Chapter 12 Lecture Notes: Metabolism – Enzyme and Gene Regulation

I. Introduction to regulation
   A. Why regulate?
      1. Bacteria are extremely efficient organisms. It is wasteful to have all 1000 – 2000 metabolic pathways on at the same time.
      2. During exponential growth all cellular components are synthesized at constant rates relative to one another (balanced growth).
      3. Thus, the cell integrates signals regarding metabolic state and environmental conditions and uses the information to effectively regulate metabolism.

   B. Patterns of regulation
      1. Feedback inhibition
         a) One of the initial steps in a pathway, which is the rate limiting step, is regulated by the pathways' end product(s)
         b) Types (see attached)
            (1) simple
            (2) cumulative – one enzyme has two separate binding sites for each branch end product and so the inhibitory effects are cumulative
            (3) sequential – each end product inhibits the first reaction of its branch and the branch point intermediate inhibits the first step in the common pathway (Fig. 12-8 too)
            (4) isoenzymes- two enzymes catalyze the same reaction but each is subject to feedback inhibition from a different branch of the pathway
         c) Many biosynthetic pathways are regulated by feedback inhibition; some catabolic pathways are also subject to feedback inhibition
      2. Positive regulation
         a) Enzymes in a pathway are not active/made unless a positive regulator (many times a precursor molecule) is present
         b) Types
            (1) Positive regulation of a "downstream" enzyme by a precursor molecule (i.e. positive regulation of pyruvate kinase by fru-1,6-bisP)
            (2) Positive regulation by intermediate in a related pathway (activation of PEP kinase by acetyl-CoA)
         c) Many catabolic pathways are positively regulated

C. Levels of metabolic regulation
   1. Control of enzyme location – metabolic channeling (see II)
   2. Control of enzyme activity (see III)
   3. Control of number of enzyme molecules (see IV-VI)
D. Control of the major metabolic fueling reactions in chemoorganoheterotrophs (see attached)

Not simple regulation because formation of 12 precursor molecules are interrelated; instead the controls work internally in each of the main fueling pathways
1. regulation by intermediates several steps further in the pathway
2. regulation by AMP/ADP/ATP
3. regulation by NAD/NADH

II. Metabolic channeling - localization of metabolites & enzymes in different parts of the cell
A. Compartmentalization - the differential distribution of enzymes and metabolites among different cell structures
B. Channeling within compartments – molecules do not easily diffuse and so cytoplasm is not a homogeneous bag of enzymes and substrates; thus, regulation can occur via levels of substrates, products, and enzymes

III. Control of enzyme activity
A. Allostery (Fig. 12-2)
1. Change in the activity of an enzyme due to the noncovalent binding of a small molecule (effector) to the effector molecule binding site.
2. Binding of effector causes a change in the active site of the enzyme which either activates or inhibits the enzymatic reaction.
   a) change in substrate binding
   b) change in reaction rate
3. Example = aspartate carbamoyltransferase ACTase (Fig, 12-3, 12-4)
   a) Role in the cell – catalyzes the condensation of caramoyl phosphate and aspartate to form carbamoylaspartate which is used for pyrimidine biosynthesis
   b) Regulator molecules
      (1) positive = ATP
      (2) negative = CTP (pyrimidine based)
B. Covalent modification
1. Change in the activity of an enzyme due to the covalent attachment of a small molecule to the enzyme.
2. Modification causes a change in the active site of the enzyme that either activates or inhibits the enzymatic reaction.
   a) change in substrate binding
   b) change in reaction rate
3. Types
   a) adenylylation
   b) phosphorylation
   c) methylation
   d) uridylylation
   e) acetylation
4. Example- glycogen phosphorylase (Fig. 12-6)
C. Glutamine synthetase regulated by allostery and covalent modification

1. Review of N incorporation
   a) Initial reactions for ammonia incorporation:
      (1) via glutamate dehydrogenase (GDH) if NH₃ >1mM
      \[ \text{a-ketoglutamate} + \text{NH}_3 + \text{NADH} + \text{H}^+ \rightarrow \text{glutamate} + \text{NAD} + \text{H}_2\text{O} \]
      (2) glutamine synthetase (GS) and glutamate synthetase if NH₃ <1mM; costs ATP

   b) Secondary reactions
      transamination of amino groups from above amino acids to other C skeletons
   c) glutamate provides N to 85% of the N containing molecules in the cell;
      glutamine provides N to 15% of the N containing molecules in the cell

