For BioResire students

# Life sciences Material

## Elite Batch

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## Metabolism: Glycolysis and its Role in Cancer (The Warburg Effect)

**Basic Principle**: Glycolysis is the ancient metabolic pathway that breaks down one molecule of glucose (6 carbons) into two molecules of pyruvate (3 carbons). It occurs in the cytoplasm and produces a net gain of 2 ATP molecules and 2 NADH molecules per glucose. Under aerobic conditions, pyruvate enters the mitochondria for the TCA cycle to produce much more ATP.

**Engaging the Basics:**

This is the primary energy-harvesting pathway for many cells and is the first step in cellular respiration. Its regulation is crucial. Key control points include the enzymes hexokinase, phosphofructokinase-1 (PFK-1), and pyruvate kinase, which are inhibited by ATP (feedback inhibition) and activated by AMP.

- **Key Output:** 2 Pyruvate, 4 ATP (net gain of **2 ATP**), 2 NADH.
- **Key Irreversible Enzymes (Regulation Points):**

1. **Hexokinase/Glucokinase:** (Glucose -> Glucose-6-P). *Inhibited by G6P.*
2. **Phosphofructokinase-1 (PFK-1):** (Fructose-6-P -> Fructose-1,6-BP). **The most important regulatory enzyme.**

- **Inhibitors:** ATP, Citrate, low pH.
- **Activators:** AMP, ADP, Fructose-2,6-Bisphosphate (F2,6BP).

3. **Pyruvate Kinase:** (PEP -> Pyruvate). *Inhibited by ATP and Alanine; Activated by F1,6BP.*

**Fate of Pyruvate: The Metabolic Branch Point**

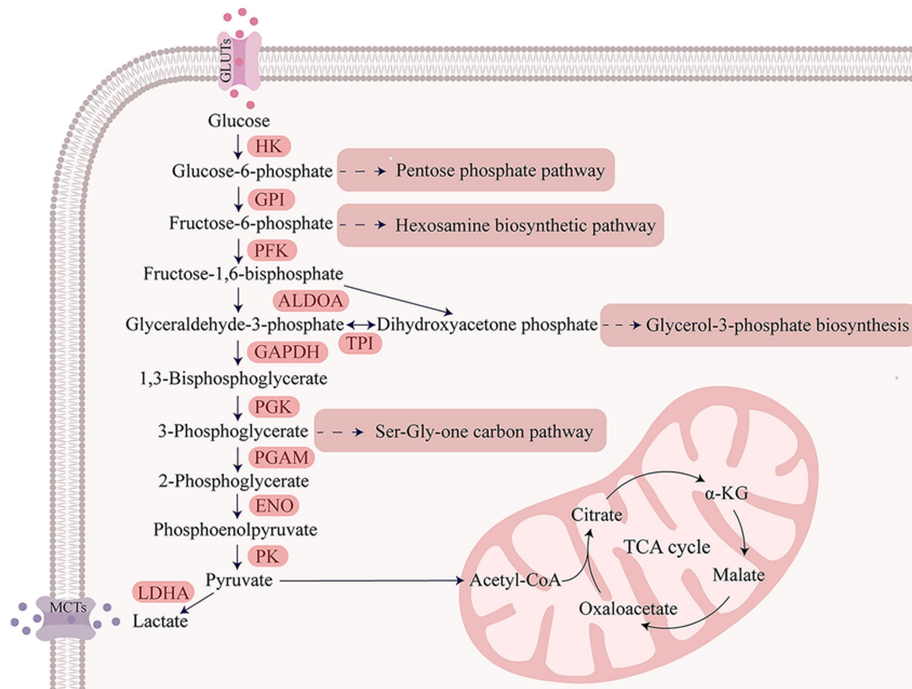
Pyruvate's fate depends on oxygen availability and cellular needs.

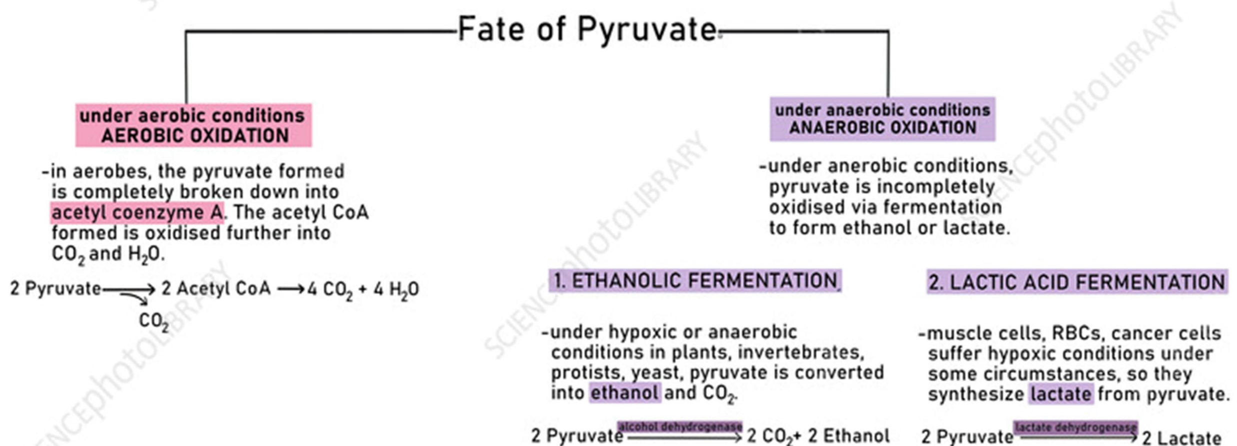
- **Aerobic Conditions:** Pyruvate is transported into the mitochondria.

1. **Pyruvate Dehydrogenase Complex (PDC):** Irreversibly converts Pyruvate to **Acetyl-CoA**. This commits the carbon skeleton to the TCA cycle for complete oxidation.

- **Regulation:** *Inhibited* by its products: NADH, Acetyl-CoA. Also inhibited via phosphorylation by Pyruvate Dehydrogenase Kinase (activated by high NADH/Acetyl-CoA).
- **Anaerobic Conditions (or in lack of mitochondria):**

1. **Lactic Acid Fermentation:** Pyruvate is reduced to **Lactate** by Lactate Dehydrogenase (LDH). This regenerates $NAD^+$ from NADH, allowing glycolysis to continue. *Occurs in skeletal muscle under strenuous exercise and in erythrocytes.*
2. **Alcoholic Fermentation:** In yeast, pyruvate is decarboxylated to acetaldehyde and then reduced to **ethanol**.




**Advanced Research: Metabolic Reprogramming in Cancer**

In the 1920s, Otto Warburg made a puzzling observation: cancer cells often consume massive amounts of glucose and convert pyruvate to lactate even in the presence of oxygen. This "Warburg Effect" or aerobic glycolysis is inefficient for energy production, so why do cancer cells do it?

Modern cancer metabolism research has revealed the answer:

**Biosynthetic Precursors**: The glycolytic intermediates are siphoned off into side pathways (pentose phosphate pathway, serine biosynthesis) to generate nucleotides, amino acids, and lipids—the raw materials to build new cancer cells.

**Rapid ATP Generation**: While inefficient per glucose molecule, glycolysis generates ATP very rapidly to meet the high energy demands of proliferating cells.

**Managing Reactive Oxygen Species (ROS):** Avoiding full oxidative metabolism helps cancer cells manage levels of potentially damaging ROS.

This understanding has led to new research avenues, such as developing tracers like $^{18}$F-FDG for PET imaging (which detects tumors based on their high glucose uptake) and designing drugs that target cancer-specific metabolic enzymes.

**Research Application:** Scientists now study "metabolic reprogramming" as a hallmark of cancer. They use techniques like Metabolic Flux Analysis (MFA) with $^{13}$C-labeled glucose to trace the precise flow of carbon in cancer cells versus normal cells, identifying vulnerabilities for new therapies.

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**The Tricarboxylic Acid (TCA) Cycle: A Comprehensive Guide**

**1. Overview and Location**

- **Other Names:** Krebs Cycle, Citric Acid Cycle.
- **Location: Mitochondrial Matrix** in eukaryotes. Cytoplasm in prokaryotes.
- **Primary Function:** To complete the **oxidative catabolism** of fuel molecules (glucose, fatty acids, amino acids) by producing high-energy electron carriers (**NADH** and **$FADH_2$**) and some GTP, which are used to generate ATP via oxidative phosphorylation.

- **Key Concept:** It is an **amphibolic pathway**—it functions in both catabolism (breakdown) and anabolism (synthesis) by providing precursors for biosynthesis.

**2. Entry Point: Pyruvate to Acetyl-CoA**

Before glucose carbons can enter the TCA cycle, pyruvate from glycolysis must be converted to **Acetyl-CoA**. This is an irreversible link reaction.

**Pyruvate Dehydrogenase Complex (PDC)**

- **Reaction:** Pyruvate + $NAD^+$ + CoA-SH → Acetyl-CoA + NADH + $H^+$ + $CO_2$
- **Location:** Mitochondrial Matrix.
- **Enzyme Complex:** A multi-enzyme complex comprising three enzymes:
    1. **Pyruvate Dehydrogenase (E1)**
    2. **Dihydrolipoyl Transacetylase (E2)**
    3. **Dihydrolipoyl Dehydrogenase (E3)**
- **Cofactors Required: Thiamine Pyrophosphate (TPP)** (on E1), **Lipoic Acid** (on E2), **Coenzyme A (CoA-SH)**, **FAD** (on E3), **$NAD^+$**.
- **Regulation:** This is a **highly regulated**, irreversible step.
    - **Inhibited by:** High levels of **ATP, Acetyl-CoA, NADH** (end-products of the cycle). Also inhibited by phosphorylation of the E1 subunit by **Pyruvate Dehydrogenase Kinase (PDK)**.
    - **Activated by:** Dephosphorylation by **Pyruvate Dehydrogenase Phosphatase (PDP)**. High levels of **AMP, $Ca^{2+}$, $NAD^+$** signal energy need and stimulate the complex.

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Citric acid cycle

Acetyl-CoA

$CH_3-C(=O)-S\text{-}CoA$

❶ **Claisen condensation**: methyl group of acetyl-CoA converted to methylene in citrate.

citrate synthase

$H_2O$ CoA-SH

Citrate

$CH_2-COO^-$
$HO-C-COO^-$
$CH_2-COO^-$

❷a **Dehydration/rehydration**: —OH group of citrate repositioned in isocitrate to set up decarboxylation in next step.

aconitase

$H_2O$

*cis*-Aconitate

$CH_2-COO^-$
$C-COO^-$
$C-COO^-$
H

$H_2O$

aconitase

❷b **(Rehydration)**

Isocitrate

$CH_2-COO^-$
$H-C-COO^-$
$HO-C-H$
$COO^-$

❸ **Oxidative decarboxylation**: —OH group oxidized to carbonyl, which in turn facilitates decarboxylation by stabilizing carbanion formed on adjacent carbon.

isocitrate dehydrogenase

$CO_2$

α-Ketoglutarate

$CH_2-COO^-$
$CH_2$
$C=O$
$COO^-$

α-ketoglutarate dehydrogenase complex

CoA-SH $CO_2$

❹ **Oxidative decarboxylation**: pyruvate-dehydrogenase-like mechanism; dependent on carbonyl on adjacent carbon.

Succinyl-CoA

$CH_2-COO^-$
$CH_2$
$C(=O)-S\text{-}CoA$

succinyl-CoA synthetase

GDP (ADP) + $P_i$

GTP (ATP)

CoA-SH

❺ **Substrate-level phosphorylation**: energy of thioester conserved in phosphoanhydride bond of GTP or ATP.

Succinate

$CH_2-COO^-$
$CH_2$
$COO^-$

succinate dehydrogenase

$FADH_2$

❻ **Dehydrogenation**: introduction of double bond initiates methylene oxidation sequence.

Fumarate

$COO^-$
CH
HC
$COO^-$

fumarase

$H_2O$

Malate

$COO^-$
$HO-CH$
$CH_2$
$COO^-$

❼ **Hydration:** addition of water across double bond introduces —OH group for next oxidation step.

malate dehydrogenase

Oxaloacetate

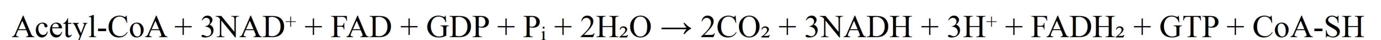
$O=C-COO^-$
$CH_2-COO^-$

❽ **Dehydrogenation**: oxidation of —OH completes oxidation sequence; generates carbonyl positioned to facilitate Claisen condensation in next step.

(3) NADH

**Net Reaction and Energy Yield per Acetyl-CoA**

**Net Reaction:**



Acetyl-CoA + $3NAD^+$ + FAD + GDP + $P_i$ + $2H_2O$ → $2CO_2$ + 3NADH + $3H^+$ + $FADH_2$ + GTP + CoA-SH

**Energy Yield per Acetyl-CoA (One Turn of Cycle):**

- 3 NADH
- 1 $FADH_2$
- 1 GTP (equivalent to 1 ATP)

**Energy Yield per Glucose Molecule:**
One glucose produces **two Acetyl-CoA** molecules.

- **Direct ATP yield:** 2 GTP → **2 ATP**
- **Reducing equivalents:** 6 NADH, 2 $FADH_2$
- These reducing equivalents are used in oxidative phosphorylation to produce:
  - NADH → ~2.5 ATP each → 6 NADH ≈ **15 ATP**
  - $FADH_2$ → ~1.5 ATP each → 2 $FADH_2$ ≈ **3 ATP**
- **Total ATP from TCA cycle (per glucose): ~20 ATP**
- **Grand Total (Glycolysis + Link + TCA + OXPHOS):** ~30-32 ATP/glucose.

## Regulation of the TCA Cycle

The cycle is regulated at three irreversible, exergonic steps to match cellular energy needs.

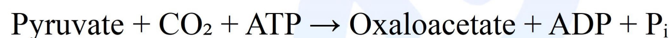
| Enzyme | Activators | Inhibitors | Rationale |
|---|---|---|---|
| Pyruvate Dehydrogenase Complex (PDC) | AMP, $Ca^{2+}$, $NAD^+$, CoA | ATP, Acetyl-CoA, NADH | High energy charge (ATP/NADH) slows fuel entry into the cycle. $Ca^{2+}$ signals muscle contraction, increasing energy demand. |
| Citrate Synthase | --- | ATP, NADH, Succinyl-CoA, Citrate | High levels of downstream products and energy signal indicate sufficient cycle activity. |

| Enzyme | Activators | Inhibitors | Rationale |
|---|---|---|---|
| Isocitrate Dehydrogenase | ADP, $Ca^{2+}$ | ATP, NADH | Key regulator. ADP signals low energy, activating the cycle's first oxidation step. |
| α-Ketoglutarate Dehydrogenase Complex | $Ca^{2+}$ | Succinyl-CoA, NADH | |

## Anaplerotic Reactions (Replenishing Cycle Intermediates

TCA cycle intermediates are constantly siphoned off for biosynthesis (e.g., oxaloacetate for gluconeogenesis, α-ketoglutarate for amino acid synthesis). **Anaplerotic reactions** replenish these intermediates.

