ANABOLISM OUTLINE:
Overview of Photosynthesis
Key experiments:
- Light causes oxygen, which is from water splitting (Hill)
- NADPH made (Ochoa)
- Separate from carbohydrate biosynthesis (Rubin & Kamen)

Light Reactions
- energy in a photon
- pigments
- HOW

Light absorbing complexes
- Reaction center
- Photosystems (PS)
- PSI – oxygen from water splitting
- PSII – NADPH
- Proton Motive Force – ATP

Overview of light reactions
Carbon Assimilation – Calvin Cycle
- Stage One – Rubisco Carboxylase/Oxygenase
- Glycolate cycle
- Stage Two – making sugar
- Stage Three – remaking Ru 1,5P2

Overview and regulation
Calvin cycle connections to biosyn.
C4 versus C3 plants
Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
Precursors

Gluconeogenesis
- reversible steps
- irreversible steps – four enzymes
two steps to PEP

Glycogen Synthesis
- UDP-4-lic
- Glycogen synthase
- Branching

Pentose-Phosphate Pathway
- Oxidative phase
- Non-oxidative/recycling phase
- ROS and NADH/NADPH shuttles

Regulation of Carbohydrate Metabolism
- Acetyl-CoA/Pyruvate
- Pyruvate/PEP
- F6P/FBP: Fru 2,6P2
- Glc/Glc6P
- Glycogen
- Anaplerotic reactions

Regulation of Carbohydrate Metabolism
Catabolism vs. Anabolism
Regulation of Carbohydrate Metabolism

- Glycogenolysis versus Glycogen Synthesis
- Glycolysis versus Gluconeogenesis

Regulated enzymes often correspond to points in the pathways that have the same substrate and product, but a different enzyme. Also where there are junctions.

Can you name those enzymes?

Regulation of Pyruvate Kinase

- All tissues
- Allosterically activated by fructose-1,6-bisphosphate
  - increase flow through glycolysis
  - Feed-forward activation
- Allosterically inhibited by signs of abundant energy supply.
  - ATP
  - acetyl-CoA and long-chain fatty acids
  - alanine (enough amino acids)

Liver only (under hormonal control)

Inactivated by phosphorylation in response to signs of glucose depletion (low blood-glucose→glucagon) (liver only)

Glucose from liver is exported to the brain and other vital organs.

This is not the only time we’ll see hormonal control of these pathways.
Regulation of Carbohydrate Metabolism

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Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Carboxylase

- Allosteric **activation** of pyruvate carboxylase by Acetyl-CoA
  - Stimulates glucose synthesis via gluconeogenesis because plenty of acetyl-CoA signals plenty of CAC intermediates

- Notice the reciprocal control of PDH Complex by acetyl-CoA
Regulation of Carbohydrate Metabolism

Glycogenolysis versus Glycogen Synthesis

Glycolysis versus Gluconeogenesis

- Regulated enzymes often correspond to points in the pathways that have the same substrate and product, but a different enzyme.

- Can you name those enzymes?

Regulation of Phosphofructokinase-1

- Fructose-6-phosphate $\rightarrow$ fructose 1,6-bisphosphate is the commitment step in glycolysis.

- While ATP is a substrate, ATP is also a negative effector.
  - Do not spend glucose in glycolysis if there is plenty of ATP.
  - Same for citrate, if there is plenty of citrate, do not waste glucose.

- Low energy charge inhibits biosynthesis of Glc.

Is this a typo?

Homeostatic level of Fru 6-P

Bumble bees are missing an FBPase that responds to AMP.
Regulation of Carbohydrate Metabolism

Regulation of Phosphofructokinase-1 versus Fructose 1,6-bisphosphatase-1

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Fructose 2,6-(bis)phosphate (βD-Fru-2,6P₂)

- NOT a glycolytic intermediate, only a regulator.
- Produced specifically to regulate glycolysis and gluconeogenesis.
  - Activates phosphofructokinase-1 (PFK-1) (glycolysis)
  - Inhibits fructose 1,6-bisphosphatase (FBPase-1) (gluconeogenesis)

Enzyme for synthesis and degradation of Fru 2,6P₂ done with a dual-function enzyme: PFK-2/FBPase-2
Regulation of Carbohydrate Metabolism

Regulation of Glycolysis and Gluconeogenesis by Fru-2,6P₂

- Without Fru₂,6P₂, STOP glycolysis, GO gluconeogenesis.
- With Fru₂,6P₂ (130 nM), Go glycolysis.
- With Fru₂,6P₂ (1300 nM), Stop gluconeogenesis.

