Part III => METABOLISM and ENERGY

§3.3  Glycogen Metabolism
§3.3a Glycogen Breakdown
§3.3b Glycogen Synthesis
§3.3c Glycogen Regulation
Section 3.3a:
Glycogen Breakdown
(Glycogenolysis)
Synopsis 3.3a

- Peripheral organs such as brain and erythrocytes demand a constant supply of glucose as a source of energy—implying that excess glucose must be stored and released as needed during starvation/fasting.

- In animals, excess glucose (from the hydrolysis of starch in the food) is converted via glycogenesis and largely stored in the liver in the form of a branched polymer called “glycogen”.

- In the liver, the breakdown of glycogen via glycogenolysis is needed to generate a constant supply of glucose for peripheral organs during starvation/fasting.

- Glycogen breakdown (glycogenolysis) in the liver involves a series of conversions:
  \[ \text{glycogen} \rightarrow \text{G1P} \rightarrow \text{G6P} \rightarrow \text{glucose} \]

- During sustained starvation/fasting (> 12 hr), glycogen becomes usurped—this is when gluconeogenesis kicks in to make glucose available from non-carbohydrate sources such as proteins (glucogenic amino acids), triglycerides (glycerol) and other metabolites (pyruvate/lactate).

- While the major role of glycogen breakdown in the liver cells (hepatocytes) is for the release of glucose into the bloodstream, glycogenolysis in the muscle cells (myocytes) solely serves to meet local energy demands needed for muscle contraction and rapid bursts of muscular activity.
Overview of Glucose Metabolism

- Glycogen
  - glycogen breakdown (glycogenolysis)
  - glycogen synthesis (glycogenesis)

pentose phosphate pathway

- Glucose
- Glucose-6-phosphate
  - glycolysis
  - glucose synthesis (gluconeogenesis)

- Pyruvate
- Amino acids
- Lactate
- Acetyl-CoA
- Citric acid cycle
- Ribose-5-phosphate
- Recall that (from §1.3):

  - glycogen is comprised of repetitive glucose (α-D-glucopyranose) units joined together via (α1→α4) glycosidic linkages in a head-to-tail fashion
  - additional glucose units are added via the (α1→α6) glycosidic linkages to generate a branched biopolymer, with branch points on average occurring at every 10 residues or so
  - glycogen is the principal fuel storage or energy reservoir in animals—deposited as insoluble granules largely in liver but also present in small quantities in virtually all cells—glycogen can comprise as much 10%(w/w) of liver!
Glycogenolysis: Reducing and Non-Reducing Ends

- Glycogen breakdown involves sequential removal of glucose units from the non-reducing ends (C4-OH) of branched polymer toward the reducing end (C1-OH)—ie the end harboring the aldehyde group @ C1 in the linearized form of glucose that becomes reduced to -OH upon cyclization!

- While glycogen has only ONE reducing end, it harbors as many non-reducing ends as there are branch points or linkages—one non-reducing end on every branch!

- Being a branched polymer thus allows rapid breakdown of glycogen into individual glucose units for the generation of energy—the enzymes involved can simultaneously set up a “shop” at each end of the branch point!
**Glycogenolysis: Metabolic Pathway**

1. **Glycogen phosphorylase**—cleaves glycosidic bonds (that are at least five units away from a branch point, thereby leaving behind 4-mer limit branches) via substitution of a phosphate group (phosphorolysis—cf hydrolysis) to yield glucose-1-phosphate (G1P):
   
   \[
   \text{glycogen (n)} + \text{HPO}_4^{2-} \leftrightarrow \text{glycogen (n-1)} + \text{G1P}
   \]
   
   where \( n \) is the number of residues in glycogen.

2. **Glycogen debranching enzyme**—transfers the terminal three glucose residues (in the context of a trisaccharide unit) of the 4-mer limit branches to the non-reducing end of another branch (thereby enabling glycogen phosphorylase to finish off glycosidic bonds that it otherwise cannot access)—the enzyme also hydrolyzes the remaining glucose residue at the branch point.

3. **Phosphoglucomutase**—converts G1P to glucose-6-phosphate (G6P), which can then enter glycolysis (energy production) or channeled into the pentose phosphate pathway (synthesis of nucleotides, amino acids, fatty acids).

