PLANT RESPIRATION

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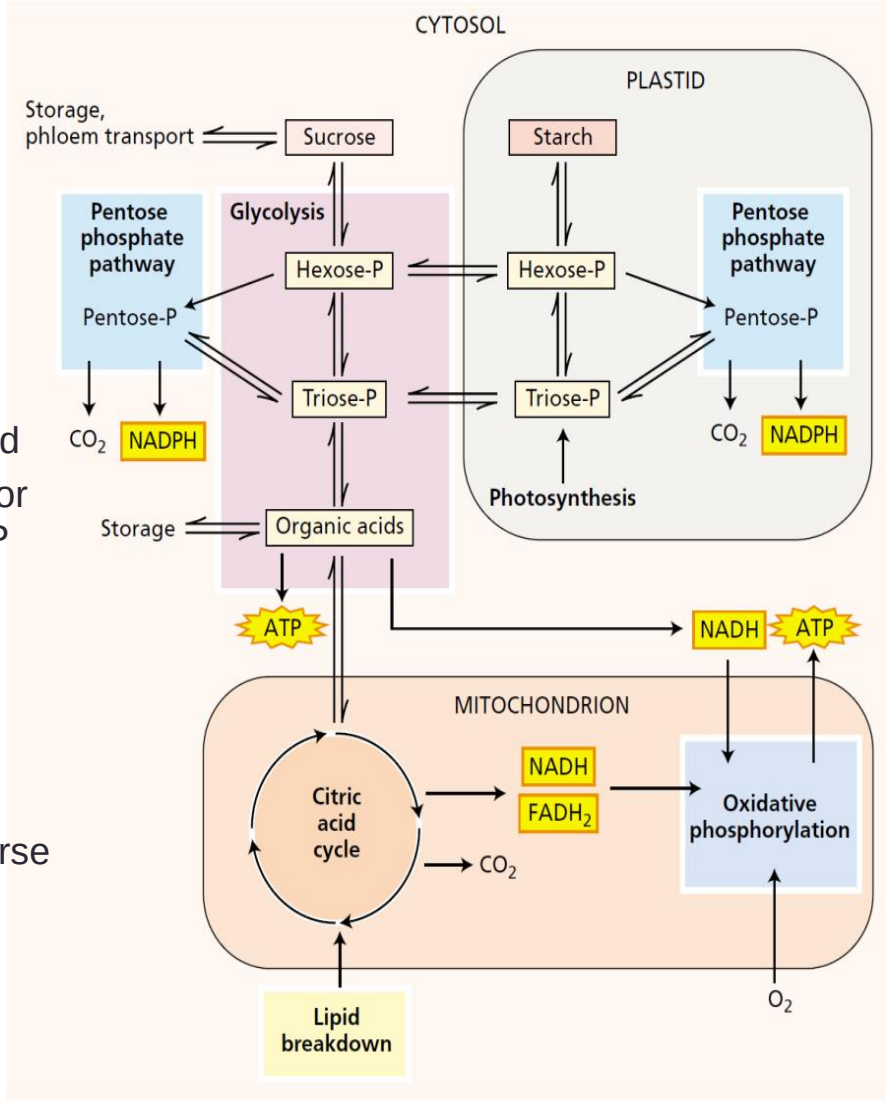
Overview

- Aerobic (oxygen-requiring) respiration is common to nearly all eukaryotic organisms
  - in plants
  - in animals and
  - In lower eukaryotes.
- Aerobic respiration:
  - Reduced organic compounds are mobilized and
  - Subsequently oxidized in a controlled manner.
- During respiration:
  - Fee energy is released and
  - Transiently stored in a compound (ATP).
  - These can be readily utilized for the maintenance and development of the plant.
- The Most commonly substrate for respiration: **Glucose**.
- Plant cell: The **reduced carbon** is derived from sources such as
  - The disaccharide: sucrose and
  - The triose phosphates: starch degradation and photosynthesis,
  - Fructose-containing polymers (fructans), and
  - Other sugars
  - Lipids (primarily triacylglycerols),
  - Organic acids

Overview of respiration

- In the cytosol:
  - Glycolysis and
  - The pentose phosphate pathways
- The plastid convert sugars to organic acids: via
  - Hexose phosphates and
  - Triose phosphates,
  - Generating NADH or NADPH and ATP.
- In the mitochondria:
  - The organic acids are oxidized by Citric acid cycle, and
  - The NADH and $FADH_2$ produced provide the energy for ATP synthesis by the electron transport chain and ATP synthase in oxidative phosphorylation.
- In gluconeogenesis,
  - Carbon from lipid breakdown is broken down in the glyoxysomes,
  - Metabolized in the citric acid cycle, and
  - Then used to synthesize sugars in the cytosol by reverse glycolysis.



Chemical Standpoint

- Plant respiration can be expressed as the oxidation of the 12-carbon molecule sucrose and the reduction of 12 molecules of O_2:

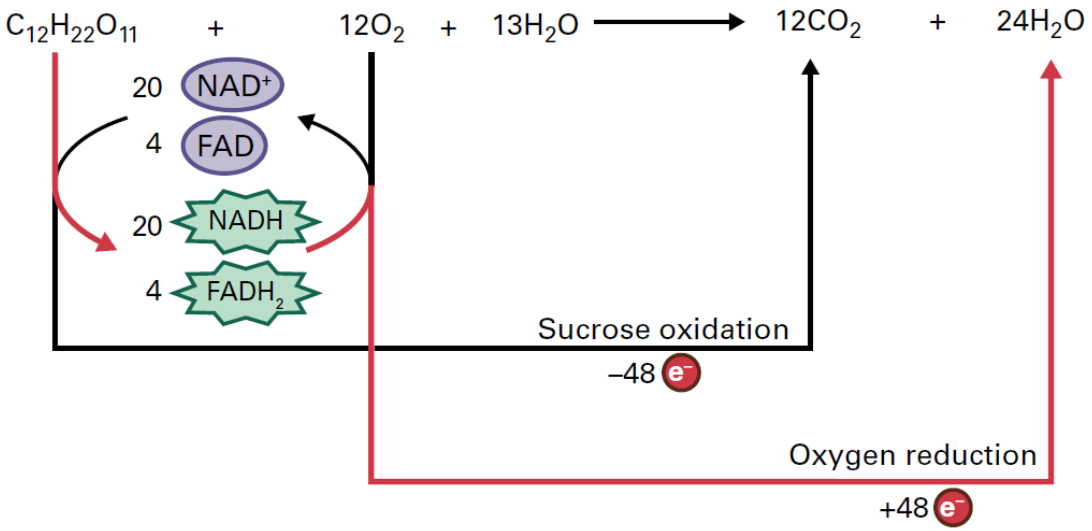
$$C_{12}H_{22}O_{11} + 13\ H_2O \rightarrow 12\ CO_2 + 48\ H^+ + 48\ e^-$$

$$12\ O_2 + 48\ H^+ + 48\ e^- \rightarrow 24\ H_2O$$

- Net reaction:

$$C_{12}H_{22}O_{11} + 12\ O_2 \rightarrow 12\ CO_2 + 11\ H_2O$$

- The oxidation of carbohydrate to carbon dioxide (CO2) is coupled to the reduction of oxygen (O2) to water (H2O).
- The free energy released during this process is linked to the synthesis of ATP.

