Metabolism

Metabolism is the chemical change of compounds by organisms
- Evolutionarily created to improve fitness
- Catalyzed by enzymes

Various classes
- Energy (anabolism of ATP & other energetic compounds)
- Matter (anabolism of amino acids, nucleotides, lipids, etc.)
- Detoxification (catabolism of dangerous compounds)

Substrates and products are “metabolites.” All but input and output are “intermediates”

Metabolic pathways

A series of chemical reactions involved in the metabolism of a compound (or compounds).
Each step is a single enzymatic reaction

Fluxes of metabolites can be
- Linear (series of transformations mapping single substrate to a single product)
- Branching (single substrate, multiple products)
- Cyclic (multiple substrates, multiple products)

Pathways are regulated in various ways to control the fluxes of metabolites.
Pathways form densely interconnected graph

- Intermediates in one pathway are often inputs to another.
  - Intermediates in energy metabolism are inputs to synthesis of most amino acids
  - Nucleotides (esp. adenosine) are inputs in energy metabolism (e.g. to make ATP, NAD, etc.)

Universality of pathways

- Much intermediary metabolism is widely shared throughout life. Broad functional categories are nearly universal:
  - Protein translation, modification, transport and degradation
  - DNA replication, transcription & repair
  - Energy production & conversion
  - Cell division, chromosome & membrane synthesis
  - Cell motility, secretion and signal transduction
  - Metabolism & transport of
    - Carbohydrates
    - Amino Acids
    - Inorganic ions
    - Lipids
    - Nucleotides
    - Coenzymes

Diversity of pathways

- Most organisms exhibit some diversity from the “universal” intermediary metabolism
- Sometimes the differences are major:
  - E.g. some Archaea run the oxidative Krebs cycle (more shortly) backwards to create ATP in highly reducing environments
- Usually the differences are in the presence or absence of particular enzymes, making alternative pathways through the network.
- Different niches require different metabolisms.
Our first pathway: Glycolysis

- Breakdown of a hexose (6C sugar) into lactate (or, in organisms like yeast, into ethanol and CO₂), producing a net gain of two ATPs.
- Eleven steps in 3 groups:
  - Lyase, splitting 6C sugar into two 3C sugars
  - Oxidation of 3C sugar to produce ATP
  - Reduction of 3C sugar (allowing recycling of NADH)
- Lots of phosphorylation, in order to
  - Create ATP, increase reactivity of intermediates for recognition, create irreversibility, impede glucose transport

Initial phosphorylations

- First step is an irreversible phosphorylation catalyzed by hexokinase, consuming one ATP
- Then isomerization to Fructose (reversible) by phosphoglucone isomerase
- Then irreversibly add another phosphate (consuming one ATP) by phosphofructokinase

Splitting the sugar

- The fructose is split into two three carbon sugars (triose) by aldolase
- The dihydroxyacetone product is converted to a second glyceraldehyde by triose phosphate isomerase
- These trioses may have been the original inputs
Oxidizing the triose, producing ATP

- Glyceraldehyde 3-phosphate dehydrogenase and an NAD cofactor oxidize and phosphorylate the trioses.
- Phosphoglycerate kinase creates the ATP. Happens for both trioses, so 2 moles

Hydrolysis produces more ATP

- Phosphoglyceromutase moves the phosphate
- Enolase catalyzes a dehydration
- And pyruvate kinase irreversibly phosphorylates another (pair of) ADPs to ATPs.

Finally, we get the electron back

- Lactate dehydrogenase breaks a C=O double bond, reducing NAD to NADH, conserving the reductive potential for reuse.
- In aerobic metabolism, this step is not taken, but instead the pyruvate goes on for further processing.
**Glycolysis summary**

- Costs 2 ATPs to produce 4 (2 from each triose) for a net gain of 2 moles of ATP for each mole of glucose.
- Can be run backward, called gluconeogenesis, using different enzymes for irreversible steps.
  - Direction is regulated by phosphofructokinase versus fructose-1,6-bisphosphatase (which reverses it). Don't want both, since that would produce energy consuming futile cycles!
- Other sugars (and starch) can be fed into this pathway, and intermediates used elsewhere

**Glycolysis feeds....**

- Pyruvate to
  - TCA for more ATP
  - Synthesis of
    - Acetyl CoA
    - Tryptophan
    - Lysine
    - Alanine, Tyrosine
- α-D-glucose-1P to
  - Nucleotide synthesis
  - Starch metabolism
- Etc!

