

Syllabus for BASIC METABOLIC PRINCIPLES

The video lecture covers basic principles you will need to know for the lectures covering enzymes and metabolism in Principles of Metabolism and elsewhere in the curriculum. Much of this material is covered in introductory biochemistry courses as well as in MCAT reviews for the biological and physical sciences. Therefore for many of you this video is a refresher.

Learning Objectives for the video lecture are given below. After reading the learning objectives you may proceed to the video directly or take the pre-video quiz that is at the beginning of the video. If you make a score of 80% or better on the pre-quiz then you probably do not need to watch the video module. The syllabus that follows is NOT a verbatim script of the video, but it does hit the major points. It is provided mostly for those of you who prefer to read the material instead of watching the video.

Learning Objectives

Be able to describe the key properties of a metabolic pathway.

- Be able to describe the various modes of regulation of metabolic pathways as they are presented in this lecture.
- Be able to explain the key properties of coenzymes, and know the types of group carried by each coenzyme discussed in this lecture.
- Be able to explain what isozymes are and why they are important in medicine.
- Be able to describe the important characteristics of anabolic pathways and catabolic pathways.
- Be able to explain why ATP is called the energy currency of the cell, why phosphoanhydride bonds are "high energy" bonds, and what the term substrate-level phosphorylation means.
- Know the molecules discussed in this lecture that can transfer phosphate groups to ADP or receive phosphate groups from ATP.
- Understand what is meant by the term "coupled reaction" and the role of ATP hydrolysis and redox reactions in reaction coupling.
- Be able to explain in broad terms how reduced electron carriers are used to generate ATP via the electron transport system in mitochondria.
- Understand the role of thioesters and acetyl-CoA in metabolism.
- Be able to describe the reactions catalyzed by kinases, phosphatases, dehydrogenases, synthases/synthetases, transferase, and isomerases.

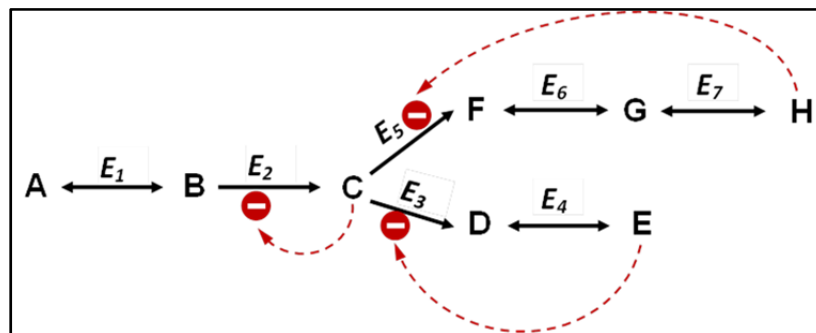
I. Metabolic Pathways And Enzymes

Intermediary metabolism is the name given to the sequential chemical reactions that degrade, synthesize, or interconvert small organic molecules inside living cells. A metabolic pathway is a series of biochemical reactions that are interconnected by their intermediates (A – E below); these reactions are catalyzed by enzymes such that the product of one enzymatic reaction becomes the substrate for the next enzyme in the pathway.



Note that some of the arrows in the pathway point in both directions, indicating that these reactions are reversible; for the reactions catalyzed by E1, E3, and E4, the direction is determined by the concentration of substrate and product for that particular reaction. In contrast, the arrow for the reaction catalyzed by E2 points only in the forward direction, indicating that the reaction is irreversible, i.e. it catalyzes only the conversion of B to C and not the reverse reaction. In biochemistry the first irreversible step in a metabolic pathway is called the committed step. The **committed step in a metabolic pathway is usually the slowest step in the pathway and it is also the most common step where regulation occurs. Thus the committed step controls the overall flow of materials through a particular metabolic pathway.**

