I. Glycogen Phosphorylase

Glycogen phosphorylase catalyzes the following reaction:

\[
\text{Glycogen}_n + \text{Pi} \rightarrow \text{Glucose-1-phosphate} + \text{glycogen}_{n-1}
\]

Glycogen phosphorylase is a dimer of two identical subunits. Each subunit has an active site which contains a pyridoxal cofactor covalently attached via a Schiff base. The active sites are located in the center of each subunit. This enzyme is allosterically regulated.

AMP↑
ATP↓
Glucose-6-phosphate↓

The binding sites for these allosteric effectors is located near the subunit interfaces.

This enzyme binds inorganic phosphate cooperatively. This allows the enzyme’s activity to increase by great amounts over a narrow range of substrate concentrations.

Glycogen phosphorylase generates glucose-1-phosphate which is isomerized into glucose-6-phosphate and enters the glycolytic pathway to produce ATP. This end product ATP is a feed back inhibitor of glycogen phosphorylase. Glucose-6-phosphate is an allosteric inhibitor of the enzyme. ATP and glucose-6-phosphate produce a negative effect on the cooperativity of substrate binding. As shown above the
substrate saturation curve is shifted to the right by ATP requiring a lot higher concentration of Pi to produce half of the maximal velocity.

AMP is also an allosteric effector of glycogen phosphorylase. It competes for the same allosteric binding site as ATP but stimulates glycogen phosphorylase by having a positive effect on the cooperativity of substrate binding. Increase in the cellular concentration of AMP is an indicator that the energy status of the cell is low and more ATP via glycolysis needs to be produced. The reciprocal changes of ATP and AMP concentrations combined with their competition for the allosteric binding site with opposite effects provide a mechanism for rapid and reversible control over glycolysis.

Glycogen phosphorylase is a typical allosteric enzyme.
- It is composed of 2 identical subunits (multiple subunits)
- The dimer has symmetry
- The enzyme exist in only two conformations designated R and T.
- These conformations are in equilibrium $R \leftrightarrow T$
- The substrates bind when the enzyme is in the R state.
- Positive allosteric effectors bind to the R state and stabilize it shifting the equilibrium to the left.
- Negative allosteric effectors bind to the T state and stabilize it shifting the equilibrium to the right.

In the T-state, the active site is buried which lowers the affinity of the enzyme for the substrates. In addition, Asp-283 faces the active site so that there is electrostatic repulsion between this residue and the substrate inorganic phosphate.

In the R-state, the active site is exposed to the solvent facilitating substrate binding. In addition, Asp-283 is displaced from the active site and replaced by Arg-569 which produces favorable electrostatic interactions between the negatively charged inorganic phosphate.

The allosteric controls allow the cell to adjust to normal metabolic demands. In crisis conditions in which ATP is needed immediately, these allosteric controls are overridden by reversible covalent phosphorylation of glycogen phosphorylase by the enzyme, phosphorylase kinase. The reversible covalent modification of Ser-14 converts the enzyme from a less activated, allosterically regulated form $b$ to a more active, allosterically unresponsive form $a$. Thus the covalent modification is like a permanent allosteric transition.
The phosphorylation of Ser-14 causes dramatic conformational changes in phosphorylase. Upon phosphorylation, residues 10 through 22 swing through an arc of 120° moving from the interior of the protein to the subunit interface. This movement allows the phosphorylated serine residue to electrostatically interact with two cationic Arginine residues. The phosphorylation of the serine residue essentially locks the enzyme into the R-state.

The black lines are the unphosphorylated enzyme, phosphorylase \( b \), the yellow lines are the phosphorylated enzyme, phosphorylase \( a \).

The phosphorylation is reversible. The dephosphorylation is carried out by the enzyme called \textbf{phosphoprotein phosphatase 1}.

\section*{II. Glycogen Synthase}

Glycogen Synthase is a typical allosteric enzyme.