2. GS enzyme = 12 identical subunits (Fig. 12-7)

3. Regulation by covalent modifications
   a) Level 1: adenylylation of each of the 12 subunits of GS by adenyl transferase (AT)
      (1) adenylylated (+AMP) = less active
      (2) deadenylylated = more active
   b) Level 2: regulation of AT
      (1) AT bound to PII = catalyzes adenylylation of GS
      (2) AT bound to PII-UMP = catalyzes deadenylylation of GS
c) Level 3: regulation of PII uridylylation by bifunctional enzyme (UT/UR) which responds to cellular cues about the N level
   (1) UT/UR = uridylyl transferase (puts on UMP) and uridylyl removase (removes UMP)
   (2) cellular cues for low N = low glutamine and high a-ketoglutarate

\[
\begin{align*}
\text{AKG} & \quad \text{glutamine} \\
+ & \quad - \\
\text{UT*/UR} & \quad \text{PII} \\
\text{UT/UR}\hat{\star} & \quad \text{PII-UMP} \\
+ & \quad - \\
\text{glutamine} & \quad \text{AKG}
\end{align*}
\]

4. All together

4. Allosteric regulation
   Feedback regulation via binding of AMP, CMP, trp, or his, which all require glutamine for synthesis: only when GS is less active because during these times GS is only required for synthesis of 15% of cell components that contain N (such as these) whereas when GS is active (low N) it is responsible for the incorporation of N for the entire cell
IV. Control of number of enzyme molecules via transcriptional regulation: Introduction
A. Regulation of transcriptional initiation by sigma factors (see notes for chapter 11)

B. Regulation of transcriptional initiation by transcriptional regulator proteins (see V)
   1. negative control = mRNA synthesis proceeds more rapidly in the absence of a controlling factor (repressor protein); in other words repressor negatively affects transcription
      a) relief by induction (Fig. 12-10)
         (1) small molecule (inducer) binds repressor and inactivates it → TRANSCRIPTION OCCURS
         (2) typically for catabolic pathways where inducer is molecule or derivative of the molecule to be broken down
      b) relief by derepression (Fig. 12-11)
         (1) small molecule (corepressor) has to bind repressor for it to be active (able to repress); if the small molecule is not present then the repressor is inactive (and unable to repress) → TRANSCRIPTION OCCURS
         (2) typically for anabolic pathways where corepressor is end product
   2. positive control = mRNA synthesis proceeds more rapidly in the presence of a controlling factor (activator protein)

C. Regulation of transcriptional elongation by attenuation (see VI)

D. Regulation by antisense RNA
   1. Regulatory RNA molecule that has a nucleotide sequence complementary to another mRNA molecule and specifically binds to the target RNA to block nucleic acid activities (replication, transcription, or translation)
   2. Examples – see text
V. The lactose operon (an inducible operon under both negative and positive control; regulation at the level of transcriptional initiation).
Francois Jacob and Jacques Monod 1950s

A. Introduction
1. *E. coli* can use lactose (glc + gal) as a carbon source (although glucose is preferred).
2. The enzyme β-galactosidase (β-gal for short) cleaves lactose into glc and gal and is encoded by the *lacZ* gene. (Fig. 12-9)
3. The enzyme lactose permease transports lactose into the cell and is encoded by the *lacY* gene.
4. The genes are transcribed from a polycistronic mRNA along with the *lacA* gene.
5. There is a promoter region that controls *lac* expression.
6. There is an operator region to which a repressor binds.
7. The entire unit (POZYA) is called an operon (a set of adjacent genes transcribed as one polycistronic mRNA plus the adjacent regulatory signals)
B. Negative regulation

1. Original observation was that β-gal was not synthesized unless lactose (or a non-metabolizable analogue of lactose called IPTG) was in the growth media. This also applied to permease synthesis. In otherwords, β-gal synthesis was INDUCED in response to the presence of lactose or IPTG. What was the mechanism behind this regulation?