4. **Glucose-6-Phosphatase**—since G6P cannot pass through the cell membrane, it is first hydrolyzed (primarily in the liver) to glucose by glucose-6-phosphatase:
   
   \[
   \text{G6P} + \text{H}_2\text{O} \leftrightarrow \text{glucose} + \text{Pi}
   \]

Given that it lacks glucose-6-phosphatase, the primary role of glycogenolysis in muscle is to meet local energy demands (via the provision of G6P for glycolysis) needed for muscle contraction and rapid bursts of muscular activity!
Glycogenolysis: (1) Glycogen Phosphorylase

PLP acting as an acid

PLP acting as a base

Hydrogen phosphate ($\text{HPO}_4^{2-}$) not inorganic phosphate ($\text{PO}_4^{3-}$)—recall that the ionization below occurs with pKa of 12 (see §1.1):

$$\text{HPO}_4^{2-} \leftrightarrow \text{PO}_4^{3-} + \text{H}^+$$

Glycogen phosphorylase requires PLP as a prosthetic group (covalently linked via Schiff base) that serves as a general acid-base catalyst.
Glycogenolysis: (2) Glycogen Debranching Enzyme

The enzyme transfers the terminal three ($\alpha_1-\alpha_4$)-linked glucose residues (in the context of a trisaccharide unit) from a 4-mer limit branch to the non-reducing end of another branch—thereby making them available to glycogen phosphorylase for further cleavage to G1P.

The solo ($\alpha_1-\alpha_6$)-linked glucose residue remaining at the branch point is hydrolyzed by further action of the enzyme—such free glucose residues thus bypass G1P intermediate needed for conversion to G6P by phosphoglucomutase.
Glycogenolysis: (3) Phosphoglucomutase

The active site of phosphoglucomutase harbors a phosphoserine (pS) that initially serves as a phosphate donor to the C6-OH position on G1P to generate a glucose-1,6-bisphosphate (G1,6P) intermediate. pS at the active site of phosphoglucomutase is subsequently regenerated by the transfer of the phosphoryl group at the C1-OH position on G1,6P in a balancing act with concomitant release of G6P.
Glycogenolysis: (4) Glucose-6-Phosphatase

- Glucose-6-phosphatase hydrolyzes G6P to glucose for subsequent release into the bloodstream.

- G6P hydrolysis to glucose primarily occurs in the liver—glycogen breakdown in the liver is essential for the homeostatic control of blood glucose level.

- Glucose leaves the liver via GLUT2—a bidirectional glucose transporter (see §1.6)—and is carried by the bloodstream to other cells (e.g., brain cells and erythrocytes).

- Glucose-6-phosphatase is primarily expressed in the liver and kidneys—cells of the muscle (unidirectional GLUT4) and other tissues are deficient in glucose-6-phosphatase!

- Glycogen breakdown in muscle cells solely serves to meet local energy demands—e.g., to power up muscle contraction!
Exercise 3.3a

- List the metabolic sources and fates of G6P

- Describe the structure of a glycogen molecule. What is the advantage of its branched structure?

- List the three enzymes involved in glycogen degradation and describe the type of reactions they catalyze
Section 3.3b: Glycogen Synthesis (Glycogenesis)
- In animals, excess glucose (from the hydrolysis of starch in the diet) is converted to glycogen via glycogenesis primarily in the liver cells (for homeostatic control of blood glucose level)—as well as in the muscle cells (to meet local energy demands for glucose)

- **Glycogen synthesis (glycogenesis)** involves a series of conversions:
  glucose => G6P => G1P => UDP-glucose =>==>==> glycogen

- **Uridine diphosphate glucose (UDP–glucose)** is an activated molecule that spontaneously donates a glucosyl unit to the growing chain of glycogen

- Glycogen is extended from a primer built on and by a protein enzyme called “glycogenin”
Glycogen Breakdown versus Synthesis

Breakdown

- Glucose

- Hexokinase

- Phosphoglucomutase

- Glucose-6-phosphatase

- Debranching enzyme

- Glycogen phosphorylase

- UTP

- G1P

- G6P

- Glucose

Synthesis

- Glycogen

- Branching enzyme

- Glycogen synthase

- UDP-glucose

- Inorganic pyrophosphatase

- PPi

- 2 Pi

- UDP-glucose pyrophosphorylase

- Catabolic and anabolic pathways must differ in order for them to operate simultaneously under similar physiological conditions (see §3.1)

- Thus, the pathway involved in glycogen breakdown to G1P cannot be reversed to synthesize glycogen (ΔG ≫ 0)

- G1P (from glucose) conversion to glycogen involves the activated UDP-glucose intermediate that can spontaneously donate a glucosyl unit to the growing chain of glycogen
Glycogenesis: Metabolic Pathway

1. Hexokinase
2. Phosphoglucomutase
3. UDP-glucose pyrophosphorylase
4. Glycogenin*
5. Glycogen synthase
6. Glycogen branching enzyme

Glucose

Glucose-6-phosphate (G6P)

Glucose-1-phosphate (G1P)

UDP-glucose

Oligoglucosyl-glycogenin

Glycogen (linear)

Glycogen (branched)

William Whelan (1924-date)

*Our own Professor Whelan discovered glycogenin in 1984 while working in his laboratory within the BMB department—of which he has been an highly distinguished member of since 1967—the luckiest guy indeed!

