to free repressor, and changing the affinity of the repressor for the operator. Or you can think of the effector as changing the relative concentrations of free rectangles and circles. (The direction of the change depends on whether it's an inducible or repressible operon -- see below.)

D. An example of Induction-- (see middle panel of handout 15B or Becker fig. 23-4 or Sadava 13.19 (13-17). For an animation try <http://vcell.ndsu.nodak.edu/animations/lacOperon/index.htm>. (There are multiple animations on the web; if anyone finds one they especially like, please tell Dr. M. This site has multiple animations of biological processes.) For an animation with a different slant, try http://trc.ucdavis.edu/biosci10v/bis10v/media/ch10/lac_negative.html This goes into some extra fine points, but is clear and interesting. See next time for an animation of repression.

What are the characteristics of an inducible Operon?

- Effector molecule (inducer) that binds to repressor protein **prevents** repressor from binding to operator -- decreases supply of free rectangles by converting them to circles.
- Effector (Inducer) shifts following equilibrium to **right**:

"Rectangle form" of rep. protein ("sticky" form that binds to O) ↔ "Circle form" (form that doesn't bind to O)

- **Empty** form of repressor protein (without effector) sticks to operator.

E. Constitutive Mutants & Plasmids

1. What happens if repressor protein is mutant and doesn't bind to DNA at all? Will operon be on? off? Inducible or constitutive?

2. What happens if operator is deleted? Is it the same as above?

See problem 12-3.

3. How do you test out the properties of constitutive mutants? Many experiments and problems involve having a cell with two copies of an operon. How is this possible? A bacterium has only one DNA molecule (chromosome) with one copy of each gene or operon.

Answer: Bacteria can carry mini-chromosomes called plasmids that have 'extra' genes. The 'extra' genes can be totally new or they can be additional copies of the genes already in the cell. Therefore a bacterium with a plasmid can have two copies of a gene or two copies of a whole operon -- the bacterium can have one copy on its normal chromosome and another copy on a plasmid. Such a cell is called a partial diploid (see below.) The two copies do not have to be exactly the same -- one can be normal and one mutant, or they can both be different mutants. For example, suppose a bacterium has two copies of the lactose operon. Suppose one copy is constitutive and the other is inducible, or suppose both are constitutive. What should happen when you put the two operons together? Will both be constitutive? Both inducible?

4. Use of Mutants. Study of the properties of constitutive mutants was how induction/repression was figured out by Jacob and Monod, who received the [Nobel prize in 1965](#) for their work. Now you can try it the other way -- you can use your knowledge of operon function to predict the properties of mutants, both singly and in combination. See Chap. 12 of the problem book.

5. Terminology

a. Haploid = A cell (or organism) with one copy of each chromosome. Therefore one copy of each gene. Example: bacteria.

b. Diploid = A cell (or organism) with two copies of each chromosome (usually one copy from each parent). Therefore 2 copies of each gene. Examples: mammals, higher plants.

c. Partial Diploid = A cell (or organism) that is basically haploid, but has two copies of a few genes. The 'second copy' of the few genes is usually on a plasmid. Therefore the partial diploid has one copy of most genes, but two copies of the genes on the plasmid -- one copy on the plasmid and one on the chromosome.

(See below and/or next time for how you get a bacterium with a plasmid.)

To learn how to tell the types of constitutive mutants apart, see problems 12-4 & 12-8 & Becker table 23-2.

F. Strong & Weak Promoters -- all promoters are **not** the same.

1. All Promoters are similar in structure and function -- all P's have to be able to bind RNA polymerase and serve as signals to start transcription.

2. P's can be strong or weak

a. Weak Promoter → little (or infrequent) RNA polymerase binding → low levels of transcription → low levels of corresponding protein.

b. Strong Promoter → lots of (or frequent) RNA polymerase binding → high levels of transcription → high levels of corresponding protein.

c. Why does strength of promoter matter? The strength of the promoter determines how much mRNA can be made. Actual amount of mRNA made at any time depends on both strength of promoter and extent of repression or induction.

3. Example of strong vs. weak Promoters: P of lac operon vs P of lac repressor gene

a. Promoter of lac operon is strong. P of lac operon = P for the structural genes; controls production of polycistronic mRNA → enzymes for metabolism of lactose. Since this P is strong, you make a lot of mRNA and a lot of the corresponding enzymes.

b. Promoter of lac repressor gene is weak. P of lac repressor = P for the R gene; controls production of mRNA for lac repressor → lac repressor protein. Since this P is weak, you make only a little of the mRNA, and relatively little of the repressor protein.

c. Why does this make sense? You need a lot of the

metabolic enzymes (if you are growing on lactose as a carbon and energy source) but relatively few molecules (100 or so) of repressor protein.