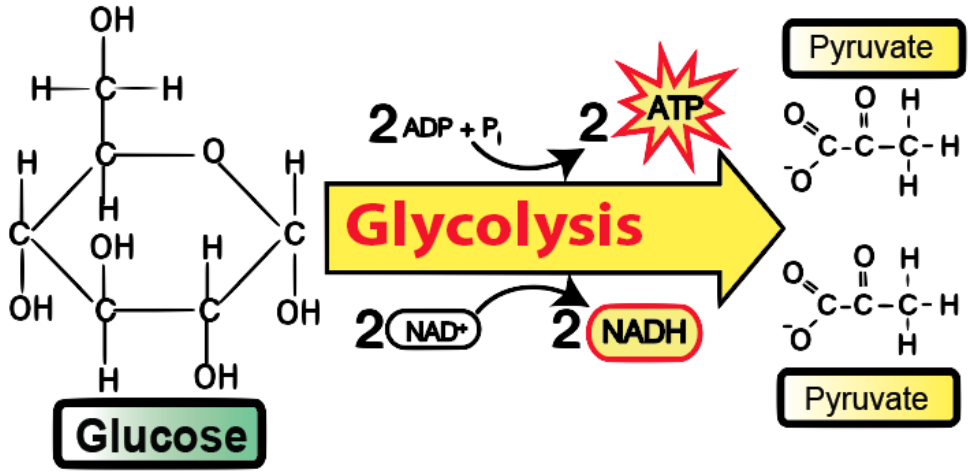


Chemical Standpoint

- Here
  - Sucrose is completely oxidized to CO_2
  - Oxygen serves as the ultimate electron acceptor, being reduced to water.
- The oxidation of sucrose in a series of step-by-step reactions.
- These reactions can be grouped into four major processes:
  a) Glycolysis,
  b) Citric Acid Cycle,
  c) The reactions of the Pentose Phosphate Pathway, and
  d) Oxidative Phosphorylation

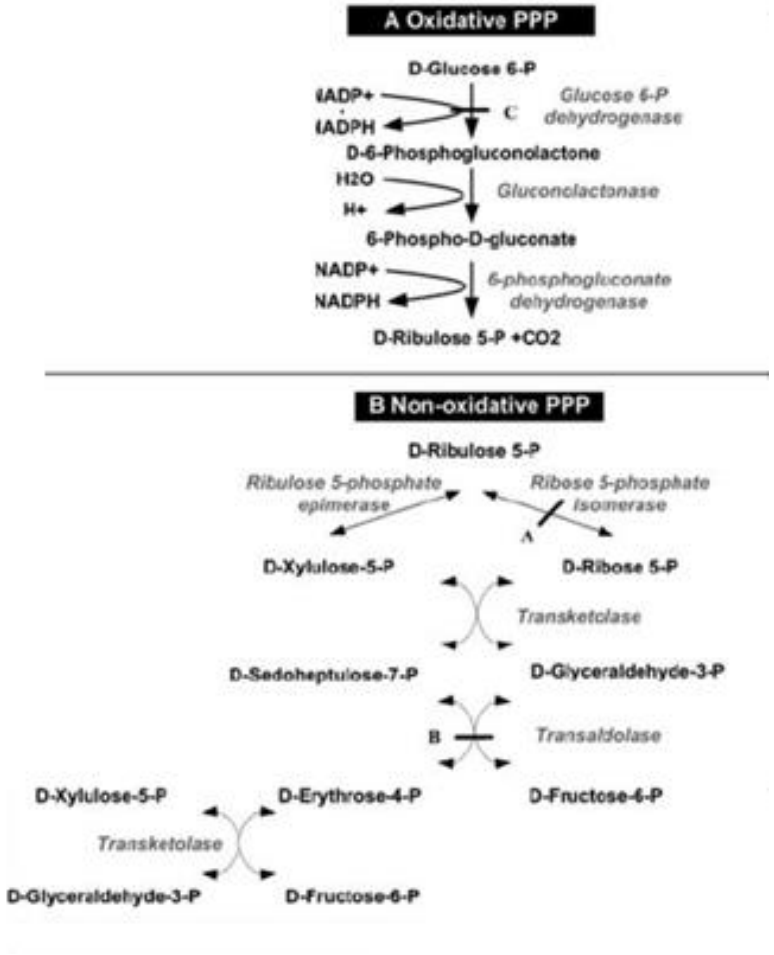
Glycolysis

- It involves a series of reactions carried out by a group of soluble enzymes located in both the
  - Cytosol and
  - The plastid.
- A sugar (sucrose): partly oxidized via
  - Six-carbon sugar phosphates (hexose phosphates) and
  - Three-carbon sugar phosphates (triose phosphates) to produce an organic acid (pyruvate).
- The process yields
  - A small amount of energy as ATP, and
  - Reducing power in the form of a reduced pyridine nucleotide (NADH).