**Most Important Anaplerotic Reaction:**



Pyruvate + $CO_2$ + ATP → Oxaloacetate + ADP + $P_i$

- **Enzyme: Pyruvate Carboxylase**
- **Activated by Acetyl-CoA.** This is crucial: if Acetyl-CoA accumulates (meaning the cycle is slow), it stimulates pyruvate carboxylase to make more oxaloacetate, "priming" the cycle to accept more acetyl groups.
- Other anaplerotic reactions involve amino acids (e.g., glutamate -> α-ketoglutarate via transamination).

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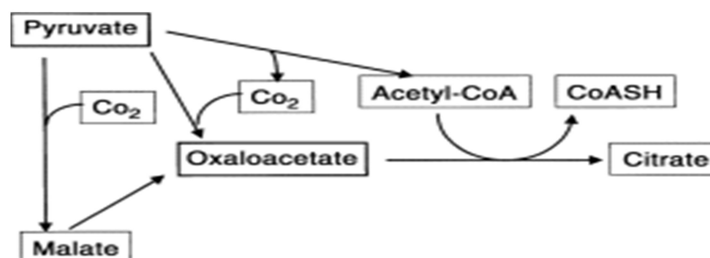


## OXIDATIVE PHOSPHORYLATION: A CSIR NET PERSPECTIVE

Oxidative Phosphorylation (OXPHOS) is the process where the energy stored in the reduced coenzymes **NADH** and **$FADH_2$** (from glycolysis, the TCA cycle, and β-oxidation) is used to synthesize **ATP**. It consists of two tightly coupled processes: the **Electron Transport Chain (ETC)**, which creates a proton gradient, and **Chemiosmosis**, which uses that gradient to power ATP synthesis.

### 1. Location and Overview

- **Location: Inner Mitochondrial Membrane** in eukaryotes. The cristae (folds) maximize surface area for this process.
- **Overall Reaction:**
  $NADH + H^+ + \frac{1}{2} O_2 + {\sim}2.5\ ADP + {\sim}2.5\ P_i \rightarrow NAD^+ + H_2O + {\sim}2.5\ ATP$
  $FADH_2 + \frac{1}{2} O_2 + {\sim}1.5\ ADP + {\sim}1.5\ P_i \rightarrow FAD + H_2O + {\sim}1.5\ ATP$
- **Key Principle:** The energy from electron transfer is used to pump protons (**$H^+$**), creating an **electrochemical proton gradient** across the inner membrane. The energy stored in this gradient (the **proton-motive force**) drives ATP synthesis.

### 2. The Electron Transport Chain (ETC)

The ETC is a series of protein complexes (I-IV) and mobile electron carriers embedded in the inner mitochondrial membrane. Electrons flow from higher energy (NADH) to lower energy ($O_2$), and this exergonic flow is coupled to the pumping of protons.

**Mobile Electron Carriers:**

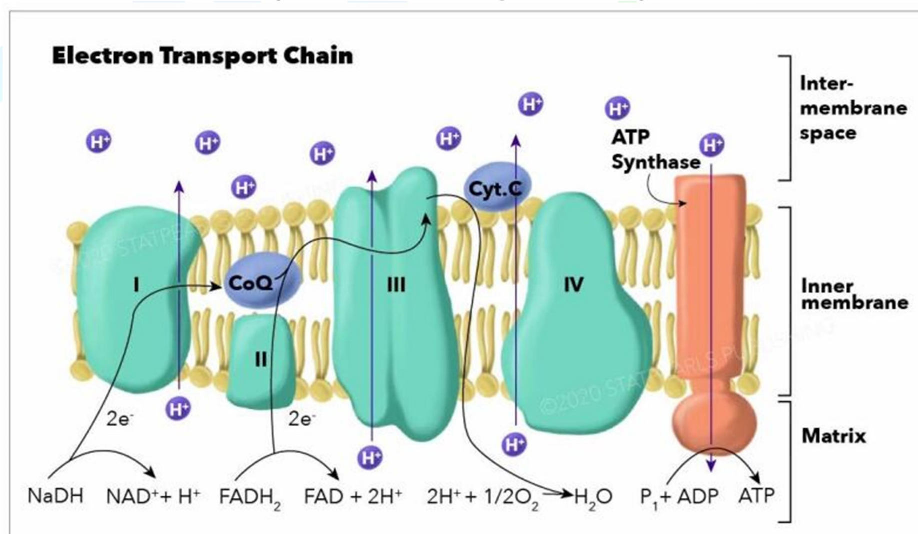
- **Ubiquinone (Coenzyme Q, Q):** Lipid-soluble, shuttles $e^-$ between Complex I/II and Complex III.
- **Cytochrome c (Cyt c):** Water-soluble, shuttles $e^-$ between Complex III and Complex IV.

**Two Entry Points:**

1. **High-energy entry:** Via **Complex I** from **NADH**.

2. **Low-energy entry:** Via **Complex II** from **$FADH_2$** (from succinate in TCA cycle). This bypasses the first proton-pumping site, which is why $FADH_2$ yields less ATP.

| Complex | Name | No. of Proteins | Prosthetic Groups |
|---|---|---|---|
| Complex I | NADH Dehydrogenase | 46 | FMN, 9 Fe-S cntrs. |
| Complex II | Succinate-CoQ Reductase | 5 | FAD, cyt $b_{560}$, 3 Fe-S cntrs. |
| Complex III | CoQ-cyt c Reductase | 11 | cyt $b_H$, cyt $b_L$, cyt $c_1$, Fe-$S_{Rieske}$ |
| Complex IV | Cytochrome Oxidase | 13 | cyt a, cyt $a_3$, $Cu_A$, $Cu_B$ |



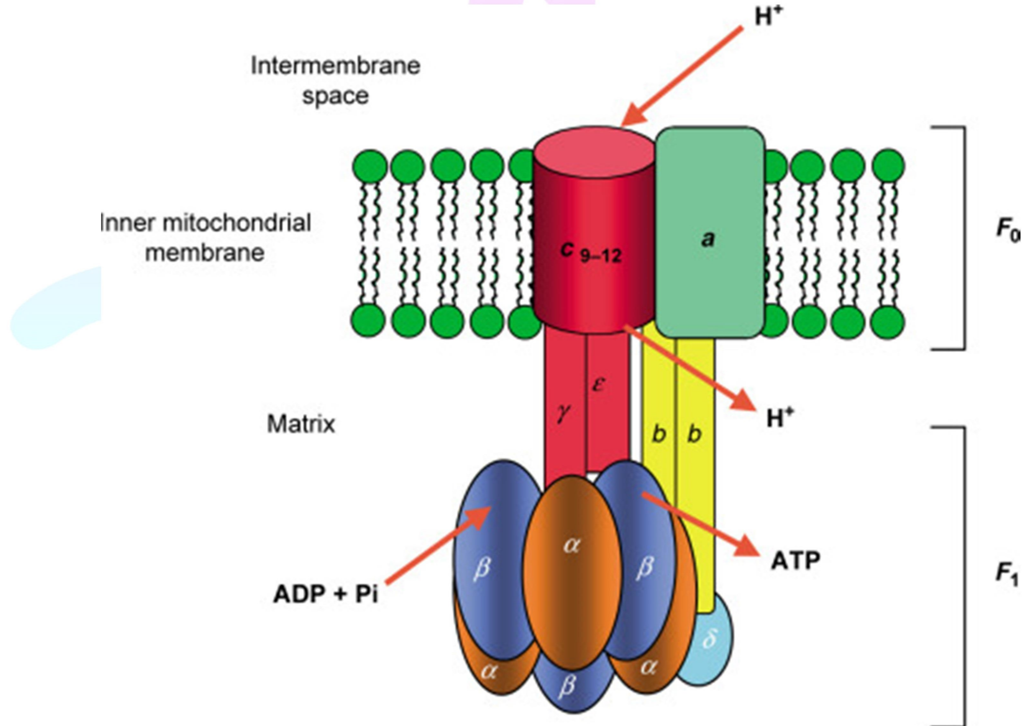

**Chemiosmosis and ATP Synthase (Complex V)**

- **Chemiosmotic Theory (Peter Mitchell, Nobel Prize 1978):** The ETC creates a **proton gradient** across the inner mitochondrial membrane. This gradient has two components:
    1. **Chemical Gradient (ΔpH):** Higher $[H^+]$ in the intermembrane space.

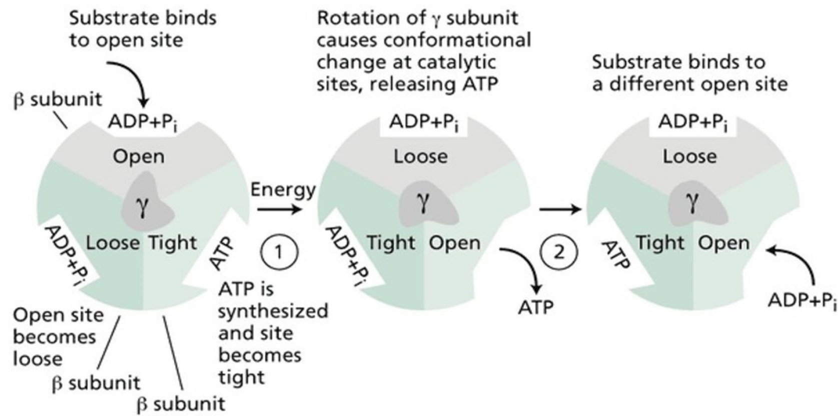
2. **Electrical Gradient (ΔΨ):** More positive charge in the intermembrane space.

- Together, these form the **Proton-Motive Force (PMF)**.

- **ATP Synthase (Complex V or $F_0F_1$ Complex):** This is a molecular motor that uses the energy of the PMF to synthesize ATP.
  - **$F_0$ Component:** Integral membrane protein complex that forms a proton channel. As protons flow down their gradient through $F_0$, it rotates.
  - **$F_1$ Component:** Peripheral membrane protein complex (in the matrix) where ATP synthesis occurs. The rotation of the $F_0$ "rotor" drives conformational changes in the $F_1$ "knob" that catalyze the formation of ATP from ADP and $P_i$.




  - **Mechanism: Binding Change Mechanism.** The three active sites of $F_1$ cycle through three conformations:
    1. **Open (O)** - low affinity, releases ATP.
    2. **Loose (L)** - binds ADP + $P_i$.
    3. **Tight (T)** - catalyzes ATP formation.




- **Stoichiometry:** It is estimated that the flow of **~4 $H^+$** is required to synthesize **1 ATP** molecule (this includes 1 $H^+$ for Pi transport into the matrix).

**P/O Ratio and ATP Yield**

The **P/O Ratio** is the number of ATP molecules synthesized per atom of oxygen reduced. It depends on the entry point of electrons.

- **For NADH:** Electrons enter at Complex I and pass through 3 proton-pumping sites (I, III, IV).
  - ~10 $H^+$ are pumped per NADH.
  - With ~4 $H^+$ needed per ATP, P/O ratio ≈ **10/4 = 2.5**
- **For $FADH_2$:** Electrons enter at Complex II and pass through 2 proton-pumping sites (III, IV).
  - ~6 $H^+$ are pumped per $FADH_2$.
  - P/O ratio ≈ **6/4 = 1.5**

**Regulation and Inhibitors**

OXPHOS is regulated by cellular energy demand: [ADP] is the key regulator. This is called Acceptor Control. High [ADP] stimulates respiration and ATP synthesis, while high [ATP] inhibits it.

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| Name | Function | Site of Action |
|---|---|---|
| Rotenone | $e^-$ transport inhibitor | Complex I |
| Amytal | $e^-$ transport inhibitor | Complex I |
| Antimycin A | $e^-$ transport inhibitor | Complex III |
| Cyanide | $e^-$ transport inhibitor | Complex IV |
| Carbon monoxide | $e^-$ transport inhibitor | Complex IV |
| Azide | $e^-$ transport inhibitor | Complex IV |
| 2,4, -Dinitrophenol | Uncoupling agent | Transmembrane $H^+$ carrier |
| Pentachloro-phenol | Uncoupling agent | Transmembrane $H^+$ carrier |
| Oligomycin | Inhibits ATP synthase | OSCP fraction of ATP synthase |

**GLUCONEOGENESIS: A CSIR NET PERSPECTIVE**

Gluconeogenesis (GNG) is a universal metabolic pathway that synthesizes **new glucose** from non-carbohydrate precursors. It is essential for maintaining blood glucose levels during fasting, starvation, or intense exercise when liver glycogen stores are depleted.

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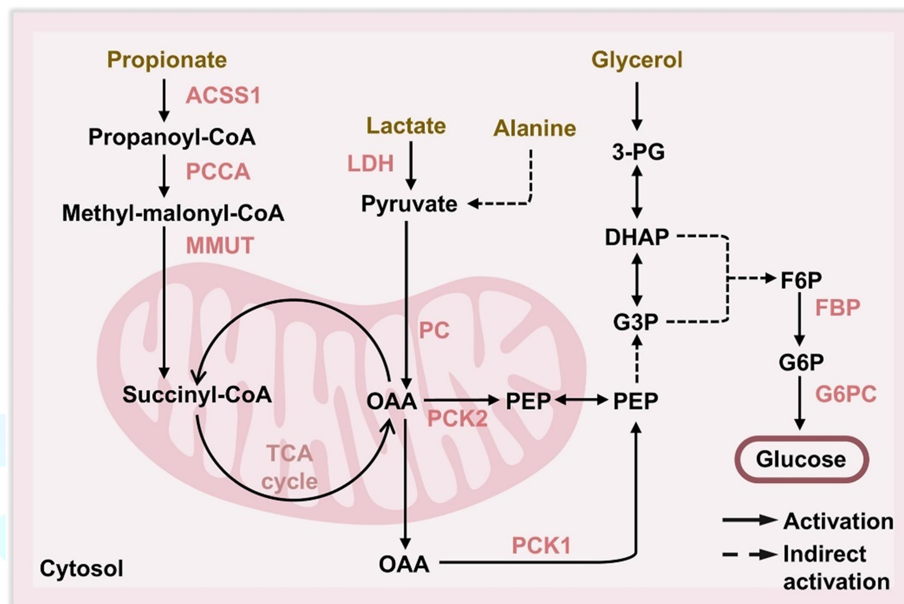
**1. Overview and Physiological Necessity**

- **Definition:** The *de novo* synthesis of glucose from non-hexose precursors.
- **Primary Location: Liver** (~90%), **Kidney Cortex** (~10%). The enzymes are located in the cytoplasm, mitochondria, and endoplasmic reticulum.
- **Why is it vital?** The human brain, erythrocytes, and renal medulla have an **absolute requirement for glucose** as their primary energy source. GNG ensures a continuous supply.
- **Precursors for GNG:** Lactate, glucogenic amino acids (especially alanine), glycerol, and all TCA cycle intermediates (e.g., oxaloacetate, α-ketoglutarate).