4. Note difference between Roles of O (operator) and P (promoter).

P determines what the maximum level of transcription is; O (plus Repressor) determines what percent of maximum is actually reached (per culture, not per cell).

a. O (by binding to repressor) determines to what extent operon is "on" -- is operon running at full throttle or is it only partially turned on (or completely off)? We usually described operons as "off" or "on." Operons can be partially turned on if there is an intermediate level of co-repressor or inducer, so that part of the repressor protein is in the "rectangle" form and part is in the "circle" form.

Note: one molecule of repressor (in the "rectangle form") per cell is not enough to shut down one operon. There has to be more than one molecule of repressor protein per operon to be sure the operator is always occupied with a repressor protein molecule.

b. P determines the maximum level of transcription
= level when operon is fully "on" and running at full throttle.

III. How is bacterial DNA passed on?

A. Introduction to cell division -- How does 1 cell make 2?

1. How do you double cell contents? Consider the central dogma -- we've covered it all -- how to double DNA, RNA and protein, and how to regulate protein synthesis. Once you double the protein (enzymes), that allows doubling of everything else, like carbs, lipids, etc. So suppose you double everything in the cell. How do you get 2 cells from 1?

2. Why distribution of DNA is the critical issue -- Making two cells from one comes down to "once the program is doubled, how are the two copies distributed to daughter cells?" Stuff that is not part of the program (not part of the genetic material) need not be divided exactly, but because of the chicken and egg problem, there must be **some** of the other material in each daughter cell. (Need some ribosomes, RNA polymerase etc. in each cell. But as long as you have some, and the genetic material, you can always make more ribosomes, enzymes

etc.)

B. How do prokaryotes do it? binary fission -- regular segregation of circular chromosome attached to membrane

1. What does the DNA (genetic information) of a bacterium look like? Each bacterium has one, circular, double stranded DNA molecule = chromosome; the chromosome is attached to the cell membrane.

2. How the Chromosomal DNA is distributed.

a. To start, you have one cell with one double stranded DNA circle attached to membrane.

b. DNA replicates by birectional DNA replication (two forks start from a single origin) → two double stranded circles, both attached to membrane. (See Becker fig. 19-4 (19-5))

c. Circles grow apart as membrane is laid down between the attachment points of DNA to membrane → two circles pushed to opposite ends of cell. (There is also an active process, other than growth of membrane, that pushes the two origins of DNA replication apart. This has only been recently discovered.)

d. To end, you need only to lay down a membrane (and wall) between the two halves of cell, each containing one circle (= complete double stranded chromosome). This → 2 complete cells.

e. Note this is not mitosis OR meiosis; it is a different process (binary fission). Mitosis and meiosis occur only in eukaryotes; they will be discussed later.

f. How will the genetic material in the two daughter cells compare? If there are no mutations it will be the same, and all descendants will be identical. All the descendants produced in this way (by asexual reproduction of a single founder) are called a clone. (Doesn't matter if "founder" is a cell, molecule, or organism.) Is there any way (besides mutation) to get new combinations of genes? To mix genes from separate clones? That requires bacterial sex.

C. Plasmids vs Fragments.

1. Bacteria can have extra pieces of DNA in addition to the chromosome.

This can happen as a result of transformation, for example.

2. What do the transferred pieces of DNA look like? The recipient cell gets only a few extra genes, not a complete 2nd set of genes or chromosomes. Two possibilities for the 'extra genes':

a. Plasmids = small circular mini-chromosomes with their own origin of replication.

b. Fragments = short linear DNAs with (virtually always) no origin of replication.

2. What difference does it make?

a. Plasmids are inherited -- Progeny get copies of the chromosome **and** the added piece (the plasmid). Therefore progeny are partial diploids or have added extra genes. Plasmids are generally replicated and passed on to all progeny, like the regular chromosome. (Some descendants may lack plasmids due to inefficient replication, distribution, etc.; this will be discussed in detail next time.)

b. Fragments are not inherited -- The added piece (the fragment) is not replicated, and is usually degraded, so only the chromosome is passed on. Progeny are haploids. Added genes on fragments are only passed on if they have been integrated into the chromosome (details next time).

Next time: Review of operons -- repression vs. induction. Then how do bacteria & viruses have sex? How is bacterial and viral DNA passed around, and how are the results of bacterial and viral crosses (i.e. sex) analyzed by complementation and recombination? (There will be a handout on all the details.)

© Copyright 2008 Deborah Mowshowitz and Lawrence Chasin Department of Biological Sciences Columbia University New York, NY.