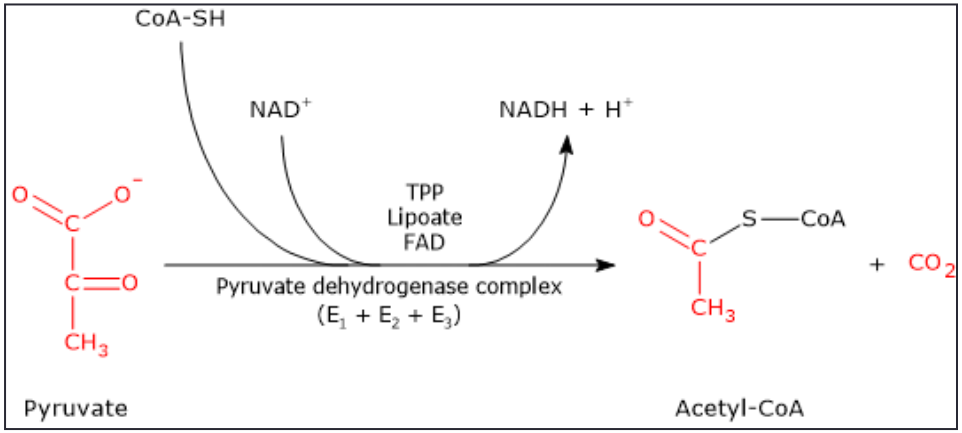
Pentose Phosphate Pathway

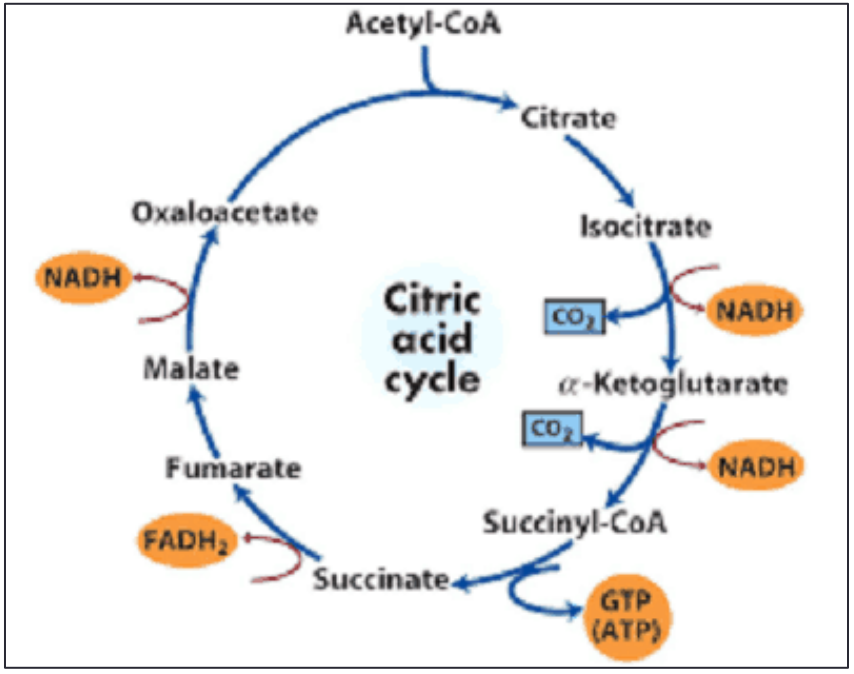
- Located: both in the cytosol and the plastid
- The six-carbon glucose-6-phosphate is initially oxidized to the five-carbon ribulose-5-phosphate.
- The carbon is lost as CO_2, and reducing power is conserved in the form of two molecules of another reduced pyridine nucleotide (NADPH).
- Ribulose-5-phosphate is converted into three- to seven-carbon sugars.



Citric Acid Cycle

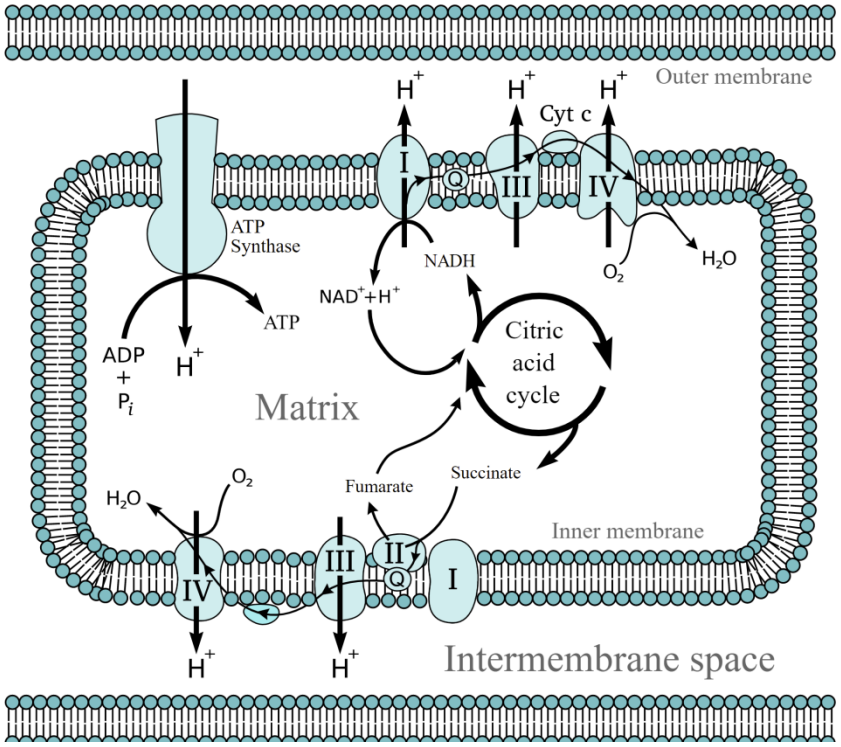
- Pyruvate is oxidized completely to CO_2.
- A considerable amount of reducing power (16 NADH + 4 $FADH_2$ equivalents per **sucrose**) is generated in the process.
- With one exception (succinate dehydrogenase), these reactions involve a series of enzymes located in matrix of the mitochondrion.
- Succinate dehydrogenase is localized in the inner of the two mitochondrial membranes.





Oxidative Phosphorylation

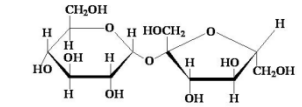
- Electrons are transferred along an electron transport chain.
- It consisting of a collection of electron transport proteins bound to the inner of the two mitochondrial membranes.
- This system transfers electrons from NADH (and related species) to oxygen, during
  - Glycolysis,
  - The Pentose Phosphate Pathway, and
  - The Citric Acid Cycle.
- This electron transfer releases a large amount of free energy
- This is conserved through the synthesis of ATP from ADP and Pi (inorganic phosphate) catalyzed by the enzyme ATP synthase.
- Collectively the redox reactions of the electron transport chain and the synthesis of ATP are called ***oxidative phosphorylation***.
- This final stage completes the oxidation of sucrose.



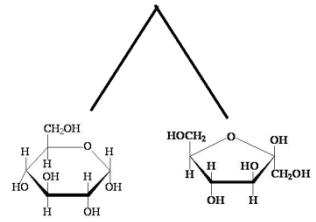
GLYCOLYSIS: A cytosolic and plastidic process

- *Greek words: glyk-, "sweet"* and *lysis*, “dissolution or splitting"
- Glycolysis is the sequence of reactions that metabolizes one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP.
- This process is anaerobic (i.e., it does not require O_2)
- Pyruvate can be further processed anaerobically (fermented) to lactate (lactic acid fermentation) or ethanol (alcoholic fermentation).
- Carbohydrates are converted to hexose phosphates.
- Hexose phosphates are then split into two triose phosphates.
- The triose phosphates are oxidized and rearranged to yield two molecules of pyruvate, an organic acid.
- Besides preparing the substrate for oxidation in the citric acid cycle, glycolysis yields a small amount of chemical energy in the form of ATP and NADH.
- When molecular oxygen is unavailable (plant roots in flooded soils):
  - Glycolysis can be the main source of energy for cells.
  - The fermentation pathways (in the cytosol), reduce pyruvate to recycle the NADH produced by glycolysis.
- The complete glycolytic pathway was elucidated by 1940, Gustav ***Embden***, Otto ***Meyerhof***, Carl Neuberg, Jacob ***Parnas***, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis is also known as the E***mbden-Meyerhof Pathway (EMP Pathway)***.