In many cases the intermediates in a metabolic pathway have no function outside of their roles as intermediates. However in some cases an intermediate in one pathway can enter multiple pathways, as diagrammed below. This is a **branched pathway** because compound C can be used in two different metabolic pathways, with the relative amount of C flowing into each branch depending upon the relative amount and activity of E_5 and E_3 . Notice that the reactions occurring at the branch points are also irreversible; in other words, once F is formed from C, all F is committed to the top branch. Similarly when D is formed, it is committed to either remain as D or to become E .. it does not become C again (at least nor via the same pathway). In other words, the reactions catalyzed by E_5 and E_3 are also committed steps for their respective branches of the pathway. **This is another general principle: in branched pathways, committed steps often occur at or near the branch point.**



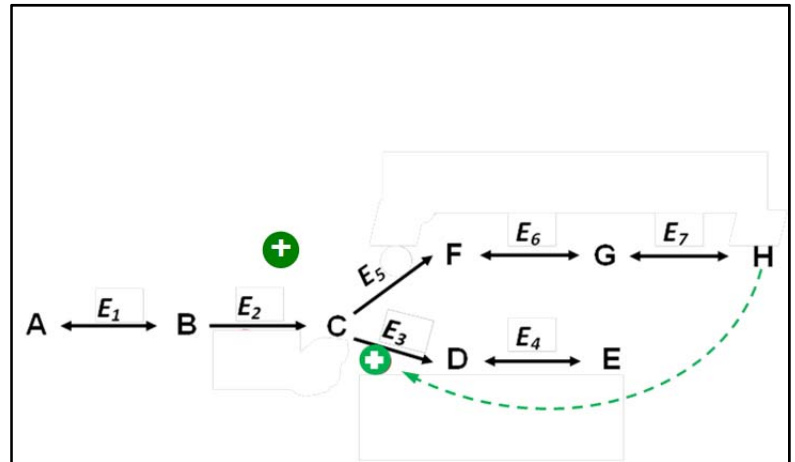
In the example shown above, the relative activities of the enzymes in the stem and branches of the pathway are regulated by **feedback inhibition**. For example, the activity of E_5 is negatively regulated by the binding of H to a regulatory site on E_5 . Similarly, the activity of E_3 is negatively regulated by binding of E to a regulatory site on that enzyme. **Most often the regulatory site on an enzyme is separate and distinct from the catalytic site on the enzyme, and binding of the inhibitor causes a change in the shape of the enzyme that leads to inhibition of substrate binding at the active site. Enzymes that undergo changes in conformation or shape as a result of the binding of small molecules are termed allosteric enzymes.**

Feedback inhibition allows precise fine-tuning of a metabolic pathway via regulation at branch points. If H begins to build up in excess of the cell's need, feedback inhibition of E_5 will decrease synthesis of F, G and H, allowing the system to equilibrate by sending more C into the branch of the pathway regulated by E_3 . Similarly

inhibition of E_3 by E will allow more C to enter the E_5 branch. If inhibition of one branch leads to the accumulation of C in excess of need, production of C can be inhibited by feedback inhibition of E_2 .

In addition to negative regulation, positive regulation is also possible. In a branched pathway in which the amounts of the end-products of both branches (H and E) need to be balanced, stimulation of enzyme E_3 by compound H can serve this purpose by diverting more of the common intermediate C into the C - D - E branch. Thus as the concentration of H increases, so does the synthesis of E .

The mechanisms described above lead to very rapid changes in enzyme and pathway activity. **Changes in gene expression are also used to regulate enzyme activity, but the effect is often slower and more long-term than allosteric regulation.** High concentrations of the substrates of certain enzymes can stimulate transcription of the genes encoding these enzymes; similarly high levels of the end-product of a pathway can repress transcription



of a key enzyme in the pathway. Regulated change in the rate of translation of mRNA and in the stability of an enzyme are other ways in which enzyme activity and metabolic pathways can be regulation.

Regulation can also occur from outside of the pathway (as in the case of E_2 here). For example some important metabolic pathways are regulated through the action of hormones on enzymes in the pathway. We will encounter this mode of regulation often during our discussion of metabolism and human disease.