- **Note:** Fatty acids (with even-numbered chains) **cannot** be net precursors for glucose because their beta-oxidation produces only acetyl-CoA, which is irreversibly committed to the TCA cycle and cannot be converted back to pyruvate.

**2. The Pathway: A Bypass of Irreversible Glycolytic Steps**

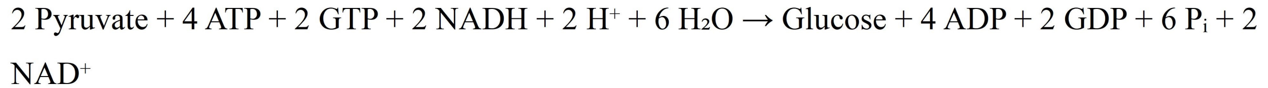
Gluconeogenesis is largely a reversal of glycolysis. However, the three irreversible steps of glycolysis (catalyzed by hexokinase, PFK-1, and pyruvate kinase) must be bypassed by four specific gluconeogenic enzymes.




| Irreversible steps in glycolysis | Corresponding key gluconeogenic enzymes |
|---|---|
| Pyruvate kinase (Step 9) | Pyruvate carboxylase; Phosphoenol pyruvate-carboxy kinase |
| Phosphofructokinase (Step 3) | Fructose-1,6-bisphosphatase |
| Hexokinase (Step 1) | Glucose-6-phosphatase |

**The Cost of Gluconeogenesis**

Synthesizing glucose is energetically expensive. The net reaction to make one glucose from two pyruvate molecules is:

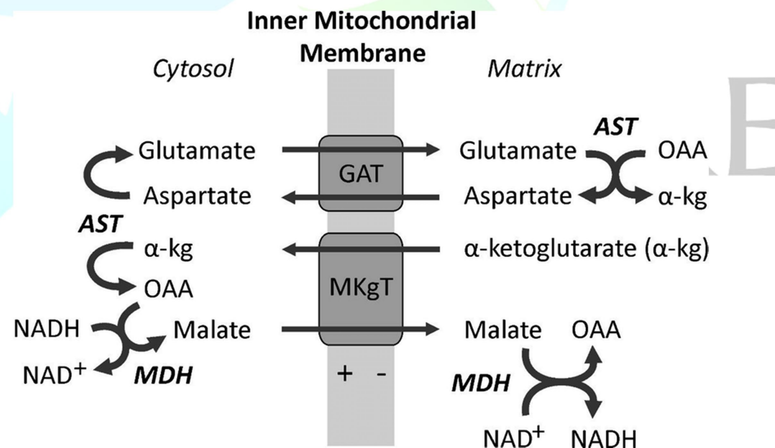


2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 $H^+$ + 6 $H_2O$ → Glucose + 4 ADP + 2 GDP + 6 $P_i$ + 2 $NAD^+$

- **Net ATP consumed per glucose synthesized: 4 ATP + 2 GTP = 6 ATP equivalents.**
- This high cost is necessary to bypass the energetically favorable steps of glycolysis and is why the pathway is tightly regulated to only run when absolutely necessary.

**4. Unique Mechanisms and Substrate Shuttles**

- **The Mitochondrial Problem:** Oxaloacetate (OAA), produced by pyruvate carboxylase in the mitochondria, cannot cross the inner mitochondrial membrane.
- **The Malate-Aspartate Shuttle (for Lactate/Pyruvate):** This is the primary shuttle.
    1. In the mitochondria, OAA is reduced to **malate** (using NADH).
    2. Malate crosses the membrane into the cytosol.
    3. In the cytosol, malate is oxidized back to OAA (generating NADH), which is then converted to PEP by PEPCK




  - **Net Effect:** This shuttle also transfers **reducing equivalents (NADH)** from the mitochondria to the cytosol, which are needed for the GAPDH step of gluconeogenesis.

**Regulation of Gluconeogenesis**

Gluconeogenesis is regulated reciprocally with glycolysis to prevent a futile cycle (where both pathways run simultaneously, consuming ATP but producing nothing).

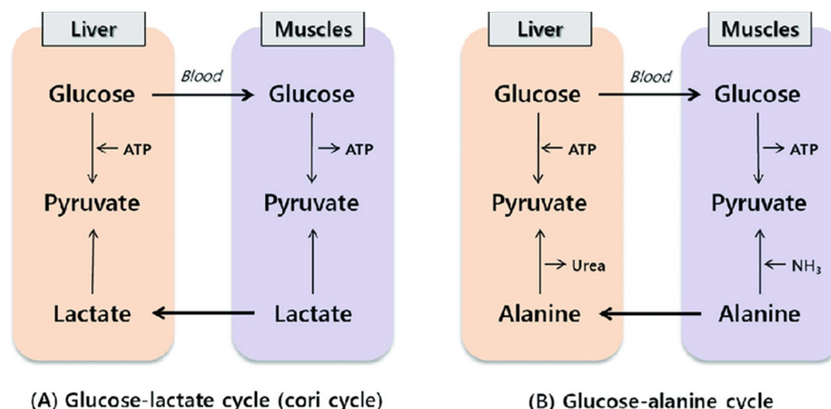
**Regulators of gluconeogenic enzyme activity**

| Enzyme | Allosteric Inhibitors | Allosteric Activators | Enzyme Phosphorylation | Protein Synthesis |
|---|---|---|---|---|
| PFK | ATP, citrate | AMP, F2-6P | | |
| FBPase | AMP, F2-6P | | | |
| PK | Alanine | F1-6P | Inactivates | |
| Pyr. Carb. | | AcetylCoA | | |
| PEPCK | | | | Glucogon |
| PFK-2 | Citrate | AMP, F6P, Pi | Inactivates | |
| FBPase-2 | F6P | Glycerol-3-P | Activates | |

**The Cori and Glucose-Alanine Cycles**

These cycles connect peripheral tissues to the liver.

- **Cori Cycle:** Lactate produced by anaerobic glycolysis in muscles and RBCs is transported to the liver. The liver uses gluconeogenesis to convert it back to glucose, which is then released back into the blood for the muscles to use.
    - **Net Energy Cost:** 6 ATP per glucose cycled. This cost is borne by the liver to recycle energy for anaerobic tissues.
- **Glucose-Alanine Cycle:** In muscles, pyruvate is transaminated to **alanine**. Alanine is transported to the liver, where it is converted back to pyruvate (for GNG) and the amino group is disposed of via the urea cycle.



(A) Glucose-lactate cycle (cori cycle) (B) Glucose-alanine cycle

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## GLYCOGEN METABOLISM: A CSIR NET PERSPECTIVE

Glycogen is a highly branched polymer of glucose that serves as the primary medium-term energy storage molecule in animals. Its synthesis (glycogenesis) and breakdown (glycogenolysis) are highly regulated to maintain blood glucose homeostasis.

### 1. Glycogenesis (Glycogen Synthesis)

#### A. Overview:

- **Goal:** To synthesize glycogen from glucose for storage.
- **Primary Tissues: Liver** (for maintaining blood glucose) and **Skeletal Muscle** (for its own energy needs).
- **Cellular Location:** Cytoplasm.

#### B. The Step-by-Step Process:

1. **Glucose Phosphorylation:**
    - Glucose + ATP -> Glucose-6-P + ADP
    - **Enzyme: Glucokinase** (in liver) or **Hexokinase** (in muscle).
    - This traps glucose inside the cell.
2. **Isomerization to Glucose-1-Phosphate:**
    - Glucose-6-P ⇌ Glucose-1-P
    - **Enzyme: Phosphoglucomutase.**
3. **Activation of Glucose:**
    - Glucose-1-P + UTP -> UDP-Glucose + $PP_i$
    - **Enzyme: UDP-Glucose Pyrophosphorylase.**
    - The reaction is driven forward by the subsequent hydrolysis of $PP_i$ to $2P_i$.
    - **UDP-Glucose** is the activated, direct precursor for glycogen synthesis.

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4. **Glycogen Chain Elongation:**
   - UDP-Glucose + $Glycogen_n$ (primer) -> $Glycogen_{n+1}$ + UDP
   - **Enzyme: Glycogen Synthase.** This is the **key regulated enzyme** of glycogenesis.
   - It catalyzes the formation of **α-1,4-glycosidic linkages**, adding glucose units to the non-reducing ends of an existing glycogen chain.
5. **Branching:**
   - **Enzyme: Branching Enzyme (Glycosyl (4:6) Transferase).**
   - It breaks an existing α-1,4 chain (at least 11 glucose units long) and transfers a block of ~6-8 glucose units to an internal location.
   - It then reattaches this block via an **α-1,6-glycosidic linkage**, creating a new branch point.
   - **Why Branch?** Branching increases the solubility of glycogen and creates more non-reducing ends, dramatically increasing the rate of both synthesis and degradation.

**2. Glycogenolysis (Glycogen Breakdown)**

**A. Overview:**

- **Goal:** To mobilize glucose from stored glycogen.
- **Primary Tissues: Liver** (releases glucose into the blood) and **Skeletal Muscle** (retains glucose-6-P for glycolysis).
- **Cellular Location:** Cytoplasm.

**B. The Step-by-Step Process:**

1. **Shortening of Chains (Phosphorolysis):**
   - $Glycogen_n$ + $P_i$ -> Glucose-1-P + $Glycogen_{n-1}$

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- **Enzyme: Glycogen Phosphorylase.** This is the **key regulated enzyme** of glycogenolysis.
- It sequentially cleaves glucose units from the **non-reducing ends** of the glycogen chain using inorganic phosphate ($P_i$). This direct use of $P_i$ produces **Glucose-1-Phosphate** without expending ATP.
- **It stops 4 glucose units away from a branch point.**

2. **Debranching:**
   - This requires a **bifunctional debranching enzyme** with two activities:
     - **Oligo (α1→4) to (α1→4) glucan transferase:** Transfers a block of 3 glucose units from the limit branch to the end of another chain, exposing the α-1,6-linked branch point glucose.
     - **Amylo-α-1,6-glucosidase:** Hydrolyzes the α-1,6-glycosidic bond, releasing a single **free glucose** molecule.
3. **Isomerization:**
   - Glucose-1-P ⇌ Glucose-6-P
   - **Enzyme: Phosphoglucomutase.**
4. **Fate of Glucose-6-Phosphate:**
   - **In Liver and Kidney:** Contains **Glucose-6-Phosphatase**. It hydrolyzes G6P to free glucose, which can be released into the bloodstream.
     Glucose-6-P + $H_2O$ -> Glucose + $P_i$
   - **In Muscle:** Lacks Glucose-6-Phosphatase. G6P is committed to **local glycolysis** to generate ATP for muscle contraction.

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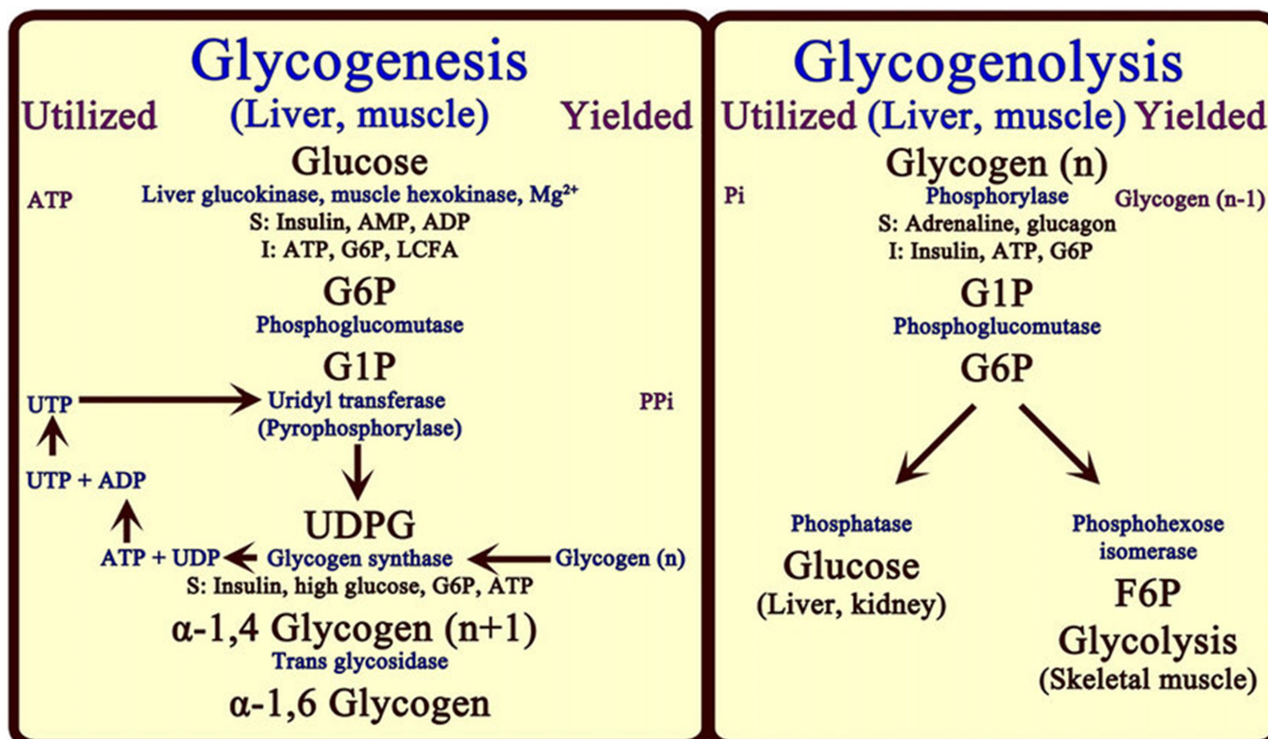


**3. Regulation: A Tale of Reciprocal Control**

Glycogenesis and Glycogenolysis are regulated **reciprocally** to prevent a futile cycle. The primary regulators are the hormones **Insulin** (fed state) and **Glucagon/Epinephrine** (fasting/stress state), which act on the key enzymes through phosphorylation/dephosphorylation.

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**PENTOSE PHOSPHATE PATHWAY (PPP): A CSIR NET PERSPECTIVE**

The Pentose Phosphate Pathway is a fundamental metabolic pathway that runs parallel to glycolysis. Its primary role is **anabolic**, not energy production. It is essential for generating reducing power and building blocks for biosynthesis.