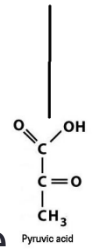
Glycolysis Converts Carbohydrates into Pyruvate



sucrose



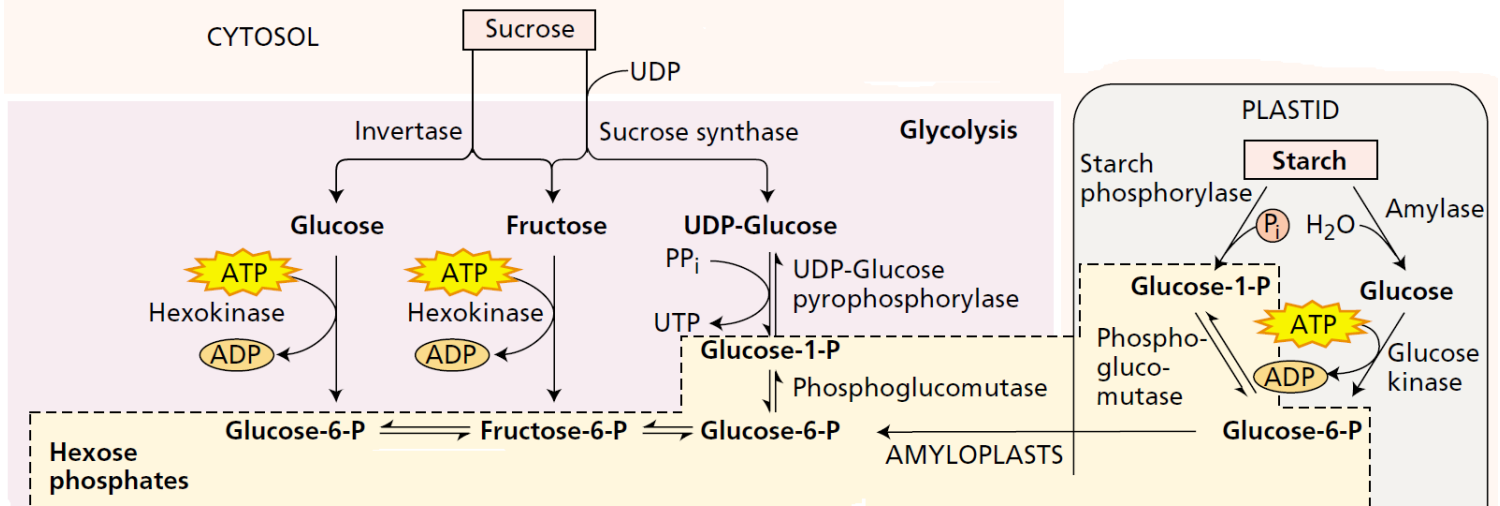
glucose fructose



Pyruvic acid

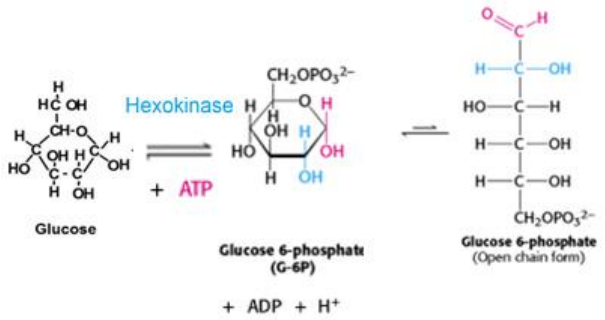
- In animals:
  - substrate of glycolysis is glucose and
  - the end product pyruvate.
- In plants sucrose is the major translocated sugar.
- Sucrose, (not glucose) the true sugar substrate for plant respiration.
- The end products of plant glycolysis include another organic acid, malate.
- In the early steps, sucrose is broken down into the two monosaccharides
  - Glucose and
  - Fructose
- These can readily enter the glycolytic pathway.
- The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps:
  - The first five constitute the ***preparatory phase***
  - The last five constitute the ***payoff phase***

- Sucrose synthase (cytosol): degrade sucrose by combining sucrose with UDP to produce fructose and UDP-glucose.
- UDP-glucose pyrophosphorylase then converts UDP-glucose and pyrophosphate (PPi) into UTP and glucose-6-phosphate.
- In some tissues, invertases (in the cell wall, vacuole, or cytosol) hydrolyze sucrose to its two component hexoses (glucose and fructose).
- The hexoses are then phosphorylated in a reaction that uses ATP.
- Plastids (chloroplasts) can also supply substrates for glycolysis.
  - Starch is synthesized and catabolized only in plastids.
  - Carbon obtained from starch degradation enters the glycolytic pathway in the cytosol primarily as
    - Hexose phosphate (which is translocated out of amyloplasts) or
    - Triose phosphate (which is translocated out of chloroplasts).
  - Plastids convert starch into triose phosphates using isozymes that convert hexose phosphates to triose phosphates.

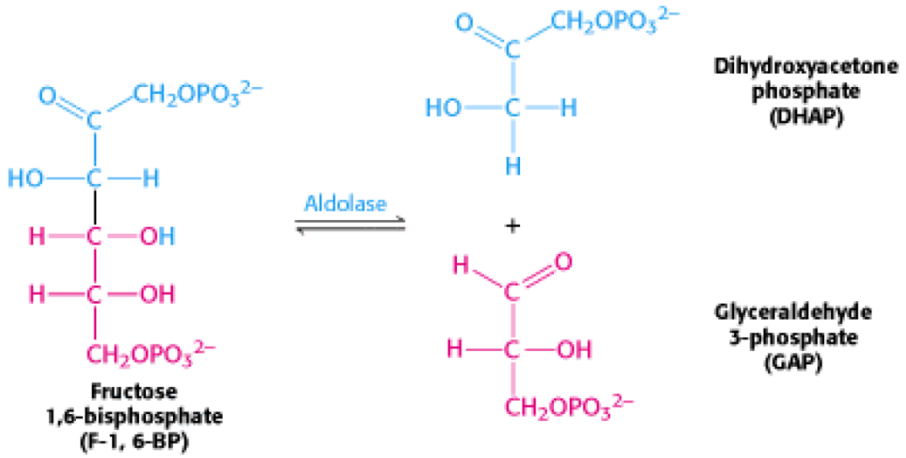


Initial Phase of Glycolysis

- Glucose is phosphorylated by ATP to form glucose 6-phosphate.
- The transfer of the phosphoryl group from ATP to the hydroxyl group on carbon 6 of glucose is catalyzed by hexokinase.