- It is composed of 4 identical subunits (multiple subunits)
- The tetramer has symmetry
- The enzyme exist in only two conformations designated R and T.
- These conformations are in equilibrium \( R \rightleftharpoons T \)
- The substrates bind when the enzyme is in the R state.
- Positive allosteric effectors bind to the R state and stabilize it shifting the equilibrium to the left.
- Negative allosteric effectors bind to the T state and stabilize it shifting the equilibrium to the right.

Glycogen synthase is allosterically inhibited by physiological concentrations of ATP, ADP and Pi. It is allosterically activated by glucose-6-phosphate.

Like glycogen phosphorylase, allosteric controls are overridden by reversible covalent phosphorylation. In this case the phosphorylated glycogen synthase, form \( b \) is less active than the original dephosphorylated form \( a \). The same phosphorylase kinase that phosphorylates glycogen phosphorylase phosphorylates glycogen synthase. Six other protein kinases phosphorylate glycogen synthase phosphorylating one or more of the nine serine residues located on its subunits. The phosphorylation is reversible. The dephosphorylation is carried out by the enzyme called \textbf{phosphoprotein phosphatase 1}.

\section*{III. Hormonal Control of Metabolism}

Glucose of course is the major fuel of the body. The brain, red blood cells, lens of the eye, kidney medulla and exercising skeletal muscle all depend on glycolysis for energy. They required an uninterrupted source of glucose.

\textbf{Insulin} and \textbf{glucagon} are the two major hormones that regulate fuel storage and mobilization.
**Insulin** is the anabolic hormone. It promotes the storage of fuels. Insulin activates the storage of glucose as glycogen in the liver and the muscles. Insulin also promotes converting glucose into triacylglycerides and the storage of triacylglycerides in the adipose tissue. Insulin also promotes protein synthesis in skeletal muscle. Insulin also promotes utilization of glucose as fuel. The release of insulin is dictated by blood glucose levels. The highest concentrations of insulin occur 40 to 45 minutes after a high carbohydrate meal. After 120 minutes the insulin level drops back to the basal levels.

The major sites of insulin action.

⊕ = Stimulated by insulin
⊖ = Inhibited by insulin

Insulin is produced by B cells of the endocrine pancreas. Insulin is a polypeptide hormone. It is synthesized as a preprohormone which is converted in the rough endoplasmic reticulum to proinsulin. Proinsulin folds into the proper conformation and the disulfide bonds are formed. It is then transported to the Golgi and stored in vesicles. In the vesicles a protease activates the insulin. Stimulation of the B cells cause the exocytosis of the insulin storage vesicles and insulin is secreted into the hepatic portal vein via the pancreas veins. Insulin is rapidly removed from circulation by the liver and kidneys and is degraded.

**Glucagon** is the hormone that promotes the mobilization of fuels. Glucagon acts to maintain glucose availability in the absence of dietary glucose by stimulating the release of glucose from the liver. Glucagon stimulates glycogenolysis and gluconeogenesis.

Glucagon also activates the mobilization of fatty acids from the adipose tissue.

The sites of glucagon action are principally in the liver and adipose tissue. The release of glucagon is suppressed by insulin and glucose. The lowest levels of glucagon occur after a high carbohydrate meal. All of glucagon’s effects are opposed by the effects of insulin. The stimulation of insulin release suppresses the release of glucagon.
Glucagon is synthesized by the type A cells of the pancreas in the form of a preproglucagon polypeptide. The preproglucagon is produced in the endoplasmic reticulum and as it enters the lumen it undergoes proteolytic cleavage to produce the mature 29 amino acid polypeptide glucagon. Glucagon is rapidly metabolized in the liver and kidneys giving it a half-life of 3-5 minutes.

Epinephrine is another hormone that is released in response to the central nervous system under times of physiological stress. Epinephrine also increases the availability of fuels. Epinephrine stimulates glycogenolysis and release of fatty acids from adipose tissues.

**Mechanisms of Hormone Action**

Hormones affect the flux of metabolites through a metabolic pathway. In order to change the flux, hormones must affect the enzymes that catalyze the rate determining steps that control the flux of metabolites through the pathway. Here are several ways hormones change the flux of metabolites through a pathway.