2. Model for negative regulation based on data below (and other data too)
   a) In the absence of lactose, LacI repressor binds to the lacO site and represses lacZYA transcription.

   b) In the presence of lactose, allolactose (an isomer of lactose) binds to LacI repressor causing a conformational change in the protein that prevents binding to lacO. Transcription of lacZYA genes can now occur.
3. Experiments that resulted in the model – analysis of regulatory mutants
   a) Identification of the repressor (LacI): One set of mutations that made β-gal regardless of whether lactose was present mapped close to lacZY but not in them. Gene was designated lacI (for controlling inducibility). Complementation studies using partial diploids (chromosomal copy of the genes plus a copy on the F plasmid) were done:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>β-gal synthesis</th>
<th>Conclusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Non-induced</td>
<td>Induced (+IPTG)</td>
</tr>
<tr>
<td>I+ Z+ Y+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>I- Z+ Y+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>I+ Z- Y+ / F I- Z+ Y+</td>
<td>-</td>
<td>+</td>
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</table>

These data suggested that LacI was a trans acting repressor of lacZ transcription. This was later confirmed by examining the lacZ mRNA levels and by in vitro transcription reactions with purified components.

b) Identification of the operator region: The second set of mutations mapped directly before lacZ. The region was designated lacO for operator region (not a gene). These mutants had the same phenotype as the lacI mutants: expression of β-gal was constitutive. Complementation studies:

<table>
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<th>Permease synthesis</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-induced</td>
<td>Induced (+IPTG)</td>
<td>Non-induced</td>
</tr>
<tr>
<td>O+ Z+ Y+</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>Oc Z+ Y+</td>
<td>+</td>
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<tr>
<td>O+ Z+ Y+ / F Oc Z- Y+</td>
<td>-</td>
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<tr>
<td>O+ Z+ Y+ / F Oc Z+ Y-</td>
<td>+</td>
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These data suggested that lacO was a cis acting site at which repression occurred. This was later confirmed with in vitro binding assays.
c) Identification of the allolactose binding site on LacI: A different mutation in *lacI* was also identified that prevented the induction of β-gal expression by IPTG. The mutation was designated *lacIS*.

Complementation studies:

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<th>Conclusion</th>
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</thead>
<tbody>
<tr>
<td>I⁺ Z⁺ Y⁺</td>
<td>-</td>
<td>+</td>
<td>β-gal expression is inducible</td>
</tr>
<tr>
<td>Iˢ Z⁺ Y⁺</td>
<td>-</td>
<td>-</td>
<td>Mutation eliminates ability of IPTG to induce</td>
</tr>
<tr>
<td>Iˢ Z⁺ Y⁺ / F I⁺</td>
<td>-</td>
<td>-</td>
<td><em>lacIS</em> mutation is dominant to <em>lacI</em></td>
</tr>
</tbody>
</table>

These data suggested that the mutation in *lacIS* destroyed the binding site for an inducer in LacI. Thus, even in the presence of IPTG or lactose, repression of *lacZ* transcription still occurs. Subsequent in vitro binding studies showed that IPTG bound LacI.

C. Positive regulation

1. Initial observation was that when glucose and lactose were both present in the media, there was no induction of β-gal expression until all the glucose was metabolized. This phenomenon was dubbed catabolite repression because it was presumed that a catabolic breakdown product of glucose repressed lactose utilization. (Fig. 12-16)

2. Catabolite repression is modulated by cAMP (Fig. 12-13) levels in the following way:
   - ↑ glucose = ↓ cAMP = ↓ β-gal expression = catabolite repression
   - ↓ glucose = ↑ cAMP = ↑ β-gal expression

3. cAMP-CRP complex binds to the lac promoter and aids RNA polymerase in activation of transcription. (Fig. 12-14)
   a) When ↑ glucose: ↓ cAMP = ↓ cAMP-CAP = no binding to the promoter = no activation of β-gal expression. This makes sense because glucose (not lactose) is the preferred carbon source.
   b) When ↓ glucose: ↑ cAMP = ↑ cAMP-CAP = binding to the promoter = activation of β-gal expression.
4. How do glucose levels control cAMP levels?
   a) cAMP levels are controlled by the enzyme adenylate cyclase. In order to be active, adenylate cyclase must be phosphorylated by Enzyme III of the PTS system for glucose transport. Enzyme III also phosphorylates glucose.

   \[
   \uparrow \text{glucose} = \uparrow \text{in Enzyme III donating phosphate to glucose} = \downarrow \text{in Enzyme III donating phosphate to adenylate cyclase} = \downarrow \text{cAMP levels}
   \]

   \[
   \downarrow \text{glucose} = \downarrow \text{in Enzyme III donating phosphate to glucose} = \uparrow \text{in Enzyme III donating phosphate to adenylate cyclase} = \uparrow \text{cAMP levels}
   \]

   b) non-phosphorylated Enzyme III (because \(\uparrow \text{glucose}\)) binds to lactose permease and prevents lactose uptake

5. Experiments resulting in the model
   a) genetic analysis of mutants that showed decreased b-gal
      (1) Adenylate cyclase (\(\text{cy}a\)) gene – makes cAMP
      (2) \(crp\) gene (encoding CAP or CRP)
   b) in vitro binding and transcription studies
VI. The tryptophan operon (a repressible operon; regulation at the level of transcriptional initiation, feedback inhibition, and premature transcriptional termination)

A. The trp operon encodes genes that are required for the synthesis of tryptophan (Trp) when it is not available in the growth medium.