Coenzymes

Coenzymes are complex nonprotein organic molecules that participate in enzymatic catalysis by providing essential functional groups. They:

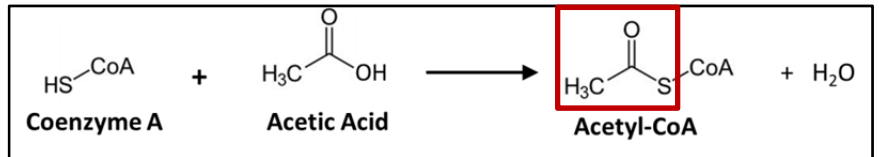
- have specificity in that each coenzyme is involved in catalyzing a specific type of reaction for a class of substrates with certain common structural and chemical features.
- carry biologically important chemical groups in an “activated” (high energy) form
- usually bind transiently to enzymes during catalysis (there are a few coenzymes that are bound to proteins at all times; these are usually called prosthetic groups)
- are used over and over again in a catalytic manner
- are small relative to the enzymes to which they bind
- are usually derived from vitamins. Symptoms of vitamin deficiencies reflect the loss of specific enzyme activities that require the coenzyme.

In general, coenzymes can be divided into two general classes: activation – transfer coenzymes (activate and carry groups as they are transferred from one substrate to another) and oxidation-reduction coenzymes (carry electrons between substrates). ATP and Coenzyme A are activation – transfer enzymes and NAD, NADP and FAD and their reduced forms are oxidation-reduction coenzymes.

Examples of Coenzymes Mentioned in this Lecture		
Carrier Molecule	Group Carried in Activated Form	Vitamin Source of Coenzyme
ATP	Phosphoryl	
NADH and NADPH	Electrons	Niacin
FADH ₂	Electrons	Riboflavin
Coenzyme A	Acyl	Pantothenate

Thioesters and Coenzyme A

Thioesters (red box in figure), especially those involving Coenzyme A, are very important coenzymes that participate in transfer of acyl and acetyl groups from glycolysis to the Citric Acid Cycle and to the fatty acid synthesis pathway. The sulfhydryl group on CoA reacts with carboxylate groups on



molecules, “priming” them for further metabolism utilizing the energy released when the thioester bond is later hydrolyzed. With a ΔG° of -7.5 kcal/mol, hydrolysis of the thioester bond in acetylCoA releases more free energy than does ATP hydrolysis to ADP.

Isozymes Allow Similar Reactions to Be Differentially Regulated in Cells

Isozymes are enzymes that catalyze the same reaction but differ in amino acid sequence and in structure. Isozymes display different kinetic parameters and often different regulatory properties. For example, isozymes have different affinities for the same substrate, use different coenzymes, and have different maximum velocities. In some cases, two different isozymes are expressed in the same cell but are localized to different organelles within the tissue. Of particular importance, because they are encoded by different genes, isozymes are often expressed at differing levels in different tissue types and at different stages of development. The particular isozyme expressed in a specific tissue is the one that is the best fit in regulatory properties and activity to the needs of that tissue.

II. Cellular Energetics

Phosphate Group Transfer and ATP

The primary dietary fuels used by the human body are carbohydrates, fats, and to a lesser extent, proteins. Breakdown of these fuels (**catabolism**) involves the release of energy as chemical bonds are broken. Some of this energy is captured in either of two forms: the two phosphoanhydride bonds of **ATP** or **reducing equivalents in the form of NADH, NADPH, or FADH₂**. These molecules can be used for biosynthetic processes