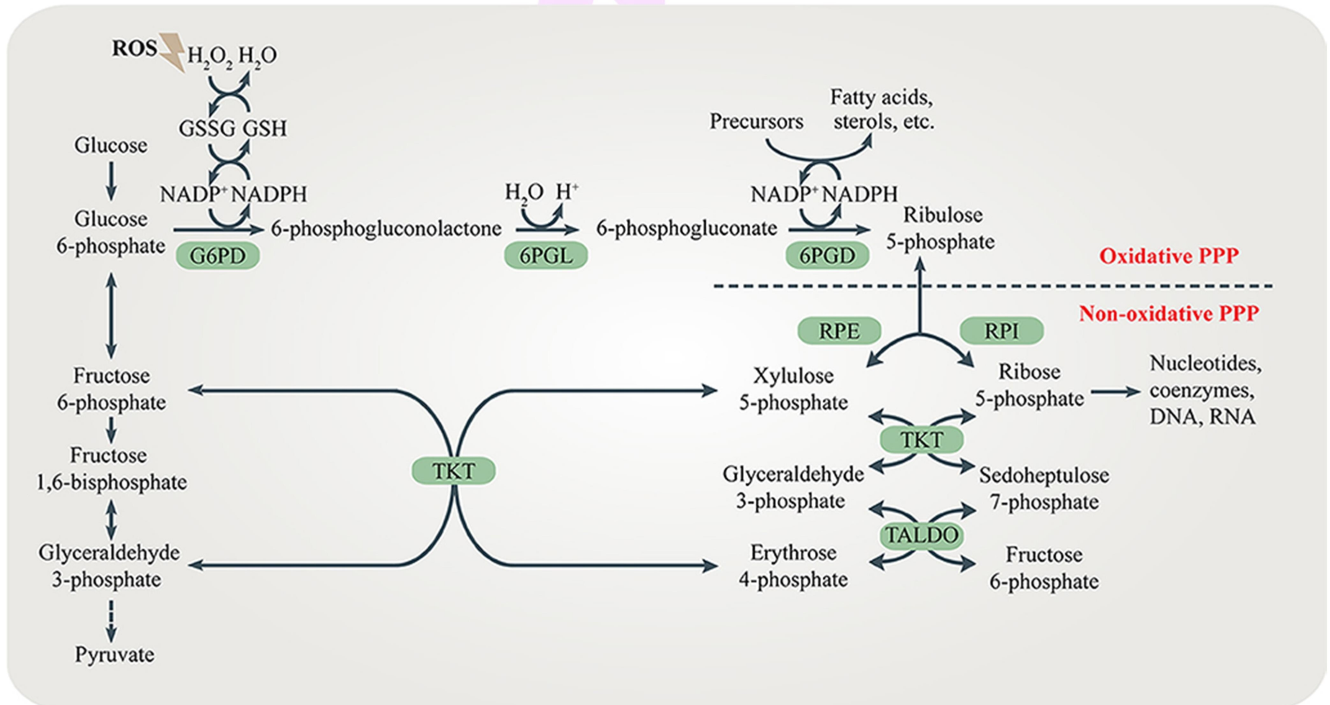
**1. Overview and Physiological Significance**

- **Location: Cytosol** of most cells, but is particularly active in tissues involved in **lipogenesis** (liver, adipose, mammary gland) and **nucleic acid synthesis** (rapidly dividing cells).
- **Key Functions:**

1. **To generate NADPH:** For reductive biosynthesis (e.g., fatty acids, cholesterol) and combating oxidative stress.
2. **To produce Ribose-5-Phosphate (R5P):** A crucial precursor for the synthesis of nucleotides (ATP, GTP, UTP, CTP) and nucleic acids (DNA, RNA).
3. **To provide metabolic flexibility:** It interconverts sugars of different carbon lengths (C3, C4, C5, C6, C7), allowing the cell to adapt to different needs.

### 2. The Two Phases of the PPP

The pathway operates in two distinct phases: the **Oxidative Phase** (irreversible) and the **Non-Oxidative Phase** (reversible).




The key enzymes and transactions are:

- **Isomerization:**
    - Ribulose-5-P ⇌ Ribose-5-P (Enzyme: **Ribose-5-Phosphate Isomerase**)
    - Ribulose-5-P ⇌ Xylulose-5-P (Enzyme: **Phosphopentose Epimerase**)
- **Carbon-Carbon Bond Transfers:** Catalyzed by **Transketolase** and **Transaldolase**.

- **Transketolase:** Transfers a 2-carbon unit (requires **Thiamine Pyrophosphate (TPP)** as a cofactor).
    - e.g., Xylulose-5-P + Ribose-5-P ⇌ Glyceraldehyde-3-P + Sedoheptulose-7-P
- **Transaldolase:** Transfers a 3-carbon unit.
    - e.g., Sedoheptulose-7-P + Glyceraldehyde-3-P ⇌ Erythrose-4-P + Fructose-6-P
- Another Transketolase reaction can then use Erythrose-4-P:
    - Xylulose-5-P + Erythrose-4-P ⇌ Glyceraldehyde-3-P + Fructose-6-P

**Net Result of Non-Oxidative Phase:**
The pathway can be balanced to produce different outputs based on the cell's relative need for NADPH, R5P, and ATP.

---

### 3. Modes of Operation (The Key to Understanding PPP)

The PPP is remarkably flexible. The fate of Glucose-6-P depends on the cell's needs, controlled by the relative activities of G6PD and the non-oxidative phase enzymes.

| Cellular Need | Pathway Emphasis | Net Reaction (per 3 G6P molecules) | Outcome |
|---|---|---|---|
| **1. More NADPH than R5P** (e.g., in lipogenic cells) | **Oxidative Phase + Recycling** | 3 G6P + $6NADP^+$ → 2 F6P + 1 GAP + 6NADPH + $3CO_2$ + $3H^+$ | Maximum **NADPH** production. Pentoses are recycled to glycolytic intermediates. |
| **2. More R5P than NADPH** (e.g., in | **Mostly Non-** | 3 F6P + 2 GAP + ATP → 3 R5P + 2 | **R5P** is made from glycolytic intermediates **without producing** |

| Cellular Need | Pathway Emphasis | Net Reaction (per 3 G6P molecules) | Outcome |
|---|---|---|---|
| dividing cells) | **Oxidative Phase** | E4P + ADP + $P_i$ | **NADPH**. |
| **3. Balanced need for both** | **Oxidative Phase only** | 3 G6P + $3NADP^+$ → 3 R5P + 3NADPH + $3CO_2$ + $3H^+$ | Direct production of **R5P** and **NADPH** in a 1:1 ratio. |

---

**4. Regulation of the PPP**

The PPP is primarily regulated at the first step to control the flux of glucose-6-P down this pathway versus glycolysis.

- **Primary Regulation: Glucose-6-Phosphate Dehydrogenase (G6PD)**
  - **The main regulator is the level of $NADP^+$/NADPH.**
  - **$NADP^+$** is a strong **activator**. High [$NADP^+$] signals a need for reducing power.
  - **NADPH** is a strong **competitive inhibitor**. High [NADPH] signals that the cell's reductive needs are met, shutting down the oxidative phase.
- **Hormonal and Transcriptional Control:**
  - **Insulin** induces the synthesis of G6PD and other lipogenic enzymes, increasing PPP flux to support fat synthesis.

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**5. Clinical Correlation: G6PD Deficiency**

- **Most common human enzyme deficiency,** affecting millions worldwide. It is an X-linked recessive disorder.

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- **Pathophysiology:** The deficient enzyme cannot regenerate sufficient **NADPH** to maintain reduced **Glutathione (GSH)** levels. GSH is a critical antioxidant that protects cells from reactive oxygen species (ROS).
- **Trigger:** Exposure to oxidative stressors (e.g., certain drugs like primaquine, antimalarials; fava beans; infections) causes a hemolytic crisis in red blood cells.
- **Why RBCs?** Mature RBCs have no nucleus or mitochondria. The PPP is their **only source of NADPH** to combat oxidative stress. Without it, hemoglobin oxidizes, denatures, and forms Heinz bodies, leading to RBC lysis (hemolysis).

## LIPID METABOLISM: A CSIR NET PERSPECTIVE

Lipid metabolism encompasses the breakdown (catabolism) and synthesis (anabolism) of various lipids, primarily focused on fatty acids and cholesterol. Its primary functions are energy storage, membrane structure, and signaling.

### I. Digestion & Absorption of Dietary Lipids

- **Site:** Small intestine.
- **Process:**
    1. **Emulsification:** Bile salts (from liver) break large fat globules into smaller droplets, increasing surface area.
    2. **Enzymatic Digestion: Pancreatic lipase** hydrolyzes triacylglycerols (TAGs) into monoacylglycerols and free fatty acids.
    3. **Micelle Formation:** Products + bile salts form mixed micelles for absorption by intestinal epithelial cells.
    4. **Resynthesis & Export:** Inside enterocytes, TAGs are resynthesized and packaged with cholesterol and apolipoproteins into **chylomicrons**.
    5. **Transport:** Chylomicrons enter the **lymphatic system** (lacteals) before reaching the bloodstream

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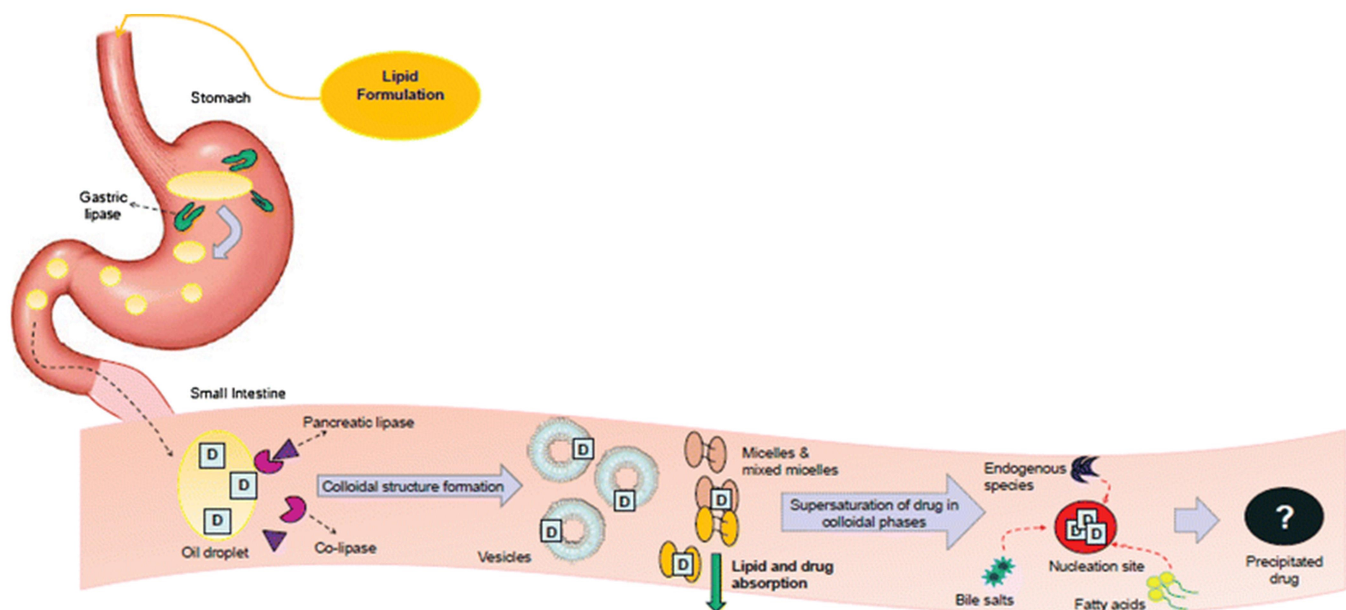
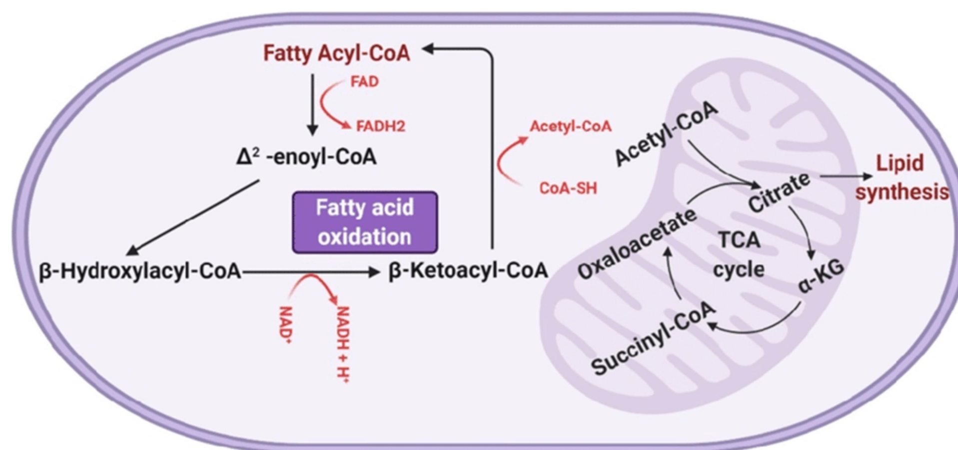



## II. Fatty Acid Catabolism (β-Oxidation)

This is the primary process for breaking down fatty acids to generate energy (ATP).

- **Location:** Mitochondrial Matrix.
- **Activation (Cytosol):** Before oxidation, a fatty acid must be activated.
  - Fatty Acid + CoA-SH + ATP → Acyl-CoA + AMP + $PP_i$
  - **Enzyme: Acyl-CoA Synthetase (Thiokinase).** This step consumes the equivalent of **2 ATP** (as AMP is equivalent to ATP → ADP → AMP).



- **Carnitine Shuttle:** Long-chain Acyl-CoA cannot freely diffuse into mitochondria. The carnitine shuttle transports it across the inner membrane.
  1. **CPT-I (Carnitine Palmitoyl Transferase I):** Exchanges carnitine for CoA on the fatty acid, forming **Acyl-carnitine**. (**CPT-I is a key regulatory enzyme** – inhibited by Malonyl-CoA).
  2. **Translocase:** Shuttles Acyl-carnitine across the inner membrane.
  3. **CPT-II:** On the matrix side, converts Acyl-carnitine back to Acyl-CoA.
- **β-Oxidation Spiral (Four Repeating Steps):** Each cycle shortens the fatty acid by two carbons, producing 1 Acetyl-CoA, 1 NADH, and 1 $FADH_2$.
  1. **Oxidation:** Acyl-CoA -> trans-$\Delta^2$-Enoyl-CoA. *Enzyme: Acyl-CoA Dehydrogenase* (Produces **$FADH_2$**).
  2. **Hydration:** trans-$\Delta^2$-Enoyl-CoA -> L-3-Hydroxyacyl-CoA. *Enzyme: Enoyl-CoA Hydratase*.
  3. **Oxidation:** L-3-Hydroxyacyl-CoA -> 3-Ketoacyl-CoA. *Enzyme: L-3-Hydroxyacyl-CoA Dehydrogenase* (Produces **NADH**).
  4. **Thiolysis:** 3-Ketoacyl-CoA -> Acetyl-CoA + Acyl-CoA (shortened by 2C). *Enzyme: β-Ketothiolase*.
- **Energy Yield (Palmitate, C16:0):**
  - Number of cycles: (n/2) - 1 = 7 cycles
  - Products per cycle: 1 $FADH_2$, 1 NADH, 1 Acetyl-CoA
  - Total Products: **7 $FADH_2$, 7 NADH, 8 Acetyl-CoA**
  - ATP from OXPHOS: (7 $FADH_2$ × 1.5) + (7 NADH × 2.5) = **10.5 + 17.5 = 28 ATP**
  - ATP from TCA: 8 Acetyl-CoA × 10 ATP/Acetyl-CoA = **80 ATP**
  - **Gross ATP: 108 ATP**
  - **Net ATP: 108 - 2 (activation cost) = 106 ATP**

### III. Fatty Acid Anabolism (De Novo Synthesis)

This pathway synthesizes new fatty acids from excess dietary carbohydrate.

