Glycolysis

- The Glycolytic pathway describes the oxidation of glucose to pyruvate with the generation of ATP and NADH
- It is also called as the Embden-Meyerhof Pathway
- Glycolysis is a universal pathway; present in all organisms: from yeast to mammals.
- In eukaryotes, glycolysis takes place in the cytosol
- Glycolysis is anaerobic; it does not require oxygen
- In the presence of O_2, pyruvate is further oxidized to CO_2. In the absence of O_2, pyruvate can be fermented to lactate or ethanol.
- Net Reaction: Glucose + $2NAD^+$ + 2 Pi + 2 ADP =

  2 pyruvate + 2 ATP + 2 NADH + 2 H_2O

The 3 stages of Glycolysis

- **Stage 1** is the investment stage. 2 mols of ATP are consumed for each mol of glucose
- Glucose is converted to fructose-1,6-bisphosphate.
- Glucose is trapped inside the cell and at the same time converted to an unstable form that can be readily cleaved into 3-carbon units.
- In **stage 2** fructose-1,6-bisphosphate is cleaved into 2 3-carbon units of glycerladehyde-3-phosphate.
- **Stage 3** is the harvesting stage. 4 mols of ATP and 2 mols of NADH are gained from each initial mol of glucose. This ATP is a result of substrate-level phosphorylation
- Glyceraldehyde-3-phosphate is oxidized to pyruvate

Step-wise reactions of glycolysis

- **Reaction 1**: Phosphorylation of glucose to glucose-6 phosphate.
- This reaction requires energy and so it is coupled to the hydrolysis of ATP to ADP and Pi.
- Enzyme: hexokinase. It has a low Km for glucose; thus, once glucose enters the cell, it gets phosphorylated.
- This step is irreversible. So the glucose gets trapped inside the cell. (Glucose transporters transport only free glucose, not phosphorylated glucose)
- **Reaction 2**: Isomerization of glucose-6-phosphate to fructose 6-phosphate. The aldose sugar is converted into the keto isoform.
- Enzyme: phosphoglucomutase.
- This is a reversible reaction. The fructose-6-phosphate is quickly consumed and the forward reaction is favored.

Step-wise reactions of glycolysis (continued)

- **Reaction 3**: is another kinase reaction. Phosphorylation of the hydroxyl group on C1 forming fructose-1,6- bisphosphate.
- Enzyme: phosphofructokinase. This allosteric enzyme regulates the pace of glycolysis.
- Reaction is coupled to the hydrolysis of an ATP to ADP and Pi.
- This is the second irreversible reaction of the glycolytic pathway.
- **Reaction 4**: fructose-1,6-bisphosphate is split into 2 3-carbon molecules, one aldehyde and one ketone: dihyroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).
- The enzyme is aldolase.
- **Reaction 5**: DHAP and GAP are isomers of each other and can readily inter-convert by the action of the enzyme triose-phosphate isomerase.
- GAP is a substrate for the next step in glycolysis so all of the DHAP is eventually depleted. So, 2 molecules of GAP are formed from each molecule of glucose

Step-wise reactions of glycolysis (continued)

- Upto this step, 2 molecules of ATP were required for each molecule of glucose being oxidized
- The remaining steps release enough energy to shift the balance sheet to the positive side. This part of the glycolytic pathway is called as the **payoff** or **harvest stage.**
- Since there are 2 GAP molecules generated from each glucose, each of the remaining reactions occur twice for each glucose molecule being oxidized.
- **Reaction 6:** GAP is dehydrogenated by the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). In the process, NAD^+ is reduced to NADH + H^+ from NAD. Oxidation is coupled to the phosphorylation of the C1 carbon. The product is 1,3-bisphosphoglycerate.

Step-wise reactions of glycolysis (continued)

- **Reaction 7**: BPG has a mixed anhydride, a high energy bond, at C1. This high energy bond is hydrolyzed to a carboxylic acid and the energy released is used to generate ATP from ADP. Product: 3-phosphoglycerate. Enzyme: phosphoglycerate kinase.
- **Reaction 8**: The phosphate shifts from C3 to C2 to form 2-phosphoglycerate. Enzyme:phosphoglycerate mutase.
- **Reaction 9**: Dehydration catalyzed by enolase (a lyase). A water molecule is removed to form phosphoenolpyruvate which has a double bond between C2 and C3.
- **Reaction 10**: Enolphosphate is a high energy bond. It is hydrolyzed to form the enolic form of pyruvate with the synthesis of ATP. The irreversible reaction is catalyzed by the enzyme pyruvate kinase. Enol pyruvate quickly changes to keto pyruvate which is far more stable.