**Glycogenesis: (1) Hexokinase**

- First step of glycolysis (see §3.2)

- Transfer of the terminal phosphoryl group of ATP to glucose to generate G6P

- Catalyzed by hexokinase (expressed in all tissues) or glucokinase (exclusively expressed in the liver and pancreas)

- **Glucokinase (an isozyme) is an isoform of hexokinase** that is specialized for the conversion of glucose to G6P specifically for the synthesis of glycogen as opposed to glycolysis

- **Glucokinase** ($K_M \approx 10\text{mM}$) exhibits 100-fold higher $K_M$ for glucose than hexokinase ($K_M \approx 0.1\text{mM}$)—ie glucokinase only kicks into action when cellular levels of glucose begin to build up!

- Recall that the blood glucose (180g/mol) level is $\approx 1\text{mg/ml}$ (or $\approx 5\text{mM}$)
Glycogenesis: (2) Phosphoglucomutase

The active site of phosphoglucomutase harbors a phosphoserine (pS) that initially serves as a phosphate donor to the C1-OH position on G6P to generate a glucose-1,6-bisphosphate (G1,6P) intermediate.

- Forward step involved in glycogen breakdown (black arrows)
- Reverse step involved in glycogen synthesis (red arrows)

- pS at the active site of phosphoglucomutase is subsequently regenerated by the transfer of the phosphoryl group at the C6-OH position on G1,6P in a balancing act with concomitant release of G1P
Glycogenesis: (3) UDP-Glucose Pyrophosphorylase

- In order to initiate polymerization of glucose units, UTP is used as a source of energy to activate glucose in the form of UDP-glucose—wherein the UDP moiety not only contributes to the binding energy of UDP-glucose substrate to the enzymes (involved in the next two steps) but will also serve as an excellent leaving group upon nucleophilic attack!

- Nucleophilic attack by O atom of the phosphoryl group of G1P on the α-phosphate group of uridine triphosphate (UTP) results in the concomitant release of uridine diphosphate glucose (UDP-glucose) and pyrophosphate (PPi)

- Subsequent hydrolysis of PPi to Pi by inorganic pyrophosphatase drives the reaction to completion by not only generating copious amounts of free energy (-33 kJ/mol) but also via shifting the equilibrium in the direction of UDP-glucose

- UTP serves as the free energy source for glycogen synthesis—it is replenished from UDP and ATP through a phosphoryl-transfer reaction mediated by nucleoside diphosphate kinase:
  
  \[
  \text{UDP} + \text{ATP} \leftrightarrow \text{UTP} + \text{ADP}
  \]
Glycogenesis: (4) Glycogenin

- In addition to UDP-glucose, glucose polymerization into glycogen also requires a short glucose oligo or a glucose primer covalently attached to a protein called “glycogenin”

- Glycogenin is a protein enzyme involved in the generation of a covalently-bound oligoglucosyl primer (4-8 glucose units) absolutely required for the initiation of longer glycogen chains

- Synthesis of such oligoglucosyl-glycogenin primer begins with the transfer of a glucosyl unit from UDP-glucose—via the formation of an oxonium ion intermediate—to a tyrosine residue (Y194) in glycogenin

- This step is repeated to add up to as many as eight (α1→α4)-linked glucose units covalently bonded at their reducing end (C1-OH) to Y194 in glycogenin

- Glycogenin forever remains covalently attached to each glycogen molecule in a “till-death-us-do-part” manner—ie glycogen is essentially a “proteoglycan”—why not a glycoprotein?!

Hasta la muerte!
Glucosyl unit of UDP-glucose is transferred to the C4-OH group (via the formation of an oxonium ion intermediate) on the non-reducing end of oligoglucosyl-glycogenin primer to form an (α1→α4) glycosidic bond—this step is extensively repeated to generate a linear chain of glycogen (bound to glycogenin).
Glycogen synthesis involves several key enzymes and steps to create glycogen, a storage form of glucose.

- **Glycogen synthase** only generates (α1→α4)-linked linear chains of glycogen.

- In order to generate a branched polymer, the glycogen branching enzyme catalyzes the formation of (α1→α6)-linked branch points.