What controls PFK-2/FBPase-2?

Regulation of Carbohydrate Metabolism

Regulation of Fru-2,6-P₂ Levels

Structurally, this enzyme, with its two activities is different than those in glycolysis and gluconeogenesis (i.e., they are conjoined, rather than independent) and are regulated via phosphorylation.

Drop relieves the activation of PFK-1, effectively inhibiting glycolysis. Drop increases activity of FBPase-1, stimulating gluconeogenesis.
Regulation of Carbohydrate Metabolism

Regulation of Fru-2,6-P₂ Levels

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Regulation of Carbohydrate Metabolism

Glycogenolysis vs. Glycogen Synthesis

Glycolysis vs. Gluconeogenesis

• Regulated enzymes often correspond to points in the pathways that have the same substrate and product, but a different enzyme.

• Can you name those enzymes?
There Are Four Isozymes of Hexokinase (I-IV)

- Isozymes are different enzymes that catalyze the same reaction.
  - Typically share similar sequences
  - May have different kinetic properties
  - Can be regulated differently
- HK I is expressed in all tissues, to different levels.
- HK IV (glucokinase) is only expressed in the liver and pancreas.
  - Has higher \( K_m \), so responsive to higher [glucose]
  - Not inhibited by glucose-6-phosphate, so can function at higher [glucose]
  - Functions to clear blood glucose at higher [glucose] for storage as glycogen
- Glc activates release/Fru inhibits

Regulation of Carbohydrate Metabolism

Glycolysis versus Gluconeogenesis

- Regulated enzymes often correspond to points in the pathways that have the same substrate and product, but a different enzyme.
- Can you name those enzymes?
Glycogen phosphorylase cleaves glucose residues off glycogen, generating glucose-1-phosphate (Glc 1P).

- Phosphorylation activates glycogen phosphorylase-b
  - Phosphorylase-b Kinase
  - Accentuated by allosteric binding of AMP (muscle only)

- Dephosphorylation inhibits glycogen phosphorylase-a
  - Phosphoprotein phosphatase-1 (PP1)
  - Accentuated by allosteric binding of Glc (in liver only)
Regulation of Carbohydrate Metabolism

Regulation of Glycogen Synthase

• Glycogen synthase adds glucose residues to glycogen using UDP-Glc.
  • Phosphorylation inhibits glycogen synthase-a
    – Its complicated, responding to multiple signals
    – Example: First Casein Kinase-2 (CKII), then Glycogen Synthase Kinase-3 (GSK3)

• Dephosphorylation activates glycogen synthase-b
  – Phosphoprotein phosphatase-1 (PP1) (in liver it’s a different PP)
  – PP1 is bound to GS-b
• Also, feedforward control by glucose and Glc-6P
  – Binding causes a conformation favorable for PP-1 binding
  – Binding does not allow GSK-3 access to phosphorylation sites

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Regulation of Carbohydrate Metabolism

Glycogen Phosphorylase Cascade

• Glucagon/epinephrine signaling pathway **activated** when there is a NEED for energy
  – starts phosphorylation cascade via cAMP
  – cAMP activates PKA
  – PKA activates phosphorylase-b kinase
  – this kinase activates glycogen phosphorylase
  – Massive degradation of glycogen
    • In muscle, Glc1P → Glc6P → glycolysis
    • In liver, Glc1P → Glc6P → Glc

• There is reciprocal **inhibition** of GS
  – PKA phosphorylates Gu, which is bound to PP1 on GS, thus dissociating it.
  – PKA also phosphorylates PP1-inhibitor protein, which binds and inactivates the free PP1, thus leaving GS-© and inactive