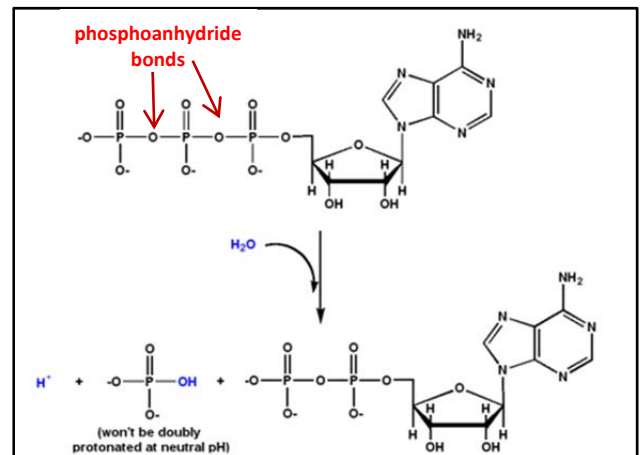
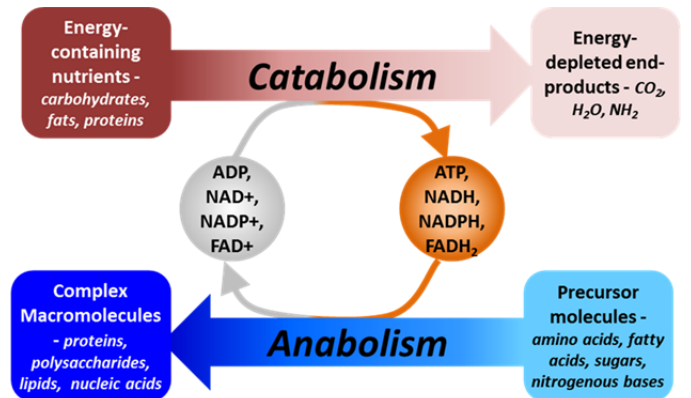
(**anabolism**) within cells. In anabolic reactions, precursor molecules are converted to larger molecules and energy is expended (in the form of **ATP hydrolysis or oxidation of NADPH to NADP⁺ or of FADH₂ to FAD⁺**) in the process.

The two phosphoanhydride bonds of ATP are often termed “high energy” bonds. We can understand this by realizing that the negative charges on phosphate groups cause these groups to repel each other in solution; bringing them together to form phosphoanhydride bonds requires significant energy to overcome this repulsion. This bonding energy is released when a phosphoanhydride bond in ATP is hydrolyzed.

Phosphate groups on other molecules may also release energy when the bond linking them to the molecule is broken. If we compare the amount of energy released upon hydrolysis of several of the more important phosphorylated compounds in the cell, we see that hydrolysis of ATP to ADP lies about in the middle. *This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups.* In cells, hydrolysis of compounds with a more negative ΔG° than ATP release sufficient free energy to drive formation of ATP from ADP (i.e., ADP accepts a phosphate group).

The formation of ATP by phosphate group transfer without the involvement of the electron transport chain (see next section) and O₂ is termed **substrate-level phosphorylation**. Similarly, hydrolysis of ATP provides sufficient energy to transfer phosphate groups to glucose or glycerol (i.e., ATP donates a phosphate group). This is the chemical basis for calling ATP “the energy currency of the cell”;

energy is “acquired” by bond formation when a phosphate group is transferred from a high energy compound to form ATP and is “spent” in the addition of phosphate groups by ATP to form lower energy compounds. *In*

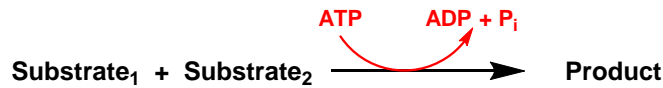


Free Energy of Hydrolysis of Some Phosphorylated Compounds

Compound	ΔG° (kcal/mol)
Phosphoenol pyruvate	-14.0
Creatine phosphate	-10.3
ATP (to ADP)	-7.3
Glucose 1-phosphate	-5.0
Glucose 6-phosphate	-3.3
Glycerol 3-phosphate	-2.2

most cases the addition of a phosphate by ATP to relatively simple compounds such as glucose or glycerol activates that compound for the next step in that compound's metabolism.