- **Location:** Cytosol (in contrast to oxidation in mitochondria).
- **Key Enzyme: Fatty Acid Synthase (FAS):** A multi-enzyme complex. The growing fatty acid chain is attached to an **Acyl Carrier Protein (ACP)** domain.
- **Source of Carbon:** Acetyl-CoA (from pyruvate or citrate).
- **Committing Step:** Acetyl-CoA is carboxylated to form **Malonyl-CoA**.
  - Acetyl-CoA + $HCO_3^-$ + ATP → Malonyl-CoA + ADP + $P_i$
  - **Enzyme: Acetyl-CoA Carboxylase (ACC).** This is the **key regulated enzyme** of fatty acid synthesis. It requires **biotin**.
- **Repeating Cycle (Elongation):** Each cycle adds 2 carbons from Malonyl-CoA to the growing chain. The cycle involves:
  - Condensation
  - Reduction (Uses **NADPH**)
  - Dehydration
  - Reduction (Uses **NADPH**)
- **Final Product:** After 7 cycles, the C16 fatty acid **Palmitate (16:0)** is released.
- **Source of NADPH:** The PPP is the major supplier of the NADPH required for synthesis.

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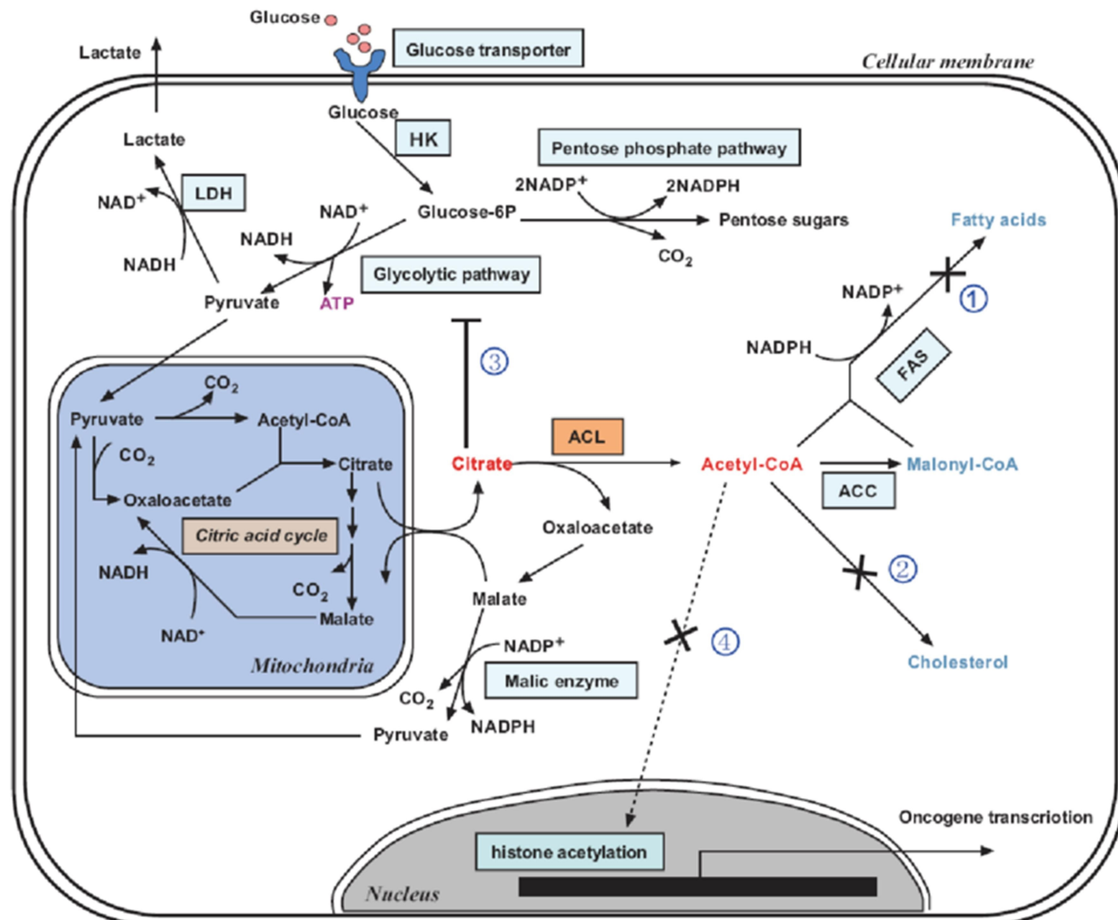



## IV. Regulation of Fatty Acid Metabolism

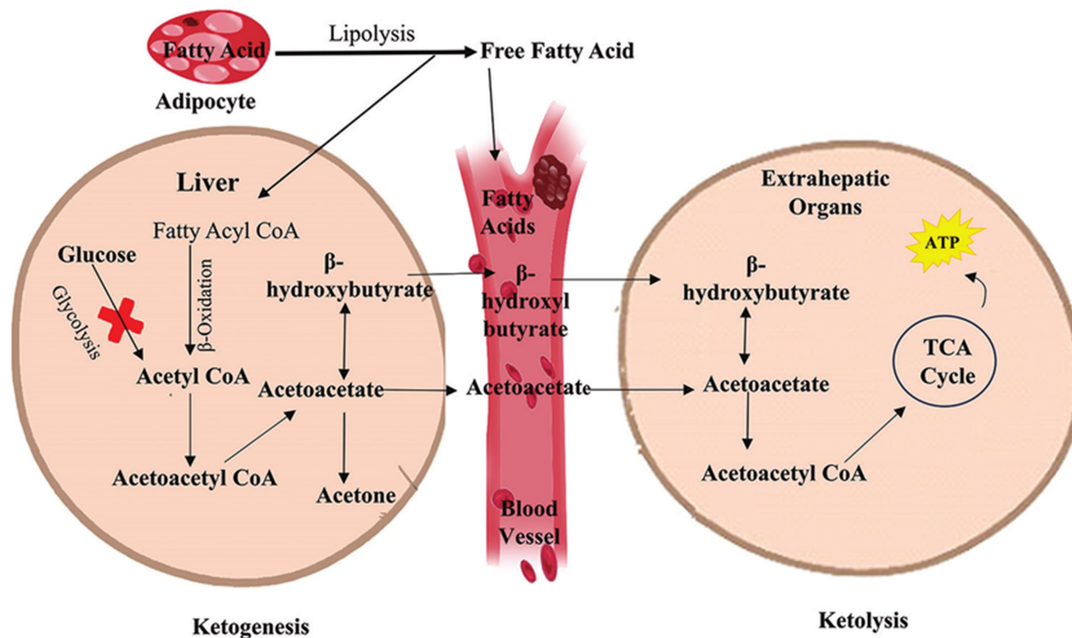
The pathways are reciprocally regulated to prevent a futile cycle.

| Factor | β-Oxidation (Catabolism) | Fatty Acid Synthesis (Anabolism) |
|---|---|---|
| **Hormones** | **Glucagon, Epinephrine** (↑cAMP ->PKA) | **Insulin** (↑dephosphorylation) |
| **Key Allosteric Regulator** | **Malonyl-CoA** (potent inhibitor of **CPT-I**) | **Citrate** (activates **ACC**), **Palmitoyl-CoA** (inhibits ACC) |

| Factor | β-Oxidation (Catabolism) | Fatty Acid Synthesis (Anabolism) |
|---|---|---|
| **Energy Charge** | **High AMP** activates | **High ATP/Citrate** activates |
| **Dietary State** | **Fasting/Starvation** | **Fed state** (high carb) |

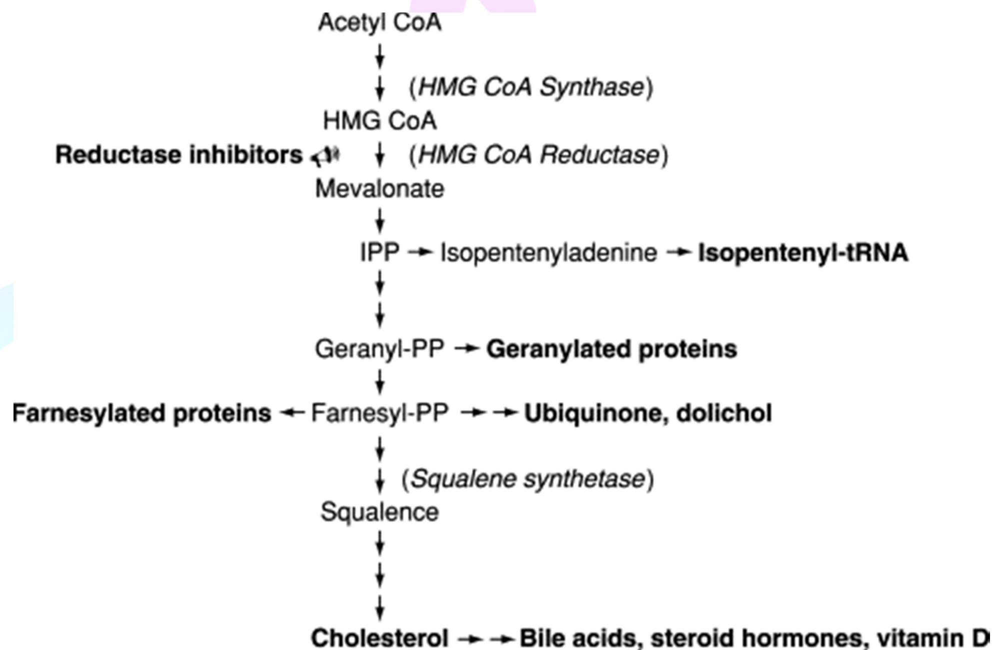
**V. Ketogenesis**

- **When?** During prolonged fasting/starvation or uncontrolled diabetes, when hepatic oxaloacetate is depleted for gluconeogenesis, making the TCA cycle slow.
- **Where?** Liver mitochondria.
- **What?** The liver converts Acetyl-CoA into **ketone bodies** (acetoacetate, β-hydroxybutyrate, acetone) for export to peripheral tissues (brain, heart, muscle) as an alternative fuel.
- **Significance:** Spares glucose for the brain and allows muscles to use fat-derived energy.




**VI. Cholesterol Metabolism**

- **Synthesis:** All 27 carbons of cholesterol are derived from **Acetyl-CoA**. The pathway occurs in the cytosol and ER. The **rate-limiting enzyme is HMG-CoA Reductase**.
- **Regulation of HMG-CoA Reductase:**
    - **Transcriptional Control:** Sterol Regulatory Element-Binding Proteins (SREBPs).
    - **Covalent Modification:** Phosphorylation (by AMPK) inactivates it; dephosphorylation activates it.
    - **Pharmacological Inhibition: Statins** are competitive inhibitors of HMG-CoA Reductase, used to lower blood cholesterol.




- **Fate of Cholesterol:**
    - Component of cell membranes.
    - Precursor for bile acids, vitamin D, and steroid hormones (cortisol, aldosterone, estrogen, testosterone).

**VII. Lipid Transport: Lipoproteins**

Lipoproteins are complexes of lipids and apolipoproteins that transport water-insoluble lipids in the blood.

| Lipoprotein | Primary Function | Core Lipid | Apolipoproteins |
|---|---|---|---|
| **Chylomicrons** | Transport **dietary** TAG from intestine to tissues. | TAG | B-48, C-II, E |
| **VLDL** | Transport **newly synthesized** TAG from liver to tissues. | TAG | B-100, C-II, E |
| **LDL** | Deliver cholesterol to **peripheral tissues**. "Bad Cholesterol." | Cholesteryl Esters | B-100 |
| **HDL** | **Reverse cholesterol transport** (from tissues back to liver). "Good Cholesterol." | Protein, Phospholipids | A-I |

- **Lipoprotein Lipase (LPL):** Enzyme on capillary walls that hydrolyzes TAG in chylomicrons and VLDL. Activated by **Apo C-II**.

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## Amino acid Metabolism

Protein metabolism encompasses the breakdown of dietary proteins, the catabolism of amino acids for energy, the synthesis of new proteins, and the conversion of amino acids into specialized nitrogenous products. The central theme is the management of Nitrogen.

## Key Metabolic Fates of Amino Acids

| Amino acid | Product | Function |
|---|---|---|
| Amino acids | Various proteins | Structure, transport, regulation, immunity, signaling, and fuels |
| Ala, Glu and Ser | Directly | Appetite |
| Arg | NO | Kill invaded microorganisms |
| Arg | NO | Facilitate neurological function and development |
| Arg | NO | Regulate vascular tone, blood flow, osmolarity in gill, and cell signaling |
| Arg and Met | Spermine | Induce larval intestinal maturation |
| Arg, Met, and Gly | Creatine | High energy storage; antioxidant |
| Cys, Glu, and Gly | Glutathione | Antioxidant and cell signaling |
| Glu and Gln | Directly | Ammonia removal |
| Glu | GABA | Promote metamorphosis |
| Glu | GABA | Regulate food intake |
| Gln | Directly | Increase growth, feed efficiency and gut development |
| Gln | Directly | Fuel for macrophage; Cell signaling |
| Gln, Gly, and Asp | Nucleotides | Genetic information storage and expression, biosynthesis, immunity and reproduction |
| Gly | Directly | Increase hepatic T4 5′monodeiodinase |
| Gly | Directly | Osmoregulation |
| His | Directly and carnosine | Protection against pH change |
| Leu | HMB | Immunity modulation; Cell signaling |
| Lys and Met | Carnitine | Lipid transporter on mitochondrial membrane |
| Met | Choline | Structure in membrane; neurotransmitter; betaine synthesis |
| Proline | P5C | Redox regulation; Cell signaling |
| Proline | Hydroxyproline | Enhance growth; Collagen function |
| Phe and Tyr | T4, T3 | Influence metamorphosis |
| Phe and Tyr | T4, T3 | Enhance growth performance |
| Phe and Tyr | T4, T3 | Influence pigmentation |
| | Melanin | Influence pigmentation |
| Phe and Tyr | Epinephrine, norepinephrine | Neurotransmitters that modulate stress responses |
| Phe and Tyr | Dopamine | Down-regulated immunity |
| Trp | Serotonin | Modulate cortisol release, behavior and feeding |
| Trp | Melatonin | Improve testicular development |
| Taurine | Directly | Osmotic pressure regulation |
| Taurine | Directly | Hardness adaptation |
| Taurine | Directly | Gut development |
| Taurine | Directly | Retinal development |

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**Protein Digestion & Absorption**

- **Goal:** Break down dietary proteins into amino acids and small peptides for absorption.
- **Process:**
    1. **Stomach: Pepsin** (activated from pepsinogen by HCl) begins proteolysis in the acidic environment.
    2. **Small Intestine:** Pancreatic proteases (secreted as zymogens) do the bulk of digestion.
        - **Endopeptidases:** Cleave internal peptide bonds.
            - **Trypsin:** Cleaves after Lys and Arg. *Master activator*—it activates all other pancreatic zymogens.
            - **Chymotrypsin:** Cleaves after Phe, Trp, Tyr.
            - **Elastase:** Cleaves after small, neutral amino acids (Ala, Gly, Ser).
        - **Exopeptidases:** Cleave terminal amino acids.
            - **Carboxypeptidases A & B:** Remove C-terminal amino acids.
    3. **Absorption:** Free amino acids and di/tri-peptides are absorbed by specific transporters on enterocytes. Peptides are hydrolyzed to amino acids inside the cell.