1. If the substrate concentration is rate limiting, then hormones may alter the concentration of the substrate to increase or decrease the rate of flux.
2. Hormones may promote the reversible phosphorylation of the flux controlling enzymes to change the conformation of the enzyme’s active site either activating or deactivating the enzyme.
3. Hormones may promote the dephosphorylation of the flux controlling enzymes.
4. Hormones can affect the concentrations of allosteric effectors.
5. Hormones can induce or repress genes to change the amount of enzyme present in the cell.
The phosphorylation and dephosphorylation mediated events occur rapidly enabling cells to respond instantly to changes in the environment. In may take hours for the induction or repression of a gene to affect the flux.

**Hormonal Signal Transduction**

Hormones initiate their action by binding to specific receptor sites on the target cells. The hormone is the first messenger. It binds to the receptor, and the message of the hormone is transmitted to intracellular enzymes by the receptor and an intracellular second messenger. The mechanism by which the hormonal signal is transmitted is called **signal transduction**.

Shown to the left are four types of signal transduction. In this first example binding of the hormone to the receptor is coupled to adenylate cyclase promoting the production of cAMP.

In the next example the binding of the hormone to the receptor activates a receptor kinase activity.

In this example, the binding of the hormone to the receptor is coupled to the hydrolysis of phosphatidylinositol bisphosphate.

In the last example, the binding of the hormone to the receptor is coupled to gated ion channels.

**Signal Transduction of Glucagon**

The receptor that binds the peptide hormone glucagon is coupled to adenylate cyclase and AMP production. Adenylate cyclase in a membrane bound enzyme that converts ATP to 3’-5-cyclic AMP, cAMP. cAMP is a regulatory molecule found in all eukaryotic cells. It is an intracellular messenger controlling a wide variety of processes.

The hormone glucagon is the **first messenger**. cAMP is the **second messenger**.

cAMP activates **cAMP-dependent Protein Kinase**, cAPK (also called **protein kinase A**) which the activity of enzymes by phosphorylating specific serine residues. Phosphorylation activates some enzymes such as glycogen phosphorylase while inactivating others such as glycogen synthase.
The **GTP binding-proteins** or **G-proteins**, couple the glucagon receptor to adenylate cyclase activity. The G-proteins are located in the plasma membrane. The G-proteins bind GTP and have dissociable subunits that interact with the membrane bound adenylate cyclase. When glucagon is not bound to the receptor, the G-protein is bound to GDP. When the G-protein has GDP bound it cannot associate with the receptor or with adenylate cyclase. Once glucagon binds to the receptor, the receptor binds the G-protein-GDP complex causing the complex to release GDP and bind GTP. When GTP binds the $\alpha$-subunit dissociates away from the $\beta\gamma$-subunits and binds to adenylate cyclase which activates the cyclase. When the GTP of the $\alpha$-subunit is hydrolyzed into GDP then the $\alpha$-subunit dissociates from adenylate cyclase and recomplexes with the $\beta\gamma$-subunits. Only when the glucagon receptor has glucagon bound can it keep adenylate cyclase active.

The cAMP produced is absolutely required to activate the cAMP-dependent protein kinase, **cAPK**. In the absence of cAMP, cAPK is an inactive tetramer consisting of two regulatory subunits and two catalytic subunits, $R_2C_2$. When cAMP binds the regulatory subunits, the subunits dissociate from the catalytically active monomers.

$$R_2C_2 \text{ (inactive)} + 4 \text{ cAMP} \leftrightarrow 2C \text{ (Active)} + R_2(\text{cAMP})_4$$

The intracellular concentration of cAMP determines the fraction of cAPK that is active.
The activated cAPK kinase phosphorylates many proteins including glycogen phosphorylase kinase and glucogen synthase.

**Phosphorylase Kinase**

Phosphorylase kinase is a protein kinase that specifically phosphorylates Ser-14 of glycogen phosphorylase b.