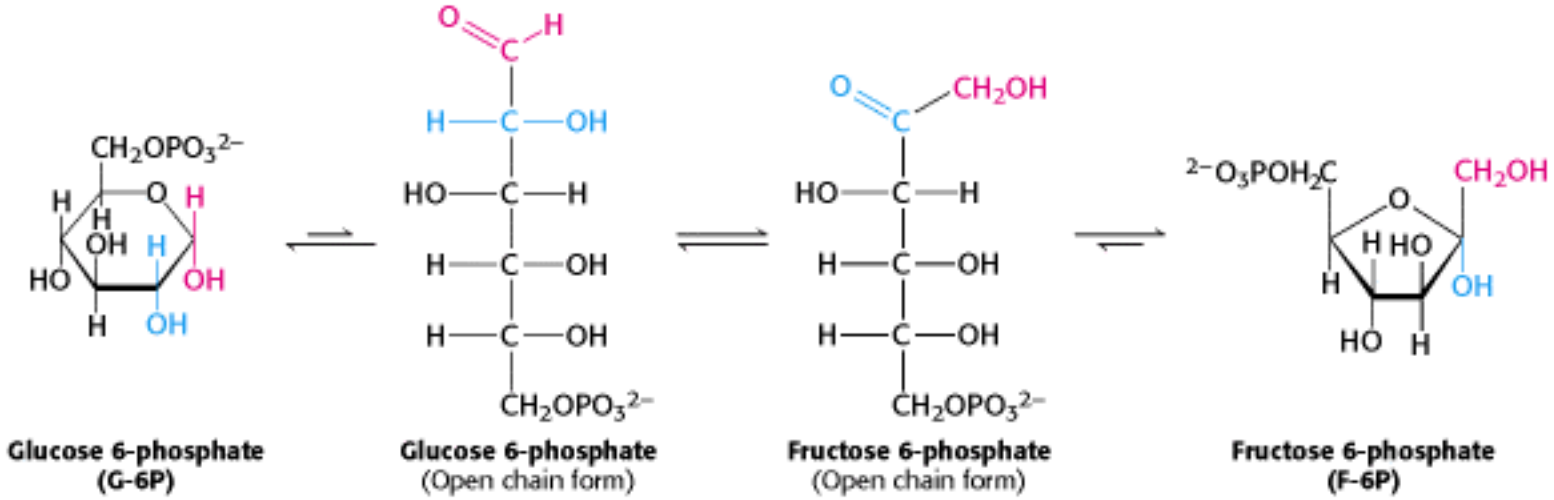


- This step is notable for two reasons:
  - Glucose 6-phosphate cannot diffuse through the membrane, because of its negative charges, and
  - The addition of the phosphoryl group begins to destabilize glucose, thus facilitating its further metabolism.

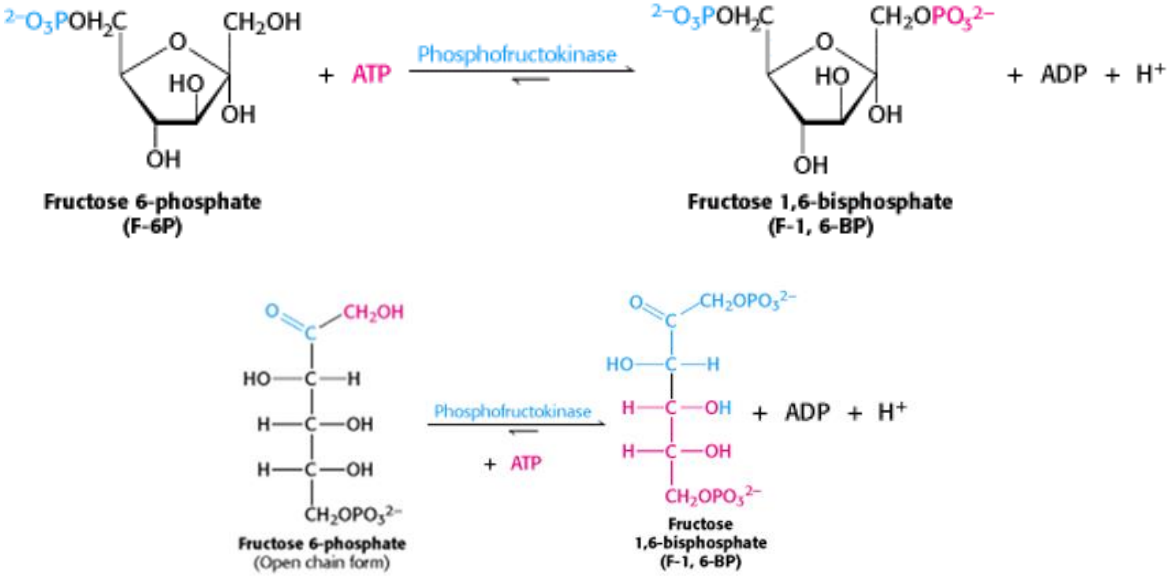


The Formation of Fructose 1,6-bisphosphate from Glucose 6-phosphate

- The next step is the isomerization of glucose 6-phosphate to fructose 6-phosphate.
- The open chain form of glucose has an aldehyde group at carbon 1, whereas the open-chain form of fructose has a keto group at carbon 2.
- Thus, the isomerization is a conversion of an aldose into a ketose.
- The reaction catalyzed by phosphoglucose isomerase includes additional steps because both glucose 6-phosphate and fructose 6-phosphate are present primarily in the cyclic forms.
- The enzyme must first open the six-membered ring of glucose 6-phosphate, catalyze the isomerization, and then promote the formation of the five-membered ring of fructose 6- phosphate.