Glycolysis: Energy balance sheet

- Hexokinase: - 1 ATP
- Phosphofructokinase: -1 ATP
- GAPDH: +2 NADH
- Phsophoglycerate kinase: +2 ATP
- Pyruvate kinase: +2 ATP

Total/ molecule of glucose: +2 ATP, +2 NADH

Fate of Pyruvate

- NADH is formed from NAD^+ during glycolysis.
- The redox balance of the cell has to be maintained for further cycles of glycolysis to continue.
- NAD^+ can be regenerated by one of the following reactions /pathways:
- Pyruvate is converted to lactate
- Pyruvate is converted to ethanol
- In the presence of O2, NAD^+ is regenerated by ETC. Pyruvate is converted to acetyl CoA which enters TCA cycle and gets completely oxidized to CO_2.

Lactate Fermentation

- Formation of lactate catalyzed by lactate dehydrogenase:
  CH_3-CO-COOH + NADH + H^+ ←→
  CH_3-CHOH-COOH + NAD^+
- In highly active muscle, there is anaerobic glycolysis because the supply of O_2 cannot keep up with the demand for ATP.
- Lactate builds up causing a drop in pH which inactivates glycolytic enzymes. End result is energy deprivation and cell death; the symptoms being pain and fatigue of the muscle.
- Lactate is transported to the liver where it can be reconverted to pyruvate by the LDH reverse reaction

Ethanol fermentation

- Formation of ethanol catalyzed by 2 enzymes
- Pyruvate decarboxylase catalyzes the first irreversible reaction to form acetaldehyde:
  CH_3-CO-COOH → CH_3-CHO + CO_2
- Acetaldehyde is reduced by alcohol dehydogenase is a reversible reaction:
  CH_3-CHO + NADH + H^+ ←→ CH_3CH_2OH + NAD^+
- Ethanol fermentation is used during wine-making

Entry of other sugars into glycolysis

- **Fructose** is phosphorylated by fructokinase (liver) or hexokinase (adipose) on the 1 or 6 positions resp.
- Fructose-6-phosphate is an intermediate of glycolysis.
- Fructose-1-phosphate is acted upon by an aldolase-like enz that gives DHAP and glyceraldehyde.
- DHAP is a glycolysis intermediate and glyceraldehyde can be phosphorylated to glyceraldehyde-3-P.
- **Glycerol** is phosphorylated to G-3-P which is then converted to glyceraldehyde 3 phosphate.
- **Galactose** has a slightly complicated multi-step pathway for conversion to glucose-1-phosphate.
- gal → gal-1-P → UDP-gal → UDP-glc → glc-1-P.
- If this pathway is disrupted because of defect in one or more enz involved in the conversion of gal to glc-1-P, then galactose accumulates in the blood and the subject suffers from galactosemia which is a genetic disorder, an inborn error of metabolism.

Regulation of Glycolysis

Enzyme	**Activator**
Hexokinase	AMP/ADP
Phosphofructokinase	AMP/ADP, Fructose-2,6-bisphosphate
Pyruvte kinase	AMP/ADP Fructose-1,6-bisphosphate

Enzyme	**Inhibitor**
Hexokinase	Glucose-6-phosphate
Phosphofructokinase	ATP, Citrate
Pyruvate kinase	ATP, Acetyl CoA, Alanine

Regulation of Hexokinase

- Hexokinase catalyzed phosphorylation of glucose is the first irreversible step of glycolysis
- Regulated only by excess glucose-6-phosphate. If G6P accumulates in the cell, there is feedback inhibition of hexokinase till the G6P is consumed.
- Glucose-6-phosphate is required for other pathways including the pentose phosphate shunt and glycogen synthesis. So hexokinase step is not inhibited unless G-6-P accumulates. (no regulation by downstream intermediates / products of metabolism)
- Actually, liver, the site of glycogen synthesis, has a homologous enzyme called glucokinase. This has a high K_M for glucose. This allows brain and muscle to utilize glucose prior to its storage as glycogen

Regulation of Phosphofructokinase

- The phosphofructokinase step is rate-limiting step of glycolysis.
- High AMP/ADP levels are activators of this enzyme, while high ATP levels are inhibitory (energy charge). In addition,
- Feed-back inhibition by Citrate, an intermediate of the TCA cycle.
- A major positive effector of phosphofructokinase is Fructose-2,6-bisphosphate. F-2,6-BP is formed by the hormone-stimulated phosphoylation of F-6-P. Thus, this is an example of allosteric feed-forward activation

Formation of Fructose-2,6-bisphosphate

- Concentration of F-2,6-BP is regulated by the action of phosphofructokinase 2 (PFK2) and fructose bisphosphatase 2 (FBPase2).
- Both enzymes are distinct domains of the same polypeptide
- When glucose levels are low, glucagon levels are high (insulin and glucagon have opposing functions). PKA is activated, which in turn inactivates PFK2 by phosphorylation. At the same time FBPase2 is activated. F-2,6-BP is converted to F-6-P which enters gluconeogensis for synthesis of glucose. In the absence of F-2,6-BP, PFK is not activated and glycolysis pauses
- When glucose levels are high, glucagon levels are low. PKA is inactive but a phosphatase dephosphorylates PFK2 and activates it. PFK2 converts F-6-P to F-2,6-BP which is a allosteric activator of PFK, the glycolytic enzyme.