Phosphorylase kinase is a 1,300 KD protein composed of four nonidentical subunits which are known as α, β, γ, and δ. The γ-subunit contains the active site while the other subunits play regulatory roles. Phosphorylase kinase is activated by Ca$^{2+}$ ions and by phosphorylation of its α and β subunits. The γ-subunit binds its own C-terminus to block the active site. Ca$^{2+}$ ion concentrations as low as 10$^{-7}$ M activate phosphorylase kinase by binding the δ-subunit which is also known as calmodulin. The binding of calcium by the calmodulin subunit causes a conformational change removing the C-terminus out of the active site of the γ-subunit and thereby activating it. The phosphorylation of the α and β-subunits by cAPK causes the enzyme to become active at much lower concentrations of Ca$^{2+}$ ions. Phosphorylase kinase phosphorylates Ser-14 of glycogen phosphorylase b to form the activated glycogen phosphorylase a. Muscle contraction increases the cystolic concentration of Ca$^{2+}$ ions. The Ca$^{2+}$ ions produced into turn stimulate the break down of glycogen into glucose.

**Calmodulin**

Calmodulin, CaM, is a free floating eukaryotic Ca$^{2+}$ ion binding protein which participates in many cellular regulatory processes. CaM has two globular domains. Each of the globular domains binds two Ca$^{2+}$ ions. The Ca$^{2+}$ ion binding sites are formed by helix-turn-helix motif known as EF hands. The binding of calcium induces conformation changes in CaM exposing a hydrophobic patch which binds to binding domain of the phosphorylase kinase γ-subunit.
Signal Transduction by Epinephrine.

Epinephrine and norepinephrine are the fight or flight hormones released into the blood by the adrenal glands. They bind to two types of receptors, $\alpha$ and $\beta$-adrenergic receptors. The $\beta$-adrenergic receptor is shown to the left. The signal transduction mechanism is the same as the glucagon receptor. Once epinephrine binds to the receptor, the receptor binds the G-protein-GDP complex causing the complex to release GDP and bind GTP. When GTP binds the $\alpha$-subunit dissociates away from the $\beta\gamma$-subunits and binds to adenylate cyclase which activates the cyclase. When the GTP of the $\alpha$-subunit is hydrolyzed into GDP then the $\alpha$-subunit dissociates from adenylate cyclase and recomplexes with the $\beta\gamma$-subunits. Only when the $\beta$-adrenergic receptor has epinephrine bound can it keep adenylate cyclase active.

When epinephrine binds to the $\alpha$-adrenergic receptor, Calcium ions are released into the cytosol increasing the concentration of $Ca^{2+}$. This reinforces the cells’ response to cAMP.

The Five Principles of Hormonal Signal Transduction

1. The specificity of action in tissues is conferred by the receptor. Ie Glucagon receptors found in liver, adipose tissue and kidney.

2. The system amplifies the signal. Glucagon present only in very small amounts in the blood. The binding of one molecule of glucagon at a receptor activates many cAPK molecules which in turn phosphorylate hundreds of enzyme molecules.

3. The metabolic responses are integrated. Glucagon stimulates the phosphorylation of enzymes that simultaneously activate glycogen catabolism and inhibit glycogen synthesis and activate inhibit glycolysis in the liver.

4. The fourth is augmentation and antagonism of signals. Glucagon and epinephrine bind to different receptors, but both increase the activity of adenylate cyclase and stimulate glycogen degradation by phosphorylation. Epinephrine and insulin are antagonistic.

5. Rapid signal termination. The rapid degradation of cAMP contribute to rapid signal termination.
Signal Transduction of Insulin

Insulin binds to a receptor on the plasma membrane of specific cells. The insulin receptor is composed of two subunits, $\alpha$-subunits to which the insulin binds and $\beta$-subunits which span the membrane and protrude out into the cytosol. The cytosolic portion of the $\beta$-subunit has tyrosine kinase activity. On the binding of insulin, the tyrosine kinase phosphorylates tyrosine residues on the $\beta$-subunits. This is called autophosphorylation. The activated tyrosine kinase also phosphorylates other substrates such as the insulin receptor substrate, IRS-1. The phosphorylated IRS-1 is a messenger involved in many cellular regulatory mechanisms.