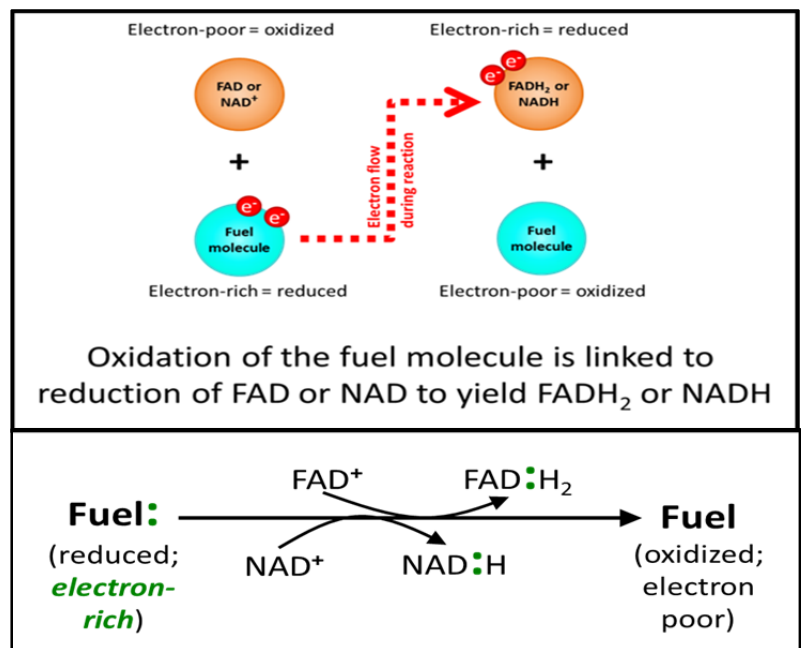
By convention reactions in intermediary metabolism that utilize ATP to supply energy for the reaction are usually written as:



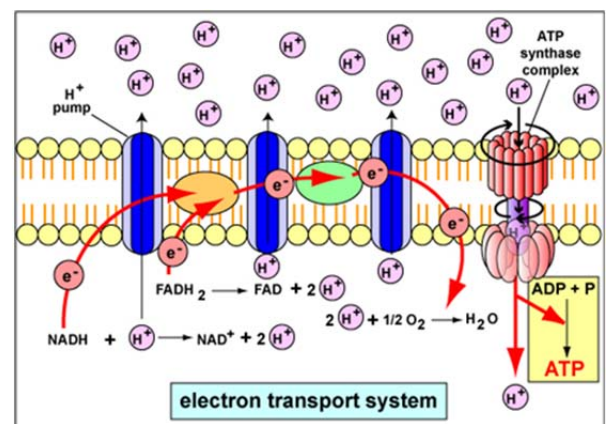
This emphasizes that ATP hydrolysis is occurring but oversimplifies what is actually happening. In reality, ATP is bound by an enzyme that catalyzes the transfer of the terminal (γ) phosphate, (or in some cases transfer of AMP) to a substrate molecule or an amino acid in the enzyme, *thereby raising the free-energy content of the substrate or enzyme*. It is the increase in free energy content that drives the chemical reaction catalyzed by the enzyme toward product formation. During the enzymatic reaction, phosphate or adenylate is displaced, generating inorganic phosphate (P_i) or AMP. There is one important class of exceptions to this: in some cases ATP is bound noncovalently to protein and ATP hydrolysis provides the energy to cycle the protein between two different conformations.

Biological Oxidations and Reductions

ATP hydrolysis provides the energy to perform most of the work within cells. A key question is: "how is energy obtained to synthesize ATP in the first place?" Ultimately the energy comes from fuel oxidation occurring in the mitochondrial matrix. At specific steps in catabolism, fuels undergo oxidation-reduction reactions in which some of their electrons (in the form of a hydrogen molecule or hydride ion) are transferred to carrier molecules, usually the coenzymes NAD^+ and FAD , reducing them to NADH and FADH or FADH_2 . These reduced carriers link fuel catabolism to ATP production.



Each carrier donates electrons to specialized electron carrier proteins located within the mitochondrial membrane; collectively these proteins comprise the electron transport chain or ETC. As electrons pass sequentially through the carrier proteins of the ETC, some of their potential energy is used to pump protons into the space between the outer and inner membrane of the mitochondrion, setting up an electrochemical gradient across the inner membrane of the mitochondrion. These protons reenter the mitochondrial matrix through a proton channel in the multisubunit ATP synthase enzyme, causing conformational changes in the



Note that as electrons are transferred from one protein to another, protons are pumped out. The proton gradient drives a conformational change in ATP synthase that leads to ATP production.

enzyme that allow it to bind ADP and phosphate (P_i), phosphorylate ADP to ATP, and release ATP. Oxygen plays an essential role in keeping the electron transport chain functioning. O_2 is a very avid electron acceptor and reduction of oxygen to water provides the large change in free energy that drives the flow of electrons through the ETC.