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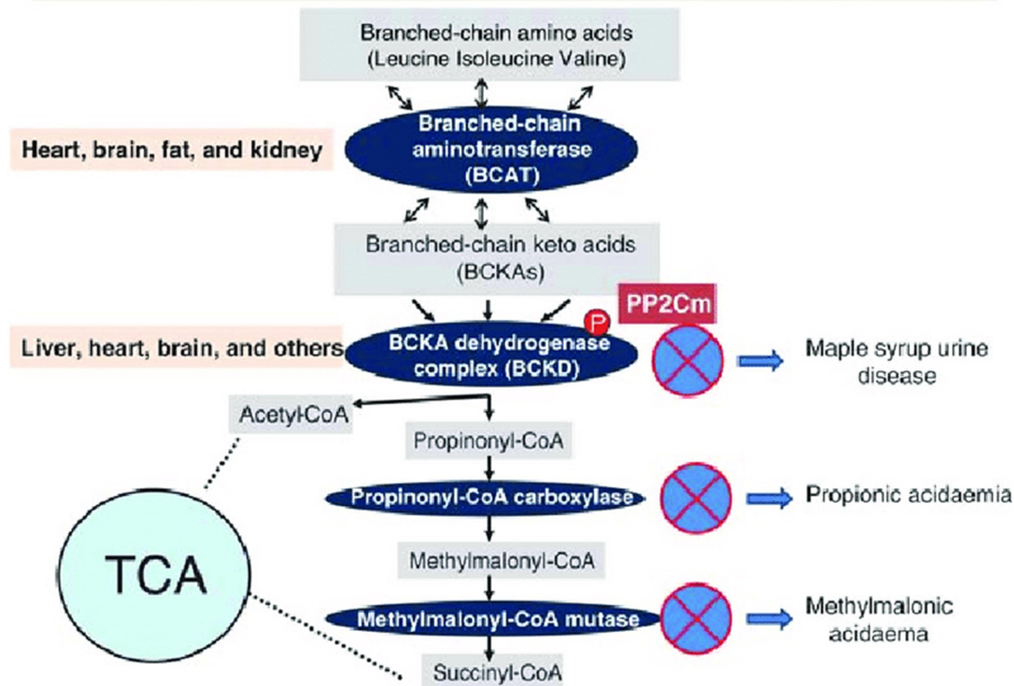
**II. General Amino Acid Catabolism**

The fate of an amino acid involves two key steps: first removing the nitrogen, then catabolizing the resulting carbon skeleton.



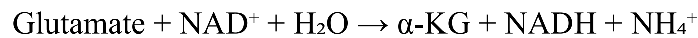

**A. Removal of Amino Nitrogen (Transdeamination)**

This is the process by which nitrogen from most amino acids is funneled into a single excretory product.

1. **Transamination (Nitrogen Collection):**
   - **Reaction:** Transfer of an α-amino group from an amino acid to an α-ketoacid.
     $\text{Amino Acid}_1 + \alpha\text{-Ketoacid}_2 \rightleftharpoons \alpha\text{-Ketoacid}_1 + \text{Amino Acid}_2$
   - **Enzymes: Aminotransferases (Transaminases)**
     - Require the coenzyme **Pyridoxal Phosphate (PLP)** (Vitamin B6 derivative).
   - **Key Example: Alanine Aminotransferase (ALT) & Aspartate Aminotransferase (AST)**
     - ALT: Alanine + α-KG ⇌ Pyruvate + Glutamate
     - AST: Aspartate + α-KG ⇌ Oxaloacetate + Glutamate

- **Net Effect:** Nitrogen from various amino acids is collected into the amino group of **Glutamate**.

2. **Oxidative Deamination (Nitrogen Release):**

   - **Reaction:** Glutamate is oxidatively deaminated, liberating free ammonia ($NH_4^+$) for excretion and regenerating α-KG.
     
     Glutamate + $NAD^+$ + $H_2O$ → α-KG + NADH + $NH_4^+$
   - **Enzyme: Glutamate Dehydrogenase (GDH)**
     - **Location:** Mitochondria.
     - **Regulation: Allosterically inhibited by ATP and GTP** (high energy); **activated by ADP and GDP** (low energy). This links nitrogen disposal to energy status.

**B. Transport of Nitrogen: The Glucose-Alanine Cycle**

- In muscles during fasting, amino acids are catabolized. The nitrogen is transferred to pyruvate (from glycolysis) to form **alanine**.
- Alanine travels to the liver.
- In the liver, alanine is transaminated back to pyruvate (which can be used for gluconeogenesis), and the nitrogen enters the urea cycle.
- **Net Effect:** Safely transports nitrogen from muscle to liver; provides substrate for hepatic gluconeogenesis.

**III. Urea Cycle (The Final Nitrogen Disposal Pathway)**

- **Goal:** To detoxify and excrete ammonia ($NH_3$/$NH_4^+$) as **urea**, a highly soluble, non-toxic compound.
- **Location:** Split between the **mitochondrial matrix** and the **cytosol** (Liver).
- **Key Nitrogen Sources:**
  1. **Ammonia** (from glutamate deamination)

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2. **Aspartate** (from transamination)

- **The Five-Step Cycle:**

1. **(Mitochondria) Carbamoyl Phosphate Synthesis:**
   $NH_3 + HCO_3^- + 2ATP \rightarrow$ Carbamoyl Phosphate + 2ADP + $P_i$
   - **Enzyme: Carbamoyl Phosphate Synthetase I (CPS-I).**
   - **This is the committed and rate-limiting step.** *Activated by N-Acetylglutamate (NAG).*
2. **(Mitochondria) Ornithine Transcarbamoylase (OTC):**
   Carbamoyl Phosphate + Ornithine → Citrulline
3. **(Cytosol) Citrulline + Aspartate → Argininosuccinate:**
   - **Enzyme: Argininosuccinate Synthetase.** Consumes 1 ATP (→ AMP + $PP_i$).
4. **(Cytosol) Argininosuccinate → Arginine + Fumarate:**
   - **Enzyme: Argininosuccinate Lyase.**
   - Fumarate can enter the TCA cycle.
5. **(Cytosol) Arginine → Urea + Ornithine:**
   - **Enzyme: Arginase.** Regenerates ornithine, which is transported back into the mitochondria.

- **Energetic Cost:** 4 ATP equivalents (2ATP for CPS-I, 1ATP → AMP for Argininosuccinate Synthase).
- **Regulation:** Primarily by **N-Acetylglutamate (NAG)**, which is synthesized in response to high arginine and glutamate levels, signaling amino acid availability.

**Urea Cycle Disorders:** Deficiencies in any cycle enzyme lead to **hyperammonemia** (elevated blood ammonia), which is neurotoxic. Symptoms include lethargy, vomiting, and brain edema.

**IV. Catabolism of Carbon Skeletons**

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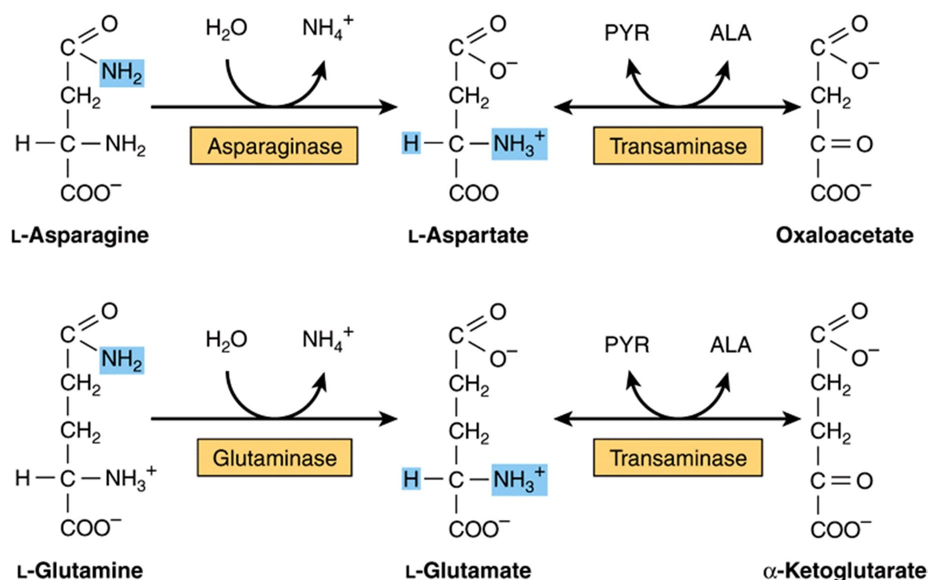
After deamination, the remaining carbon skeletons (α-ketoacids) are converted into one of seven major metabolic intermediates that feed into central pathways.




**Regulation of Protein Metabolism**

- **Overall Nitrogen Balance:** Controlled by hormones.
  - **Anabolic (Positive N-balance): Insulin, Growth Hormone, IGF-1, Testosterone.** Promote protein synthesis.
  - **Catabolic (Negative N-balance): Glucocorticoids (e.g., Cortisol).** Promote protein breakdown, especially in muscle, to provide amino acids for gluconeogenesis during stress.
- **Ubiquitin-Proteasome Pathway:** The major ATP-dependent pathway for targeted degradation of cellular proteins.

**NUCLEIC ACID METABOLISM: A CSIR NET PERSPECTIVE**

Nucleic acid metabolism encompasses the *de novo* synthesis and salvage of nucleotides, their polymerization into DNA and RNA, and the degradation of nucleic acids and nucleotides. Nucleotides are essential for energy transfer (ATP, GTP), signaling (cAMP, cGMP), and as coenzymes (NAD, FAD, CoA), in addition to their primary role as the building blocks of genetic information.

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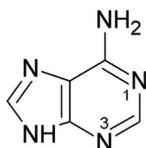
## I. Nucleotide Structure Review

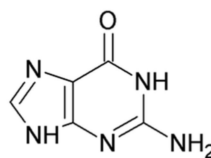
A nucleotide consists of:

1. **Nitrogenous Base:** Purine (Adenine, Guanine) or Pyrimidine (Cytosine, Uracil, Thymine).
2. **Pentose Sugar:** Ribose (in RNA) or 2'-Deoxyribose (in DNA).
3. **Phosphate Group:** One to three phosphates.

Purines

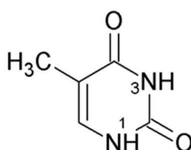



Adenine

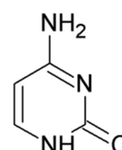



Guanine

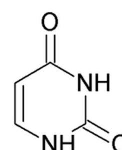
Pyrimidines




Thymine




Cytosine




Uracil

## II. *De Novo* Purine Nucleotide Synthesis

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- **Location:** Cytosol of most cells, primarily in the **liver**.
- **Key Concept:** The purine ring system is built **atom-by-atom onto a pre-existing ribose-5-phosphate** scaffold.
- **Precursor: Phosphoribosyl Pyrophosphate (PRPP)**. Formed from Ribose-5-P (from PPP) and ATP by the enzyme **PRPP Synthetase**.
- **Committing Step:** PRPP + Glutamine -> 5-Phosphoribosylamine + GLU + $PP_i$
    - **Enzyme: Glutamine-PRPPAmidotransferase.** This is the **key regulated enzyme** of purine synthesis.
- **Ring Construction:** The purine ring (Inosine Monophosphate, IMP) is assembled through a series of reactions that add carbon and nitrogen donors:

- **Glycine** (whole molecule)
- **$N^{10}$-Formyl-THF** (one-carbon unit)
- **Glutamine** (amide nitrogen)
- **Aspartate** (amino nitrogen)
- **$CO_2$**

- **Branch Point:** IMP is the common precursor for both AMP and GMP.
  - **IMP -> AMP:** Requires GTP and aspartate.
  - **IMP ->GMP:** Requires ATP and glutamine.
- **Regulation: Feedback Inhibition.**
  - **AMP** inhibits the first step in its own synthesis from IMP.
  - **GMP** inhibits the first step in its own synthesis from IMP.
  - **AMP and GMP** both inhibit the committing enzyme, Glutamine-PRPPAmidotransferase.
  - **PRPP** is a positive regulator.