The net effects of insulin are:
- Insulin reverses glucagon stimulated phosphorylation.
- Insulin produces a phosphorylation cascade which activates many protein kinases and enzymes.
- Insulin induces and represses genetic expression of enzymes,
- Insulin serves as a growth factor and stimulates protein biosynthesis
- Insulin stimulates glucose and amino acid transport in the cell.

Phosphoprotein Phosphatase-1

The level of phosphorlated enzymes is maintained by the opposition of phosphorylation by phosphatases. Phosphatases remove phosphate groups that protein kinases added and thereby reverse the effects. Phosphoprotein phosphatase-1 removes the phosphoryl groups from glycogen phosphorylase $a$ and the phosphoryl groups of the $\alpha$ and $\beta$ subunits of phosphorylase kinase.

Phosphoprotein phosphatase-1 is controlled differently in the muscle and liver. I will leave to read the text book about the regulation in the muscle.

In the liver, phosphoprotein phosphatase-1 is controlled by binding to glycogen phosphorylase $a$. It binds to glycogen phosphorylase $a$ in both the T and R states. Only in the T state however is the phosphorylated Ser-OH accessible to the phosphatase which does it duty and hydrolyzes the phosphoester bond and converts glycogen phosphorylase $a$ to glycogen phosphorylase $b$. Only high concentrations of glucose can shift the equilibrium to favor the T form of glycogen phosphorylase $a$. Thus glucose is
an allosteric inhibitor of glycogen phosphorylase \( a \). Phosphoprotein phosphatase-1 has little affinity for glycogen phosphorylase \( b \) and is released into the cytosol. The liver contains 10 times more glycogen phosphorylase than the phosphatase. Thus not until 90 % of the glycogen phosphorylase \( a \) is dephosphorylated into glycogen phosphorylase \( b \) is the phosphatase concentration high enough to dephosphorylate phosphorylase kinase or activate glycogen synthase by dephosphoryating it.

Hormonal Regulation of Glycolysis and Gluconeogenesis.

Fructose 2-6 phosphate

The enzyme that controls the flux of metabolites through the glycolytic pathway is phosphofructokinase-1. This enzyme is activates by AMP and Fructose-2,6-bisphosphate and inhibited by citrate. The enzyme that bypasses this irreversible step in the gluconeogenic pathway is fructose-2,6-bisphosphatase which is reciprocally regulated by AMP, Fructose-2,6-bisphosphate and citrate as shown.

Fructose-2,6-bisphosphate is an important allosteric regulator of this substrate cycle. The concentration of Fructose-2,6-bisphosphate depends on its rate of degradation and rate of biosynthesis. Fructose-2,6-bisphosphate is synthesized by an isozyme of PFK-1 called phosphofructokinase-2, PFK-2. The enzyme that breaks it down is called Fructose-2,6-

dphantsatase.

The weird thing is that both of these enzymes are located in different domains of the same polypeptide. One protein contains two active sites catalyzing reciprocal reactions. Wait, it gets better. These two active sites are under hormonal regulation. The bifunctional protein is phosphorylated by cAPK and
dephosphorylated by phosphoprotein phosphatase-1. When the blood glucose concentration is low, glucagon stimulates the production of cAMP which activates cAPK which phosphorylates the protein. The phosphorylated protein has activated FBPase activity and inactivated PFK-2 activity. This results in a drop in the concentration of fructose-2,6-bisphosphate increasing the net flux through the gluconeogenic pathway. Conversely when the blood glucose concentration is high, cAMP decreases Phosphoprotein phosphatase-1 dephosphorylates the protein. The dephosphorylation inactivates the FBPase activity while activating the PFK-2 activity which increases the fructose-2,6-bisphosphate concentration increasing the flux of metabolites through the glycolytic pathway.

Pyruvate Kinase
In the liver, pyruvate kinase is also under hormonal control. When the blood glucose concentration is low, glucagon stimulates the production of cAMP which activates cAPK which phosphorylates the pyruvate kinase. The phosphorylated enzyme is less active. The phosphorylated enzyme is more strongly inhibited by ATP and has a greatly Km for PEP such that under physiological concentrations of PEP, the phosphorylated pyruvate kinase inactive. The phosphorylation is reversed by phosphoprotein phosphatase-1.