Anaplerotic Reactions

• We introduced the citric acid cycle as a key **catabolic** pathway.
• It has an equal, if not more important, role in **anabolism**.
  – The biosynthesis of biological precursors has to begin with elementary materials
  – Many, if not most, of these starting points come from the several intermediates in the Kreb’s cycle.
  – This was first appreciated by Hans Kornberg: how to organisms grow on carbohydrates only?
    o The term comes from the Greek, to “fill up” or replenish
    o Recall that without this replenishment, the TCA cycle would grind to a halt
    o Anaplerotic reactions are critical

Enzyme Kornberg discovered
Anaplerotic Reactions

Kornberg Cycle = Glyoxylate Cycle

- Was intrigued by the fact that bacteria could grow very effectively on a little ammonium and phosphate salts with acetate (2C)
- From these they can synthesize all the components of the cell; DNA, DNA, proteins, membrane lipids, cytochromes, everything....
- How do you build all this from a 2-carbon compound knowing how the Kreb’s cycle works?

2 Acetyl-CoA + NAD+ ⇌ Succinate + 2 CoASH + NADH

Anaplerotic Reactions

- Purine Nucleotide Cycle
  - was first thought be be part of nucleotide degradation or synthesis
  - In muscle, its now realized as an important anaplerotic pathway

H₂O + Aspartate + GTP ⇌ NH₄⁺ + GDP + P₁ + Fumarate
Anaplerotic Reactions

- Intermediates in the citric acid cycle can be used in biosynthetic pathways.
- Must replenish the intermediates in order for the cycle and central metabolic pathway to continue.
- In animals, these 4-carbon intermediates are formed by carboxylation of 3-carbon precursors.

Pyruvate carboxylase deficiency
- an inherited metabolic disorder where anaplerosis is greatly reduced.
- What is the problem?
- How to treat this disorder?
- Other anaplerotic substrates such as the odd-carbon-containing triglyceride triheptanoin are used.

<table>
<thead>
<tr>
<th>TABLE 16.2 Anaplerotic Reactions</th>
<th>Tissue(s)/organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate + HCO₃⁻ + ATP → oxaloacetate + ADP + Pi</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td>Phosphoenolpyruvate + CO₂ + GDP → oxaloacetate + GTP</td>
<td>Heart, skeletal muscle</td>
</tr>
<tr>
<td>Phosphoenolpyruvate + HCO₃⁻ → oxaloacetate + Pi</td>
<td>Higher plants, yeast, bacteria</td>
</tr>
<tr>
<td>Pyruvate + HCO₃⁻ + NAD(P)H → malate + NAD(P)⁺</td>
<td>Widely distributed in eukaryotes and bacteria</td>
</tr>
<tr>
<td>Aspartate + GTP → NH₄⁺ + GDP + Pi + Fumarate</td>
<td>Muscle</td>
</tr>
</tbody>
</table>

ANABOLISM I: Summary

What we learned:
- Gluconeogenesis, a process by which cells can use a variety of metabolites for the synthesis of glucose
- The differences between glycolysis and gluconeogenesis
  - how they are both made thermodynamically favorable
  - how they are differentially regulated to avoid a futile cycle
- The pentose phosphate pathway, a process by which cells can generate pentose phosphates and NADPH. The pentose phosphates can be regenerated into glucose 6-phosphate, for which NO ATP is required.
- Living organisms regulate the flux of metabolites through metabolic pathways by:
  - increasing or decreasing enzyme concentrations
  - activating or inactivating key enzymes in the pathway
- The activity of key enzymes in glycolysis and gluconeogenesis is tightly and coordinately regulated via various activating and inhibiting metabolites (Fru 2,6P₂)
- Glycogen synthesis and degradation is regulated by hormones insulin, epinephrine, and glucagon that report on the levels of glucose in the body
- The citric acid cycle plays important anabolic roles in the cell: Anaplerosis
- Organisms have multiple ways to replenish intermediates that are used in other pathways: Lipid and Nitrogen biosynthesis........