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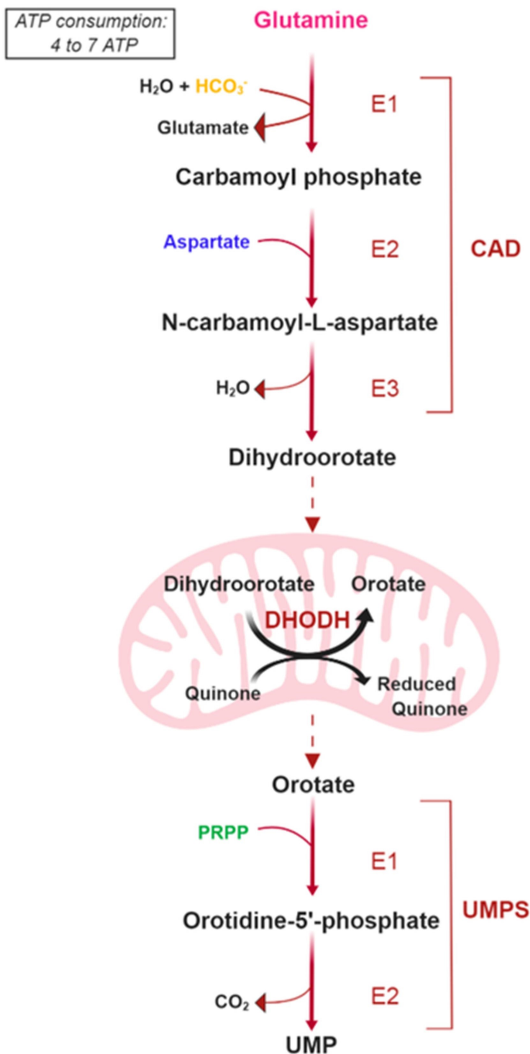
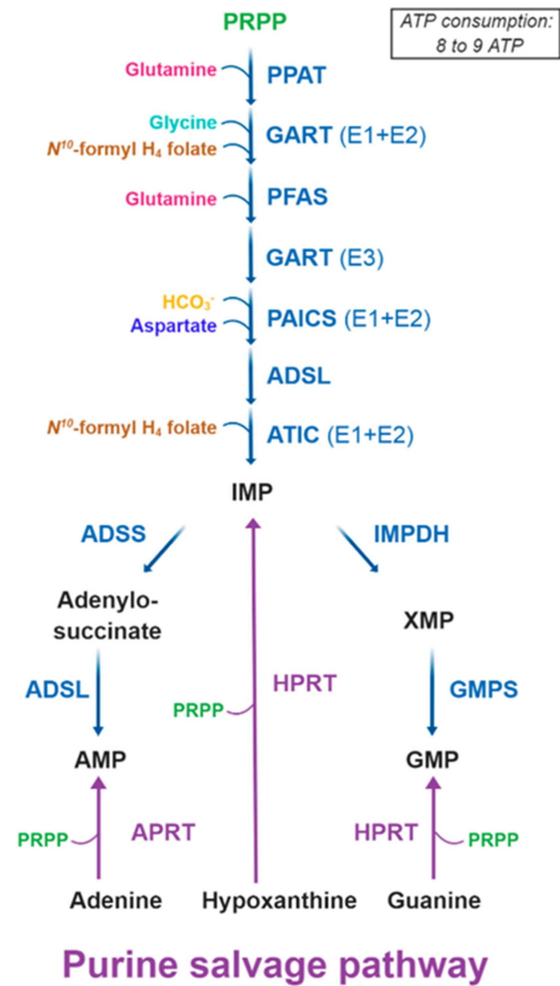
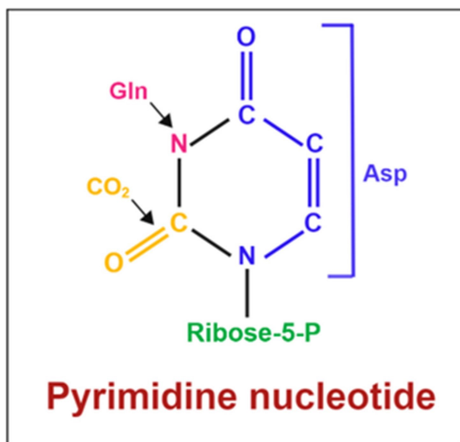
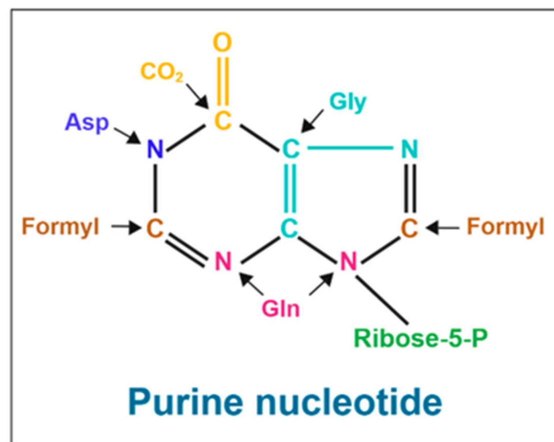

A
De novo pyrimidine synthesis
ATP consumption: 4 to 7 ATP
Glutamine
H2O + HCO3-
Glutamate
E1
Carbamoyl phosphate
Aspartate
E2
CAD
N-carbamoyl-L-aspartate
H2O
E3
Dihydroorotate
Dihydroorotate
Orotate
DHODH
Quinone
Reduced Quinone
Orotate
PRPP
E1
Orotidine-5'-phosphate
UMPS
CO2
E2
UMP


B
De novo purine synthesis
PRPP
ATP consumption: 8 to 9 ATP
Glutamine
PPAT
Glycine
N10-formyl H4 folate
GART (E1+E2)
Glutamine
PFAS
GART (E3)
HCO3-
Aspartate
PAICS (E1+E2)
ADSL
N10-formyl H4 folate
ATIC (E1+E2)
IMP
ADSS
IMPDH
Adenylo-succinate
XMP
ADSL
HPRT
PRPP
GMPS
AMP
GMP
PRPP
APRT
HPRT
PRPP
Adenine
Hypoxanthine
Guanine
Purine salvage pathway


O
Gln
N
C
C
C
Asp
CO2
C
N
O
Ribose-5-P
Pyrimidine nucleotide


O
CO2
Gly
Asp
N
C
C
N
Formyl
C
C
C
Formyl
N
N
Gln
Ribose-5-P
Purine nucleotide

### III. *De Novo* Pyrimidine Nucleotide Synthesis

- **Location:** Cytosol (in most eukaryotes).
- **Key Concept:** The **pyrimidine ring is synthesized first**, and then attached to PRPP.
- **First Step:** $HCO_3^-$ + Glutamine + 2ATP -> Carbamoyl Phosphate + Glutamate + 2ADP + $P_i$
  - **Enzyme: Carbamoyl Phosphate Synthetase II (CPS-II).**
  - **This is the key regulated enzyme** of pyrimidine synthesis. *Inhibited by UTP; activated by ATP and PRPP.*
  - **Note:** Distinct from CPS-I of the urea cycle (mitochondrial, uses $NH_3$, makes urea).
- **Ring Formation:** Carbamoyl phosphate + Aspartate -> Orotate (via dihydroorotate).
- **Attachment to Sugar:** Orotate is then attached to PRPP to form **Orotidine Monophosphate (OMP)**, which is decarboxylated to form **Uridine Monophosphate (UMP)**.
- **Conversion:** UMP is phosphorylated to UTP. UTP is aminated by **CTP Synthetase** (using glutamine) to form **CTP**.
- **Regulation:** Primarily via feedback inhibition of **CPS-II by UTP**.

### IV. Synthesis of Deoxyribonucleotides (dNTPs)

Deoxyribonucleotides are crucial for DNA synthesis and repair. They are formed by the **reduction of the ribonucleotide diphosphates (NDPs)**.

- **Enzyme: Ribonucleotide Reductase (RNR).**

  **Mechanism:** RNR catalyzes the replacement of the 2'-OH group of the ribose sugar with a hydrogen atom. This is a complex reaction that requires:

  - **Thioredoxin or Glutaredoxin** as electron donors.
  - **Thioredoxin Reductase** (uses NADPH) to regenerate reduced thioredoxin.

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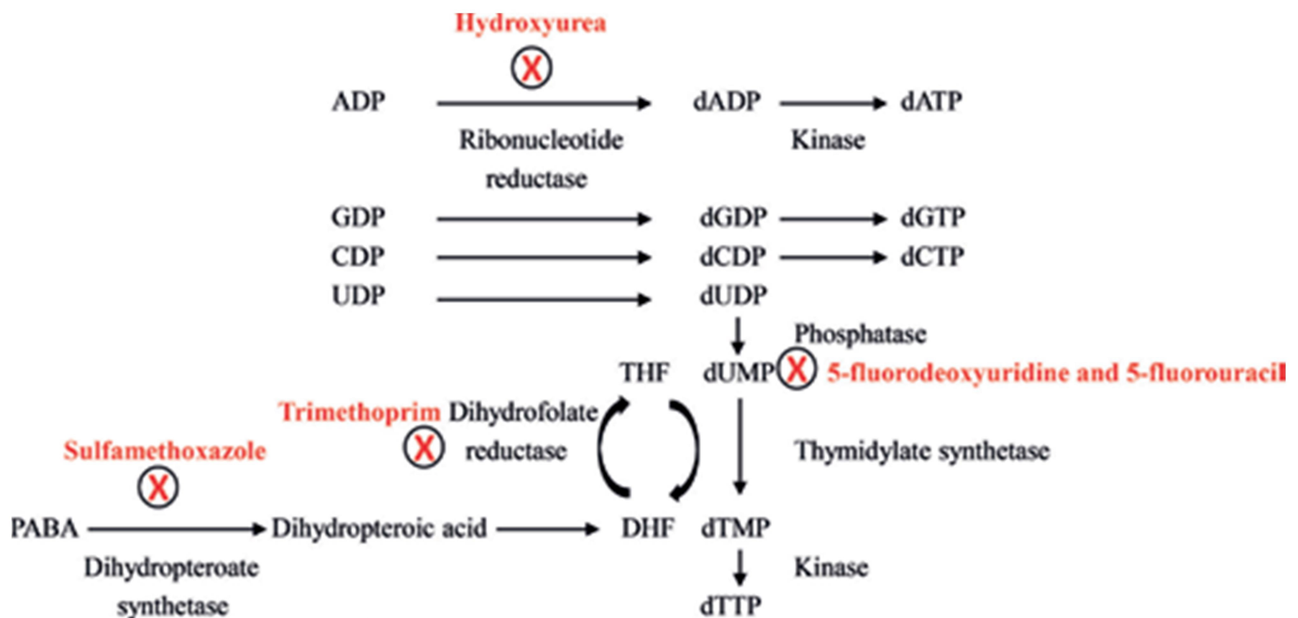
- **Strict Regulation:** The activity and substrate specificity of RNR are tightly controlled by allosteric effectors to maintain balanced dNTP pools, which is critical for genomic fidelity.
    - **Overall Activity:** Stimulated by **ATP**; inhibited by **dATP**.
    - **Substrate Specificity:** ATP, dATP, dTTP, and dGTP differentially regulate which NDP (ADP, GDP, CDP, UDP) the enzyme reduces.

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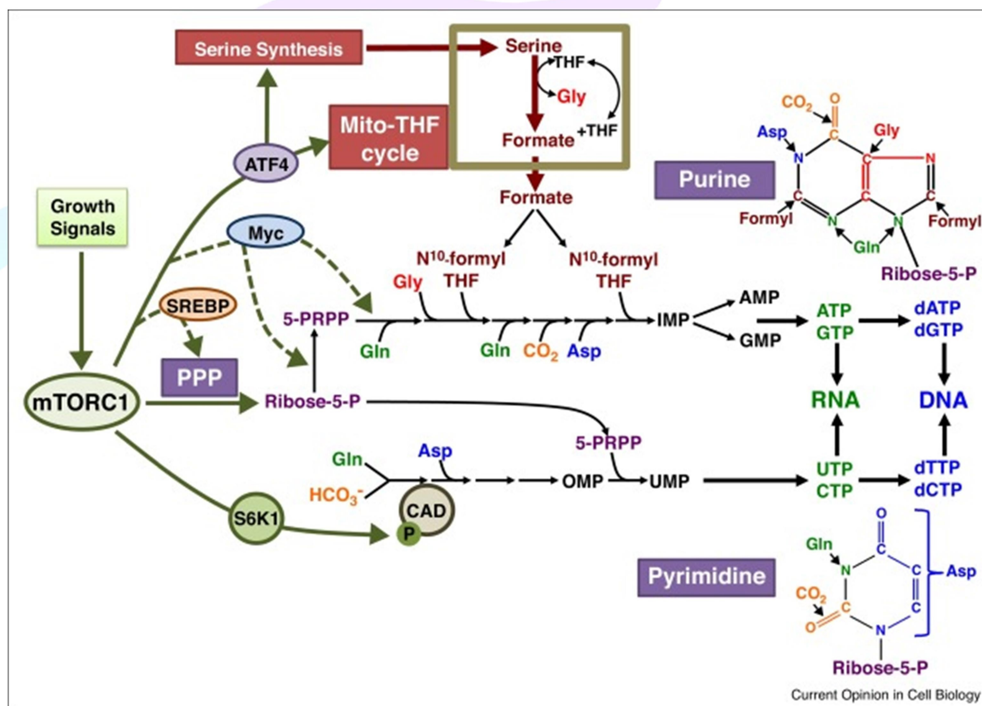
### V. Salvage Pathways

Salvage pathways recycle free purine and pyrimidine bases by converting them back into nucleotides. They are energetically cheaper than *de novo* synthesis and are especially important in tissues that cannot perform *de novo* synthesis (e.g., brain, RBCs, lymphocytes).

- **Purine Salvage:**
    - **Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT):**
      Hypoxanthine + PRPP -> IMP + $PP_i$
      Guanine + PRPP ->GMP + $PP_i$
    - **Adenine Phosphoribosyl Transferase (APRT):**
      Adenine + PRPP -> AMP + $PP_i$

- **Clinical Correlation - Lesch-Nyhan Syndrome:**
    - **Cause:** Genetic deficiency of **HGPRT** (X-linked recessive).
    - **Consequence:** Inability to salvage hypoxanthine and guanine. Leads to:
        1. **Accumulation of PRPP**, which drives *de novo* purine synthesis, resulting in **hyperuricemia** (gouty arthritis, kidney stones).
        2. **Severe neurological and behavioral disorders** (self-mutilation, mental retardation), highlighting the importance of the salvage pathway in brain development.
- **Pyrimidine Salvage:** Less significant than purine salvage, but pathways exist to convert uracil, thymine, and cytosine to their respective nucleotides.