**Fermentation**

- Fermentation is production of usable energy with organic molecules as electron acceptors.
- In *lactic acid fermentation*, the process stops at lactate, (e.g. cheese making bacteria)
- Sometimes, one additional step to converts lactate to ethanol and CO₂ reducing NAD. Called *alcohol fermentation* (e.g. in yeast).
- Fermentation is also loosely used in biotech to mean any conversion by microorganisms.
Cellular Respiration

- The advent of an oxidizing atmosphere (i.e., the oxygen proliferation of 2.2 Ga) made possible aerobic metabolism, or respiration.
- Three steps, which ultimately gain energy by moving electrons from a substrate to oxygen:
  - Glycolysis (+ the conversion of pyruvate to Acetyl CoA)
  - The Krebs (or citrate, or citric acid, or TCA) cycle
  - An electron transport chain, leading to oxidative phosphorylation to create ATP
- In eukaryotes, this takes place in special organelles called mitochondria.

Pyruvate ⇒ AcetylCoA

- Catalyzed by a multienzyme complex.
- First release of CO₂, creating a two carbon fragment.
- Oxidizes the fragment to acetate, while reducing NAD⁺ to NADH. Two moles of NADH per mole of glucose, since two pyruvates.
- Attaches coenzyme A to the acetyl group, forming acetyl CoA. This bond is unstable, making the acetyl group very reactive.

The Krebs Cycle

- Produces high energy electrons for oxidative phosphorylation, captured by NADH and FADH₃, and a GTP (like ATP, but on guanine; rapidly converted to ATP).
Inputs and outputs of the Krebs Cycle

For every glucose split in glycolysis
- Two acetyl fragments are produced
- It takes two cycles through the Krebs cycle to complete the oxidation of the fragments

For every turn through the Krebs cycle
- Two carbons enter through the Acetyl CoA, turning 4 carbon oxaloacetate to 6 carbon citrate.
- Two (different!) carbons are oxidized and leave as CO₂
- 3 NADs and one FAD (two electrons) are reduced
- One GTP is produced and then transformed into ATP
- More oxaloacetate is produced for the next round

Krebs cycle produces many intermediates for anabolism

Electron Transport Chain (ETC)

Most of the energy gained in the Krebs cycle is in the form of electrons (reduced NAD/FAD)
The electron transport chain slowly delivers these electrons to the final acceptor, oxygen.
- If all done in one step, too much energy would be liberated
Each step in the transport chain has a higher electronegativity, ending with oxygen.
Most chain constituents are cytochromes, proteins with iron cofactors (which transfers the electrons). Also, ubiquinone.
ETC creates a proton gradient

- Requires a membrane that is impermeable to $\text{H}^+ \text{ (and OH-)}$
- In Eukaryotes, this is the mitochondrial membrane
- In Prokaryotes, it is a lipid vesicle (liposome).
- The electron transport chain pumps protons out of the membrane, keeping electrons in.
- A pH and charge gradient results.

Oxidative Phosphorylation

- Production of ATPs from redox potentials is called oxidative phosphorylation.
- Proton motive force (pH and charge gradient). then powers ATP synthase (reverse direction is ATPase).
- ATP synthase allows proton back through membrane, capturing energy to make ATP. (Rotating parts!)
- Creates ~3 ATPs per NADH, and ~2 per FADH

Regulation of respiration

- Phosphofructokinase is the rate limiting step in glycolysis.
- Citrate (Krebs cycle) and ATP itself are allosteric inhibitors of phosphofructokinase.
  - Allosteric means acting in a place other than the active site
  - Increases in citrate or ATP decrease catalytic activity
- ADP and AMP are allosteric activators
  - Increases in ADP/AMP increase catalytic activity
- Negative feedback on ATP/ADP ratio
- Other regulatory mechanisms, too...
Respiration efficiency

- Produces a net gain of as many as 38 moles of ATP per mole of glucose
  - Glycolysis: 2 ATP + 2 NADH (no recycling in respiration)
  - Oxidation of pyruvate: 2 NADH per glucose
  - Krebs: 2 ATP + 6 NADH + 2 FADH per glucose
- Oxidative phosphorylation yields:
  - 10 NADH → ~30 ATP
  - 2 FADH → 4 ATP
- Uncertainties because proton motive force leaks a bit (and is used for other tasks).

Respiration summary

- Cellular respiration consumes O₂ and produces CO₂ just like breathing...
- We've covered the main catabolic pathways.
  - Others which break down fats, starch, protein, etc. all eventually feed into glycolysis or Krebs.
  - Only additional kind is neutralization of toxins (which also uses cytochromes, although different ones)
- Should have a sense of how enzymes work together in pathways to accomplish functions