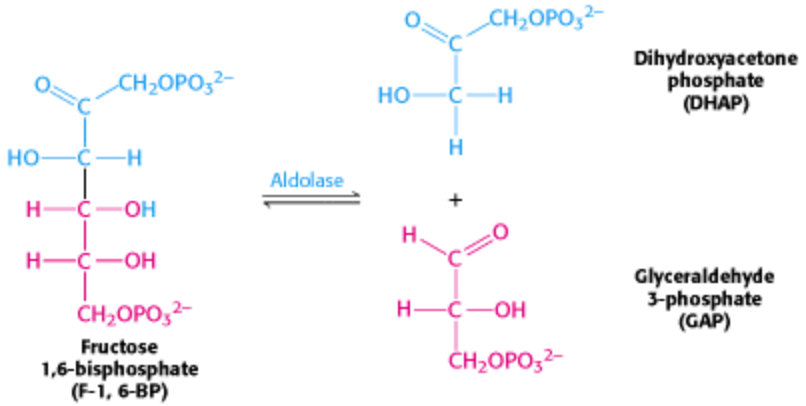


- A second phosphorylation reaction follows the isomerization step.
- Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate (F-1,6-BP).
- The prefix bis- in bisphosphate means that two separate monophosphate groups are present, whereas the prefix di- in diphosphate (as in adenosine diphosphate) means that two phosphate groups are present and are connected by an anhydride bond.
- This This reaction is catalyzed by phosphofructokinase (PFK), an allosteric enzyme that sets the pace of glycolysis.



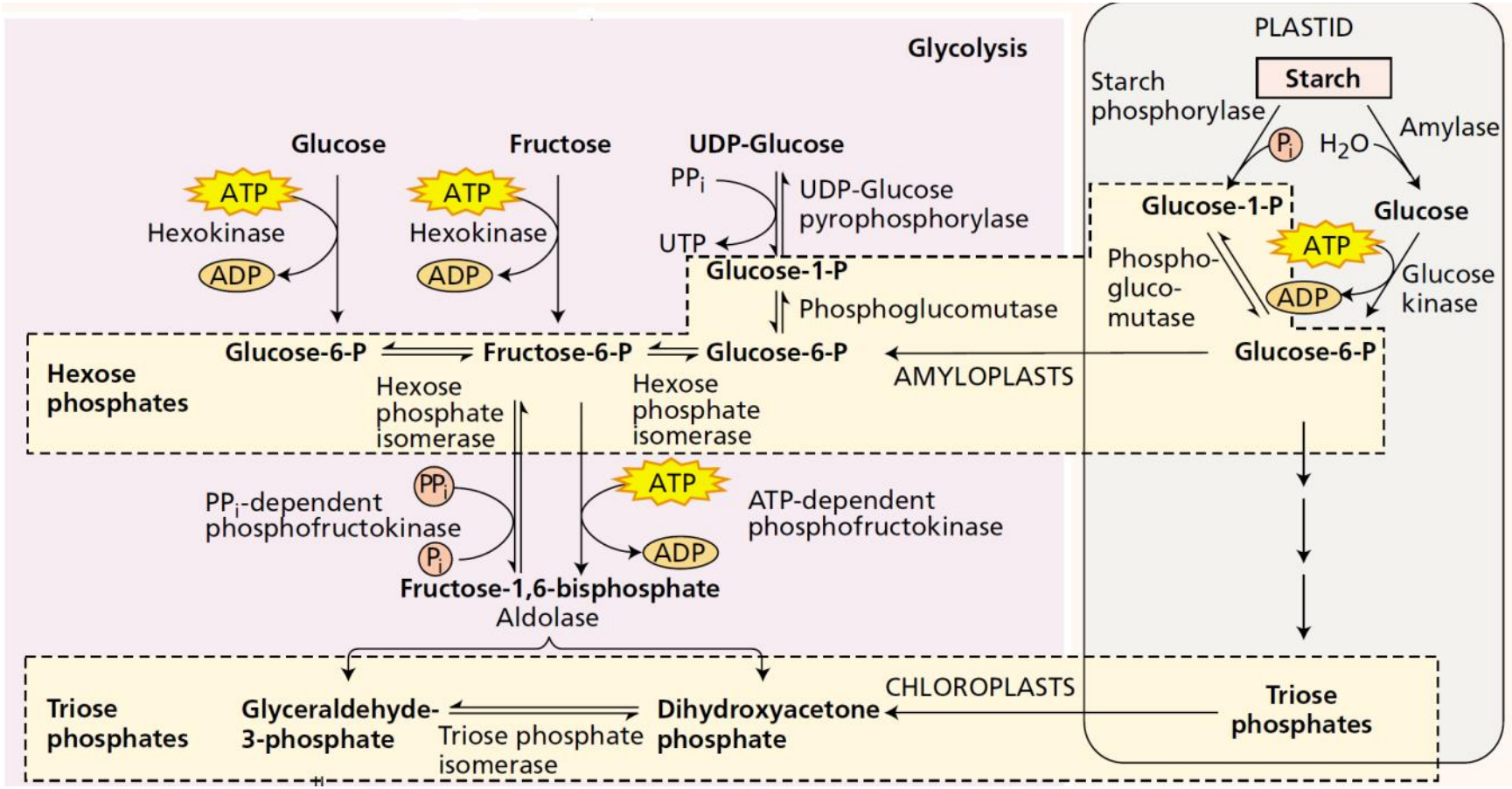
The Six-Carbon Sugar Is Cleaved into Two Three-Carbon Fragments by Aldolase

- The second stage of glycolysis begins with the splitting of fructose 1,6-bisphosphate into
  - Glyceraldehyde 3-phosphate (GAP) and
  - Dihydroxyacetone phosphate (DHAP).
- This reaction is catalyzed by aldolase.
- This enzyme derives its name from the nature of the reverse reaction, an aldol condensation.
- The reaction catalyzed by aldolase is readily reversible under intracellular conditions.