- Such branch points are generated via the transfer of the reducing end (C1-OH) of a 7-residue segment from the end of a nascent glycogen chain to the C6-OH group of a glucose unit on the same or another chain to create a branch point—which can then be extended by glycogen synthase!

- The donor nascent glycogen chain must be at least **11 residues long**.

- The new branch point created must be at least **4 residues away from a neighboring branch point**.
Exercise 3.3b

- Why must opposing biosynthetic and degradative pathways differ in at least one enzyme?

- List the three enzymes involved in glycogen synthesis and describe the types of reactions they catalyze.

- What is the free energy source for glycogen synthesis?

- Describe the role of glycogenin.
Section 3.3c: Glycogen Regulation
Synopsis 3.3c

- In the absence of any regulation or control, glycogenesis and glycogenolysis would operate simultaneously—thereby resulting in the wasteful hydrolysis of UTP.

- The opposing processes of glycogen breakdown and synthesis are under reciprocal regulation via two major mechanisms:
  1. Allosteric Control
  2. Post-Translational Modification (PTM)

- Although the above regulatory mechanisms target glycogen phosphorylase (glycogenolysis) and glycogen synthase (glycogenesis), glycogen metabolism is ultimately under the antagonistic control of endocrine hormones such as:
  1. Insulin (hyperglycemia)—promotes glycogenesis in the liver and muscle
  2. Glucagon (hypoglycemia)—promotes glycogenolysis in the liver
  3. Epinephrine/Adrenaline (exercise)—promotes glycogenolysis in the muscle and liver
**Glycogen Metabolism: Allosteric Control**

**Glycogen phosphorylase (Glycogenolysis)**
- allosterically activated by AMP
- allosterically inhibited by ATP and G6P

**Glycogen synthase (Glycogenesis)**
- allosterically activated by G6P

**Significance of Allosteric Control**

1. ATP demand exceeds supply (starvation) => low [ATP] & low [G6P] but high [AMP]:
   - glycogen phosphorylase activated
   - glycogen synthase remains inhibited
   - glycogen breakdown favored when [G6P] and [ATP] are in short supply

2. ATP supply exceeds demand (fully fed) => high [G6P] & high [ATP] but low [AMP]:
   - glycogen synthase activated
   - glycogen phosphorylase inhibited
   - glycogen synthesis favored when [G6P] and [ATP] are plentiful
Glycogen Metabolism: Role of PTM

Glycogen phosphorylase (Glycogenolysis)
- activated by phosphorylation (via phosphorylase kinase)
- inactivated by dephosphorylation (via phosphoprotein phosphatase 1)

Glycogen synthase (Glycogenesis)
- inactivated by phosphorylation (via phosphorylase kinase)
- activated by dephosphorylation (via phosphoprotein phosphatase 1)

Significance of Post-Translational Modification (PTM)
- Phosphorylase kinase and phosphoprotein phosphatase 1 act in an antagonistic manner to tightly regulate the activities of two key enzymes involved in glycogen metabolism
- Thus, net balance between glycogen synthesis and breakdown depends on the enzymatic activities of phosphorylase kinase and phosphoprotein phosphatase 1—how are these regulated?!
- Enzyme activities of phosphorylase kinase and phosphoprotein phosphatase 1 (as well as a plethora of other upstream enzymes that in turn regulate them) are ultimately under the control of hormones such as insulin, glucagon and epinephrine (adrenaline).
Endocrine hormones such as insulin, glucagon, and epinephrine (adrenaline) regulate glycogen metabolism by virtue of their ability to activate specific cell surface receptors such as insulin receptor (an RTK), glucagon receptor (a GPCR), or $\beta$-adrenoreceptor (a GPCR)—see §1.7!

Upon stimulation, these receptors turn on specific intracellular signaling cascades (such as the release of cAMP secondary messenger by GPCRs) that ultimately affect PTM of glycogen phosphorylase and glycogen synthase—thereby promoting glycogen synthesis (in response to insulin) or stimulating glycogen breakdown (in response to glucagon/epinephrine) in a highly subtle manner.

Glycogen metabolism is largely controlled by either the insulin/glucagon pair (in the liver), or insulin/epinephrine pair (in the muscle) acting in an antagonistic manner—muscle cells lack glucagon receptor since muscle glycogenolysis plays zero role in the control of blood glucose level!
Exercise 3.3c

- Summarize the effects of AMP and G6P on glycogen phosphorylase and glycogen synthase

- Summarize the effects of phosphorylation and dephosphorylation on glycogen phosphorylase and glycogen synthase

- How does regulation of glycogen metabolism differ between liver and muscle?

- Summarize the effects of insulin, glucagon, and epinephrine on glycogen metabolism