Substrate-level Phosphorylation versus Oxidative Phosphorylation

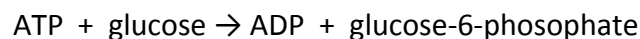
We have now learned about two ways in which ATP can be produced: substrate-level phosphorylation in which the formation of ATP is driven by transfer of a phosphate group from a higher energy compound, and oxidative phosphorylation in which the enzyme ATP synthase uses the energy generated by a chemiosmotic gradient to synthesize ATP. An important distinction between the two ATP synthesis pathways is that **oxidative phosphorylation has an absolute requirement for O_2 , because it is ultimately the reduction of O_2 to water that generates the chemiosmotic gradient within mitochondria. Substrate-level phosphorylation has no such requirement and therefore can occur under anaerobic conditions. Thus glycolysis, the first stage of glucose catabolism, can proceed in the absence of O_2 .**

GLOSSARY OF ENZYME NAMES

Metabolic pathways are much easier to understand if you know the relationship between enzyme names and the reactions they catalyze. Enzymes are named for the reaction the type of reaction they catalyze (and also the substrate, but we will save that for later). The classes of enzymes you will most often encounter, along with some representative reactions, are listed below. Being able to correlate the class name with the type of reaction catalyzed will be very useful as you study metabolism.

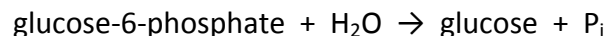
Kinase – catalyzes the addition of a phosphate to some metabolite, generally using ATP or some other nucleoside triphosphate as a phosphate donor.

Example: glucokinase or hexokinase (isozymes)



Phosphatase – catalyzes the hydrolysis of a phosphate group from some metabolite.

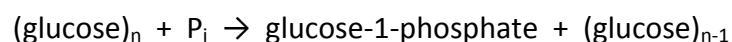
Example: glucose-6-phosphatase



Hydrolase – catalyzes the hydrolytic cleavage of some group other than phosphate. Hydrolases are often named after the bond cleaved, i.e., esterase (hydrolyzes ester bond), peptidase (hydrolyzes peptide bond), phospholipase (hydrolyzes phospholipids).

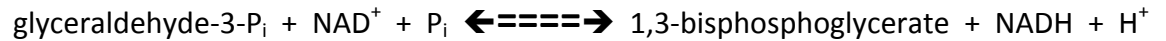
Phosphorylase – catalyzes the phosphorolytic cleavage of a bond (phosphate rather than water is attacking a nucleophile in this reaction).

Example: glycogen Phosphorylase



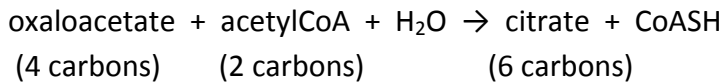
Dehydrogenase – catalyzes oxidation-reduction reactions by the transfer of hydrogens. Dehydrogenases usually use NAD⁺/NADH or FAD/FADH₂ as coenzymes.

Example: glyceraldehyde-3-phosphate dehydrogenase



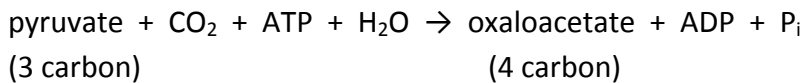
Synthetase or Synthase – catalyzes the joining of two molecules to synthesize a larger molecule.

Example: citrate synthase



Carboxylase – catalyzes the addition of CO₂ to a molecule, usually using biotin as a coenzyme.

Example: pyruvate carboxylase



Transferase – catalyzes the transfer of a functional group from one molecule to another. Names are generally descriptive. Depending upon the functional group, different coenzymes may be involved.

Example: aminotransferase (pyridoxal phosphate is coenzyme), acyltransferase (coenzyme A is coenzyme), methyltransferases.

Isomerase – converts substrate from one isomer to another.

Example: **Glucose-6-phosphate isomerase** catalyzes the conversion of glucose-6-phosphate into fructose 6-phosphate.

Decarboxylase – removes CO₂ from a molecule.

Example: pyruvate decarboxylase (requires thiamine pyrophosphate coenzyme)