Regulation of pyruvate kinase

- If glycolysis gets past the phosphofructokinase step, then regulation is at the pyruvate kinase step.
- Pyruvate kinase activity is inhibited under low glucose conditions by covalent phosphorylation
- If fructose 1,6 bisphosphate is formed, it acts a allosteric feed-forward activator and drives the pyruvate kinase reaction forward.
- Other positive effectors are AMP and ADP while ATP is a negative effector.
- Alanine, an aminoacid derived from pyruvate, is a negative effector of catabolism. Alanine levels signal the anabolic state of a cell. High alanine levels indicate that the cell has enough starting material for anabolic reactions and so catabolism (which provides the ingredients for anabolism) can be paused.

Gluconeogenesis

- Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors including pyruvate, lactate, glycerol and aminoacids
- In animals the gluconeogenesis pathway is, for the most part, the reverse of glycolysis. There are substitute or bypass reactions for the irreversible steps of glycolysis.
- Glycerol enters reverse glycolysis as DHAP by the action of glycerol kinase followed by dehydrogenase
- Lacate is converted to pyruvate by LDH. Aminoacids are converted to either pyruvate or oxaloacetate prior to gluconeogenesis.

Bypass for Puruvate Kinase

- Three steps of glycolysis are irreversible and therefore need bypass reactions for gluconeogenesis.
- **Pyruvate to PEP**: Pyruvate synthesized by glycolysis or from aa is in the mitochondria. Here, pyruvate is first converted to oxaloacetate by the enzyme pyruvate carboxylase. One carbon is supplied by CO_2 to form the 4-C oxaloacetate. The reaction is coupled to ATP hydrolysis making this a ligation reaction.
- Oxaloacetate is shuttled out to the cytoplasm where the glycolytic enzymes are located. Oxaloacetate is converted to PEP by the enzyme PEP carboxykinase. CO_2 is removed and energy in the form of GTP is utilized.
- Two high energy molecules with a total free energy change of 62 kJ/mol are used up for the formation of PEP. This is consistent with the free energy change for hydrolysis of the enoyl phosphate bond.

Bypass for PFK and Hexokinase.

- PEP can be converted to fructose-1,6 bisphosphate by reverse glycolysis.
- F-1-6 BP to F-6-P cannot proceed by reverse glycolysis since the PFK reaction is irreversible.
- Instead a different enzyme called as fructose-1,6 bisphosphatase is used. This removes the P from the 1 position. However, no ATP is formed.
- Further reverse glycolysis leads to formation of glucose-6-P
- This is converted to Glc by the action of glc-6-phosphatase since the hexokinase reaction is irreversible.
- Net Reaction for gluconeogenesis:
- 2 pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 6 H2O → glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 Pi.
- (Net reaction for glycolysis is: Glucose + 2NAD^+ + 2 ADP + 2 Pi → 2 pyruvate + 2 ATP + 2 NADH + 2 H_2O)

Regulation of Gluconeogenesis

- Fructose 1-6-bisphosphatase is co-ordinately regulated with phosphofructokinase. Thus, citrate is a positive effector and AMP and F-2,6-BP are negative effectors.
- When glucose levels are high, F-2,6-BP is high and gluconeogenesis is inhibited while glycolysis is favored. When glucose levels are low, F-2,6-BP is low and glycolysis is inhibited.
- Pyruvate carboxylase is an imp regulatory step in gluconeogenesis. Acetyl CoA and ATP are positive effectors while AMP/ADP are inhibitors
- Glycolysis and gluconeogenesis are regulated by hormones. Insulin stimulates synthesis and activity of glycolytic enzymes while glucagon turns on gluconeogenic enzymes.

Substrate cycle or Futile cycle

- A pair of non-reversible reactions that cycle between two substrates are called as a substrate cycle
- In such a cycle, there is expense of ATP without a coupled biosynthetic reaction, thus, it is also called as a futile cycle
- Eg: F-6-P + ATP $\xrightarrow{(PFK)}$ F-1,6-BP + ADP

  F-1,6-BP + H2O $\xrightarrow{FBPase}$ F-6-P + Pi
- Net: ATP + H2O → ADP + Pi + energy (heat)
- Level of substrate cycling is very minimal because of reciprocal regulation of the enzymes
- Certain organisms utilize such reactions to maintain body temperature

Cori Cycle

- Lactate is formed in the active muscle to regenerate NAD^+ from NADH so that glycolysis can continue.
- The muscle cannot spare NAD^+ for re-conversion of lactate back to pyruvate.
- Thus, lactate is transported to the liver, where, in the presence of oxygen, it undergoes gluconeogenesis to form glucose.
- The glucose is supplied by the liver to various tissues including muscle.
- This inter-organ cooperation during high muscular activity is called as the Cori cycle.