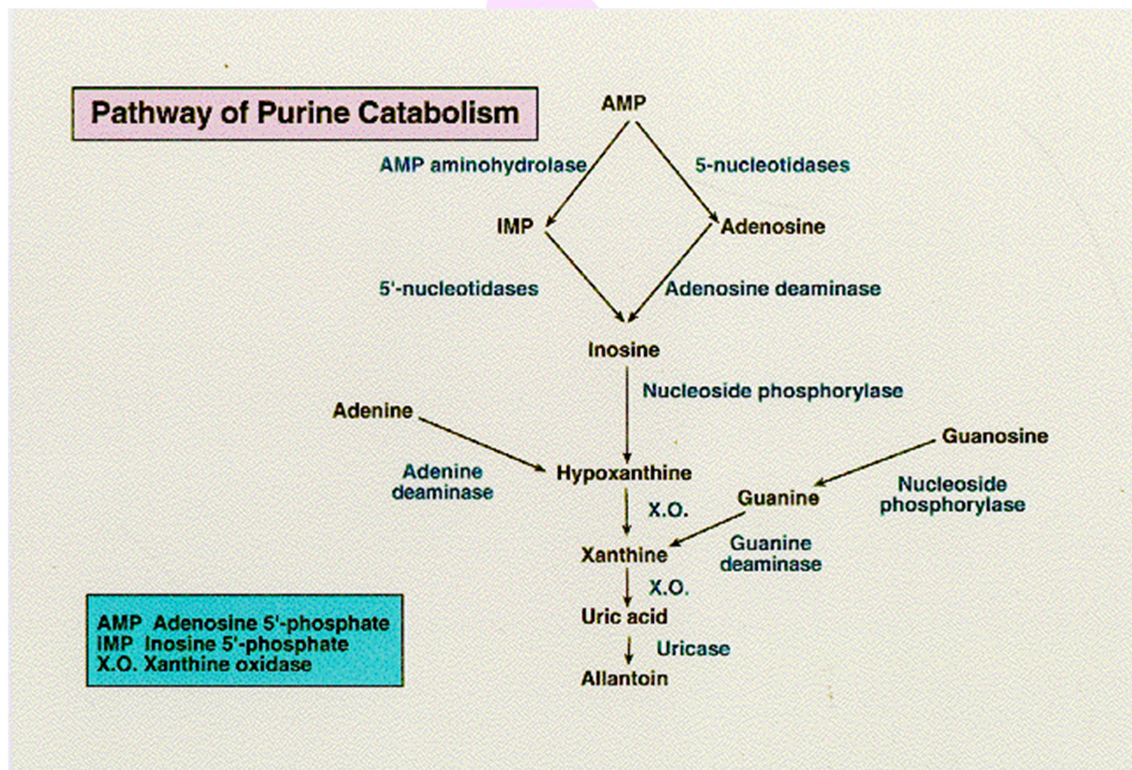



## VI. Nucleotide Degradation & Excretion

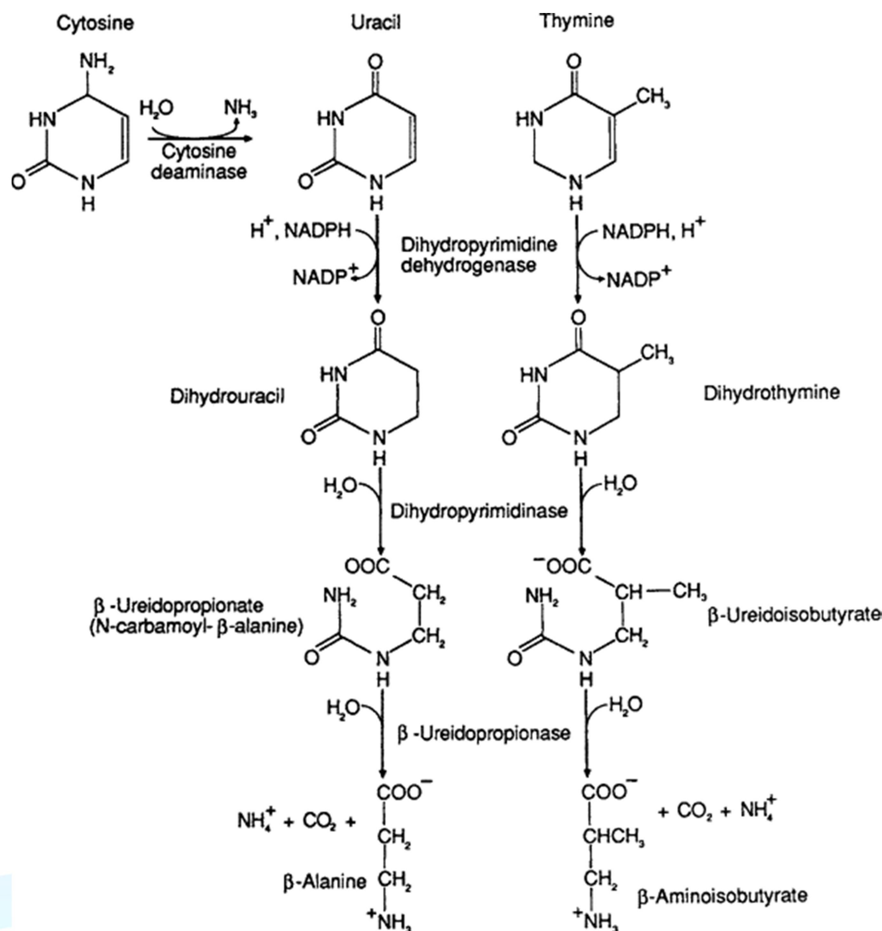
- **Purine Catabolism (→ Uric Acid):**
    1. Nucleotides -> Nucleosides -> Free bases (Adenine, Guanine).
    2. **Guanine -> Xanthine** (via Guanine Deaminase).

3. **Adenine -> Hypoxanthine -> Xanthine** (via Adenine Deaminase and Xanthine Oxidase).
4. **Xanthine -> Uric Acid** (via **Xanthine Oxidase**).

- **Final Product: Uric acid** is the final product of purine catabolism in **humans and higher apes**. It is excreted in urine.
- **Clinical Correlation - Gout:** A painful inflammatory condition caused by the deposition of **sodium urate crystals** in joints. Can be due to overproduction or underexcretion of uric acid. Treated with **Allopurinol**, a mechanism-based inhibitor of **xanthine oxidase**.




- **Pyrimidine Catabolism (→ Highly Soluble Products):**
  - Pyrimidines are degraded to highly water-soluble products like **β-alanine** (from C, U) and **β-aminoisobutyrate** (from T), which are further metabolized or excreted.
  - This is a simpler process and rarely causes pathology.




**VII. Chemotherapy Targets**

Many anticancer and antiviral drugs are analogs of nucleotides, amino acids, or folate that inhibit key enzymes in nucleotide biosynthesis.

- **Methotrexate (MTX):** A **folate analog** that inhibits **Dihydrofolate Reductase (DHFR)**, depleting tetrahydrofolate (THF) pools needed for *de novo* purine synthesis and dTMP synthesis.
- **5-Fluorouracil (5-FU):** A **pyrimidine analog** that is metabolically activated to inhibit **Thymidylate Synthase**, blocking dTMP production.
- **Hydroxyurea:** Inhibits **Ribonucleotide Reductase**, halting DNA synthesis by depleting dNTP pools.
- **6-Mercaptopurine (6-MP):** A **purine analog** that inhibits *de novo* purine synthesis.

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## BIOLOGICAL OXIDATION & BIOENERGETICS: A CSIR NET PERSPECTIVE

This field bridges the gap between the chemical reactions of metabolism (which release energy) and the physical process of trapping that energy in a usable form (ATP). It explains how cells convert the energy from food into the energy currency of life.

### I. Core Concepts: Thermodynamics in Biology

- **Bioenergetics:** The study of energy flow and transformation in living systems.
- **Biological Oxidation:** The removal of electrons (*dehydrogenation*) or hydrogen atoms from a molecule. This is the primary process by which energy is released from fuel molecules.
- **Key Thermodynamic Principles:**
  - **Free Energy Change (ΔG):** The portion of a system's energy that can perform work at constant temperature and pressure.
    - **ΔG< 0 (Exergonic):** Reaction is spontaneous, releases free energy. (e.g., Catabolism, ATP hydrolysis).
    - **ΔG> 0 (Endergonic):** Reaction is non-spontaneous, requires energy input. (e.g., Anabolism, ATP synthesis).
  - **Equilibrium Constant (K<sub>eq</sub>):** Related to ΔG by the equation: **ΔG = ΔG°' + RT ln K<sub>eq</sub>**
    - At equilibrium, ΔG = 0, so **ΔG°' = -RT ln K<sub>eq</sub>**. A large negative ΔG°' implies the reaction strongly favors products.
  - **Standard Free Energy Change (ΔG°'):** The free energy change under standard conditions (pH 7, 1M concentrations, 25°C). It indicates the *direction* and *thermodynamic potential* of a reaction.

### II. The Role of ATP: The Energy Currency

- **Structure:** Adenine + Ribose + Triphosphate chain.

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- **High-Energy Bonds:** The phosphoanhydride bonds (~ between the phosphate groups) are referred to as "high-energy" because their **hydrolysis yields a large negative ΔG** (~ -30 to -35 kJ/mol under cellular conditions).
    - ATP + $H_2O$ → ADP + $P_i$ (ΔG°' = -30.5 kJ/mol)
- **Why is ΔG so large?** Due to:
    1. **Charge Repulsion:** The four negative charges on ATP are crowded and repel each other. Relief upon hydrolysis.
    2. **Resonance Stabilization:** Inorganic phosphate ($P_i$) has greater resonance stabilization than the phosphate groups in ATP.
    3. **Solvation:** ADP and $P_i$ are better solvated (hydrated) than ATP.
- **Phosphoryl Group Transfer Potential:** The tendency of a molecule to donate its phosphoryl group. **ATP has an intermediate transfer potential**, making it the perfect energy shuttle. It can accept a phosphoryl group from high-potential compounds (like PEP) and donate it to low-potential compounds (like glucose).

### III. High-Energy Electron Carriers: Capturing Reducing Power

Energy released from oxidation is temporarily stored as high-energy electrons carried by specific coenzymes.

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| Carrier | Role | Energy Yield upon Oxidation |
| --- | --- | --- |
| **$NAD^+$ (Nicotinamide Adenine Dinucleotide)** | **Primary electron acceptor** in catabolic reactions (e.g., glycolysis, TCA cycle). Accepts a hydride ion ($H^-$), becoming **NADH + $H^+$**. | ~2.5 ATP / NADH |
| **$NADP^+$** | Used primarily for **reductive** | Not used for ATP; |

| Carrier | Role | Energy Yield upon Oxidation |
|---|---|---|
| | **biosynthesis** (anabolism). The extra phosphate allows separate regulation from $NAD^+$. | used for biosynthetic reducing power. |
| **FAD (Flavin Adenine Dinucleotide)** | Accepts two electrons as **hydrogen atoms** ($2H^+ + 2e^-$), becoming **$FADH_2$**. Used in succinate dehydrogenase (TCA) and other flavoproteins. | ~1.5 ATP / $FADH_2$ |
| **FMN (Flavin Mononucleotide)** | Prosthetic group for Complex I of the ETC. Also accepts $2H^+ + 2e^-$. | Part of the ETC machinery. |
| **Ubiquinone (Coenzyme Q, Q)** | Lipid-soluble mobile carrier in the ETC. Can accept one or two electrons. | Part of the ETC machinery. |
| **Cytochromes** | Heme-containing proteins with an Fe ion that cycles between $Fe^{2+}$ (reduced) and $Fe^{3+}$ (oxidized). Carry **one electron**. | Part of the ETC machinery. |

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**BIOENERGETICS: A CSIR NET PERSPECTIVE**

Bioenergetics is the quantitative study of energy transductions—the changes of energy from one form to another—in living cells, and the nature and function of the chemical processes underlying these transductions. It is the foundation for understanding how organisms acquire, transform, and utilize energy to perform work.

**I. Core Thermodynamic Principles**

- **First Law of Thermodynamics (Conservation of Energy):** Energy can be transferred or transformed, but cannot be created or destroyed. The total energy of the universe remains constant.
    - **Biological Implication:** The energy released by breaking chemical bonds in food (catabolism) must equal the energy stored in new bonds (anabolism) plus the energy lost as heat.
- **Second Law of Thermodynamics:** The total entropy (disorder) of the universe always increases in a spontaneous process.
    - **Biological Implication:** Living organisms create local order (low entropy) by utilizing energy from their environment and releasing heat (increasing the universe's total entropy).
- **Free Energy (G):** The component of a system's total energy that can perform work at constant temperature and pressure.
- **Free Energy Change (ΔG):** The most important thermodynamic concept in biochemistry. It predicts the direction and equilibrium of chemical reactions.
    - **$\Delta G = \Delta H - T\Delta S$** (where H is enthalpy, T is temp, S is entropy)
    - **$\Delta G < 0$ (Negative): Exergonic** reaction. Spontaneous. Releases free energy. (e.g., ATP hydrolysis, glucose oxidation).
    - **$\Delta G > 0$ (Positive): Endergonic** reaction. Non-spontaneous. Requires an input of free energy. (e.g., ATP synthesis, gluconeogenesis).
    - **$\Delta G = 0$:** The reaction is at **equilibrium**. No net flow of reactants or products.

**II. The Energy Currency of the Cell: ATP**

Adenosine Triphosphate (ATP) is the universal energy currency. Its hydrolysis drives endergonic processes.

- **Why ATP?** It has an **intermediate phosphoryl group transfer potential**.
- **Hydrolysis Reaction:** $ATP + H_2O \rightleftharpoons ADP + P_i$

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- **Standard Free Energy Change (ΔG°'): -30.5 kJ/mol** (Highly exergonic under standard conditions).
- **Why is ΔG°' so large?**
  1. **Electrostatic Repulsion:** The four negative charges on the triphosphate chain strongly repel each other. Hydrolysis relieves this strain.
  2. **Resonance Stabilization:** The products (ADP and $P_i$) are stabilized by resonance to a greater extent than the reactants (ATP).
  3. **Solvation (Hydration):** The products are more effectively solvated by water than ATP, which further stabilizes them.
- **Phosphoryl Group Transfer:** ATP's real role is not hydrolysis but **group transfer**. It donates its phosphoryl (~P) group to other molecules (e.g., glucose), making them more reactive (raising their free energy content).

### III. High-Energy Compounds: The Energy Scale

Compounds can be ranked by their **phosphoryl group transfer potential** (the negative of ΔG°' of hydrolysis). ATP sits in the middle of this scale, making it the perfect intermediary.

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| Compound | ΔG°' of Hydrolysis (kJ/mol) | Role / Context |
|---|---|---|
| **Phosphoenolpyruvate (PEP)** | -62 | **Highest potential.** Glycolysis intermediate. |
| **1,3-Bisphosphoglycerate (1,3-BPG)** | -49 | Glycolysis intermediate. |
| **Creatine Phosphate** | -43 | **Energy buffer** in muscle and nerve cells. |

| Compound | $\Delta G^{\circ\prime}$ of Hydrolysis (kJ/mol) | Role / Context |
|---|---|---|
| **ATP → ADP + $P_i$** | **-31** | **Energy Currency.** |
| **Glucose 6-Phosphate** | -14 | **Low potential.** Metabolic intermediate. |

**Strategy:** Energy from the hydrolysis of high-potential compounds (like PEP) is used to synthesize ATP from ADP. ATP hydrolysis then drives endergonic reactions like the formation of low-potential compounds (like G6P).

### IV. Biological Oxidation & Electron Carriers

Energy is released by the oxidation of fuel molecules (e.g., glucose, fatty acids). This energy is not released as heat but is captured in the form of **high-energy electrons**.

- **Oxidation:** Loss of electrons.
- **Reduction:** Gain of electrons.
- **Redox Potential ($E^{\circ\prime}$):** The tendency of a substance to gain electrons (to be reduced). A strong reducing agent (e.g., NADH) has a highly **negative $E^{\circ\prime}$**, meaning it readily donates electrons. A strong oxidizing agent (e.g., $O_2$) has a highly **positive $E^{\circ\prime}$**, meaning it readily accepts electrons.
- **Relationship between ΔG and ΔE:** $\Delta G = -nF\Delta E$
    - n = number of electrons transferred
    - F = Faraday's constant
    - ΔE = change in reduction potential
    - A **positive ΔE** leads to a **negative ΔG** (spontaneous reaction).

## About us

BioResire (NEET | CSIR NET | Biotech Internships) is a life sciences research and training organization dedicated to bridging the gap between academic learning and industry skills. We provide internships, projects, and programs in Bioinformatics, Biotechnology, Molecular Biology, Cancer Research, Neuroscience, and related fields, helping students build job-oriented scientific careers.

*"The future belongs to those who explore the unseen — where biology meets innovation and discovery begins."*