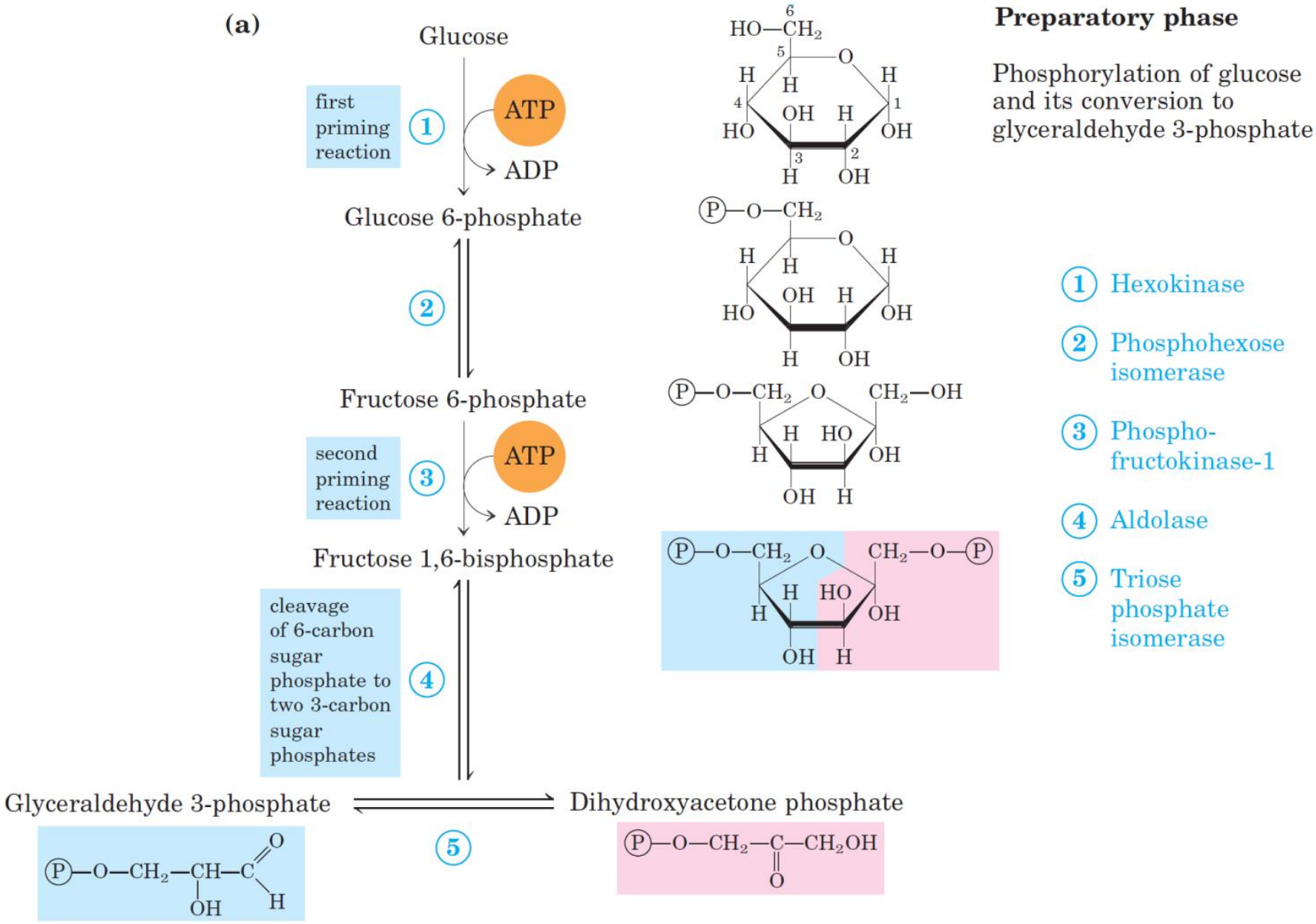


- The products of the remaining steps in glycolysis consist of three carbon units rather than six-carbon units.

- These reactions include **two** of the three essentially irreversible reactions of the glycolytic pathway catalyzed by
  - Hexokinase and
  - Phosphofructokinase
- The phosphofructokinase reaction is one of the control points.
- The products of the remaining steps in glycolysis consist of three carbon units rather than six-carbon units.



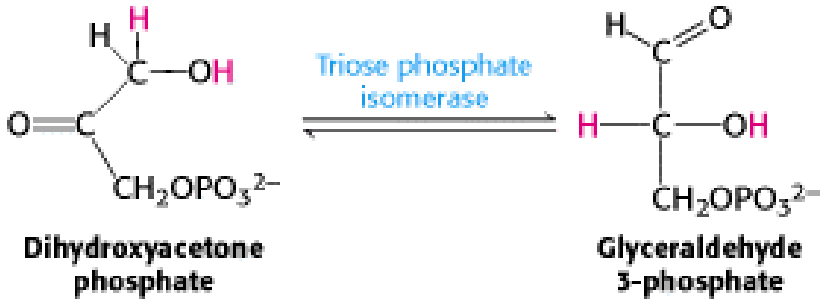
Preparatory phase



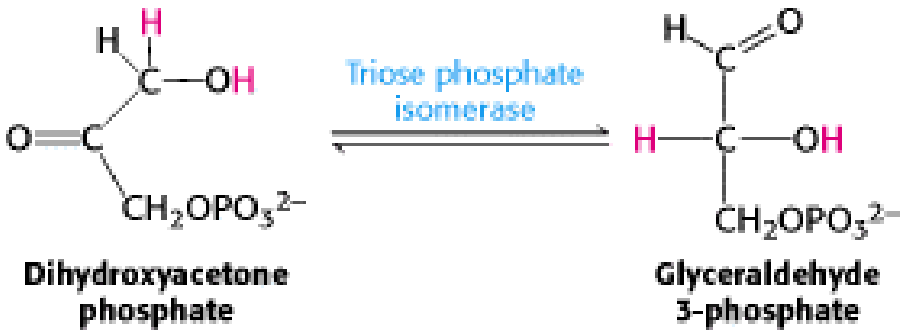
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

The energy-conserving phase of glycolysis:
Triose phosphate isomerase Salvages a Three-Carbon Fragment

- Glyceraldehyde 3-phosphate is on the direct pathway of glycolysis
- Dihydroxyacetone phosphate is not.
- Unless a means exists to convert dihydroxyacetone phosphate into glyceraldehyde 3-phosphate, a three-carbon fragment useful for generating ATP will be lost.
- These compounds are isomers that can be readily interconverted.
- Dihydroxyacetone phosphate is a ketose, whereas glyceraldehyde 3-phosphate is an aldose.
- The isomerization of these three-carbon phosphorylated sugars is catalyzed by *triose phosphate isomerase* (*TIM*).
- This reaction is rapid and reversible.
- At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate.
- However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because the subsequent reactions of glycolysis remove this product.

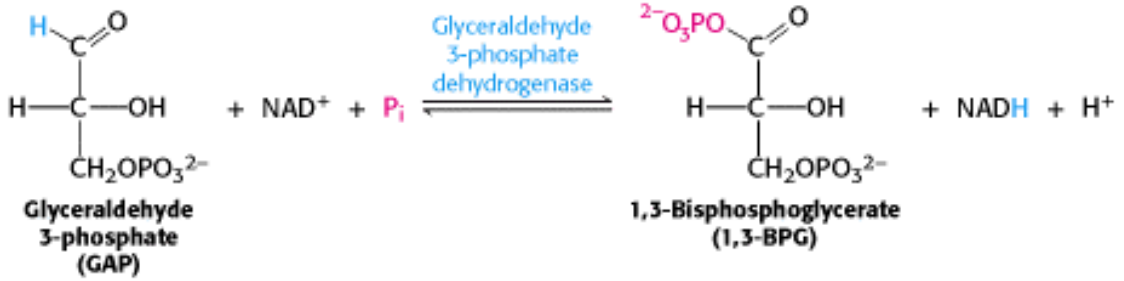


- TIM catalyzes the transfer of a hydrogen atom from carbon 1 to carbon 2 in converting DHAP into glyceraldehyde 3-phosphate, an intramolecular oxidation-reduction.
- The preceding steps in glycolysis have transformed one molecule of glucose into two molecules of glyceraldehyde 3-phosphate.
- No energy has yet been extracted, but 2 molecules of ATP have been invested.
- We come now to a series of steps that harvest some of the energy contained in glyceraldehyde 3-phosphate.

