Regulation of glycolysis (Glycolysis III)

Fates of Pyruvate (NAD regeneration)

1) Ethanol
- Alcoholic fermentation in yeast and certain bacteria
- Pyruvate -> CO_2 +acetaldehyde + NADH -> ethanol + NAD
- Recycles NADH/NAD+
- No ATP from NADH in ox phos
- Net glycolysis is 2 ATP

2) Lactate
- Pyruvate + NADH -> Lactate + NAD+
- Anaerobic conditions, cells w/o mitochondria
- Microorganisms that use lactate cause milk souring due to changes in the pH

3) CO_2 and H_20
- Pyruvate is converted to Acetyl-CoA in mitochondria and enters into citric acid cycle
-ATP produced in TCA and ox phos leads to net production of either 30 or 32 ATP depending on pathway taken.

Influx of glucose into cells
- 5 different transporters
- 12 transmembrane regions

· Glut 1 and Glut 3
- Present in all cells
- Low Km, high affinity but have a lower general rate of transport
- Basal level of entry into cells

· Glut 2 Liver and pancreatic cells (insulin release)
- Low affinity, high Km
- Sensitive to blood glucose levels
- Acts to spare glucose for brain and muscles (non gluconeogenic)

· Glut 4 Muscles and Fat
- Transport regulated by insulin
- Number of receptors on the cell membrane is altered by endocytosis into intracellular stores
- Main transporter of glucose for these cells

3 key regulatory enzymes -ΔG steps

<u>1) Hexokinase and Glucokinase (HK/GK)</u>

Glucose + ATP -> Glucose -6 phosphate + ADP

Plays a large part in blood glucose stabilization
- Hexokinase is inhibited by formation of product
- Glucokinase is not (more sensitive to glucose concentration). Liver contains GK activity - liver is not subjected to inhibition Æ liver gets some glucose even if rate of glycolysis is high
- Km of glucose of HK << GK -> tissues like brain and muscle get first "shot" at glucose

2) Phosphofructokinase (PFK)

Fructose 6-P Æ Fructose 1,6 bisphosphate

First committed step - why? carbon can enter and leave the pathway without being acted on by HK. Glycogen is converted to glucose- 6 phosphate and therefore bypasses HK. PFK is the primary control point for glycolysis.

Multi-regulated enzyme ATP, FBP , AMP, citrate and pH all act on PFK

PFK is an allosterically regulated protein.

- It is a tetramer and is found in the R and T conformations

PFK inhibition

- ATP inhibition is reversed by AMP (adenylate kinase)
  - Changes in [AMP] rather than [ATP] are the most sensitive to energy state of cell
- Low pH from lactic acid inhibits PFK
- Citrate inhibits PFK
  - Fatty acid metabolism spares glucose via ATP and citrate
- ATP binds at two sites substrate and a regulatory site
- Regulatory site binds in the T conformation. This alters the equilibrium between R-T and inhibits F6P binding (only in the R conformation). Bottom line is the one of the substrates can not bind when ATP concentrations are high enough to bind into the second site where the protein shifts to the less active T conformation

PFK activation

- Allosterically activated by fructose 2,6 bisphosphate. (F2,6 BP)
- F2,6BPactivates and reverses inhibition by ATP
  - F 2,6 BP is main regulator of PFK
  - F 2,6 BP levels (production and metabolism) are controlled by the same enzyme phosphofructokinase 2 (PFK2). Called the bifunctional enzyme
  - The PFK2 enzyme is phosphorylated by PKA and this leads to activation of PFK2 phosphatase activity and ultimately decreases in the rate of glycolysis
- AMP and ADP both bind to the R conformation and prevent a transition into the T state
- In the R state and Arg from one subunit forms an salt bridge with the phosphoryl on F6P
- The Arg only able to bind when in the R conformation. In the T conformation the Arg is removed and replaced by a negative charged amino acid
- Remember that the R-T conformation is in equilibrium and addition of agents that stabilize one form or another tremendously help shift to that form.

3) Pyruvate Kinase (PK)

Phosphoenolpyruvate + ATP -> Pyruvate + ADP

Two different isozymes: L (liver) and M (muscle) Both are tetramers.

Isozymes are proteins which catalyze the same reaction but have different kinetics, regulation, tissue types found or locations in the cell

PK activation

- PK is allosterically activated by thesubstrate PEP
- Feed forward activation by F 16 BP
- ATP and alanine inhibits
- Liver form is phosphorylated at C terminal by PKA
- The resulting phosphorylation shifts the enzyme from tetrameter (active) to monomer (inactive) form
  - This type of regulation is only observed in liver - why only in the liver and why is that important? Think in terms of energy needs during fight or flight. Muscle want ATP and liver can use other forms of energy sparing glucose for other tissues.
  - Protease treatment of C-term quickly leads to a loss in the regulation - PKA regulation lost but not PK activity

Substrate Cycling

- Additional control of glycolytic flux is provided by a substrate cycle. PFK catalyzes the opposite reaction of fructose-1,6- phosphatase (FBPase).
- The overall reaction of these two enzymes is ATP hydrolysis.
- Both enzymes in a futile cycle are usually at some level of activity. The overall flux is under hormonal and neuronal control as well as allosteric regulation by metabolites.
- Futile cycles do provide heat - the replacement of ATP lost due to the cycle results in heat. This is how bumblebees survive the cold.
- Nonshivering thermogenisis. Boy do we need this in northern MN!