B. Physiological observation was that excess Trp in the media represses trp expression. This makes sense because there is no need to make Trp if it is in the media.

C. Levels of regulation
   1. Via Feedback inhibition: When too much Trp is made, Trp binds to the first enzyme in the enzymatic pathway, thereby inactivating it. This results in a temporary shutoff of trp biosynthesis until the levels of Trp have decreased sufficiently so that Trp no longer binds to the first enzyme. This is a way to temporarily “tweak” the system to be as efficient as possible.
   2. Via repression/derepression of transcriptional initiation: Trp repressor protein (TrpR) binds to an operator in the trp operon and represses transcription. The evidence that TrpR was a repressor was similar to that for the Lac repressor, namely that deletion mutations trpR and in the operator region preceding the trp genes resulted in constitutive expression of the operon.
   3. Transcriptional attenuation (see below)

D. Transcriptional attenuation (note most of these experiments were done in a trpR deletion mutant to eliminate any repression caused by TrpR)

Charles Yanofsky et al (1970s)
   1. Observed that in a trpR mutant, there was still a 10X increase in trpE mRNA when Trp was removed from the media. Why?
   2. Found a cis acting mutation that had a 10X increase in trpE mRNA even when Trp is present in the media. Mutation was a deletion in the leader sequence (see picture above). The deleted element was designated an attenuator because its function appeared to be to reduce the amount of trpE mRNA when Trp was present.
   3. Further experiments showed that while trpE mRNA was reduced 10X, initiation of transcription and transcription of the leader was not reduced. Thus, it appeared that the 10X reduction in transcription was due to termination of transcription somewhere in the leader.
4. Now the question became, what prevents termination of transcription in the absence of Trp (or what causes termination in the presence of Trp)? Yanofsky et al proposed a model based on sequence analysis and site directed mutagenesis in the attenuator region. The model was based on the secondary structure of the leader RNA. There are two MUTALLY EXCLUSIVE possibilities for the structure of the leader – only one can exist at a time (Fig. 12-17):
   a) Hairpins can form between regions 1 and 2 AND between regions 3 and 4 (termination hairpin) OR
   b) Hairpin can form between regions 2 and 3 (antitermination hairpin)

5. Model for transcriptional attenuation (see attached):
   a) Transcription of the leader region begins. As soon as regions 1 and 2 are transcribed they form a hairpin that temporarily pauses RNAP. The ribosome hops on the mRNA and begins translating the leader. The moving ribosome disrupts the hairpin and “unpauses” the paused RNAP so that transcription resumes. Now there is a precise coupling of transcription and translation. The next events depend on whether excess Trp is present:
   b) Fig. 12-18b: If there is excess Trp in the cell (high Trp levels), the ribosome translates the leader peptide until it hits a stop codon located at the end of region 2 of the RNA. At this stop codon, the ribosome pauses thereby covering up region 2 RNA. Since region 2 RNA is covered up, it can not form the antitermination hairpin with region 3 RNA. This leaves region 3 RNA free to pair with region 4 RNA which has just been transcribed. The RNA hairpin formed by region 3 and 4 is a rho-independent termination hairpin that destabilizes the DNA:RNA hybrid causing the mRNA to dissociate from the transcription complex. TERMINATION OF TRP TRANSCRIPTION OCCURS.
   c) Fig. 12-18c: If the cell is starved for Trp (low Trp levels), the ribosome pauses at the 2 Trp codons located in region 1 of the RNA because there is not enough Trp available. Meanwhile, transcription of the leader is continuing and now region 3 has been transcribed. Region 3 RNA can form the antitermination hairpin with region 2 RNA. While this antitermination (2&3) RNA hairpin is formed, the termination hairpin (3&4) can NOT form. Thus, transcription continues through region 4 and into the trp genes. NO TERMINATION OF TRP TRANSCRIPTION OCCURS.

6. Several biosynthetic operons have regulatory mechanisms similar to this one.