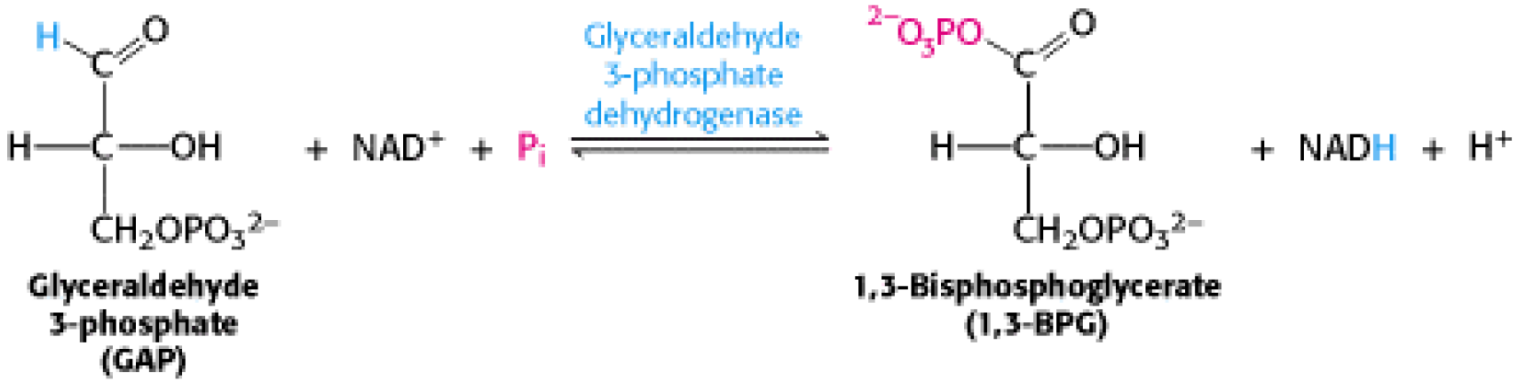


Energy Transformation: Phosphorylation Is Coupled to the Oxidation of Glyceraldehyde 3-phosphate by a Thioester Intermediate

- The preceding steps in glycolysis have transformed one molecule of glucose into two molecules of glyceraldehyde 3- phosphate, but no energy has yet been extracted.
- On the contrary, thus far two molecules of ATP have been invested.
- We come now to a series of steps that harvest some of the energy contained in glyceraldehyde 3-phosphate.
- The initial reaction is the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG)
- This reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase.

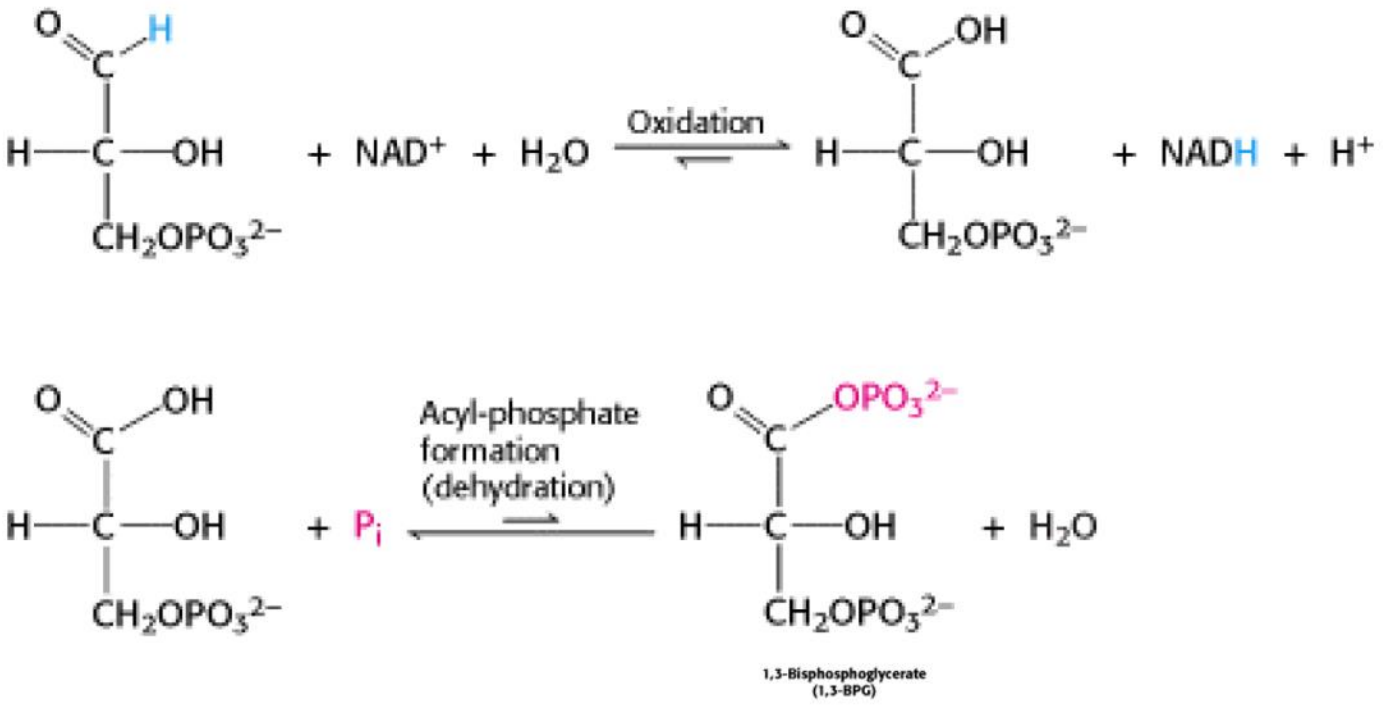


- The enzyme glyceraldehyde-3-phosphate dehydrogenase catalyzes the oxidation of the glyceraldehyde-3-phosphate to a carboxylic acid, reducing NAD+ to NADH.



- 1,3-Bisphosphoglycerate is an acyl phosphate, having a high phosphoryl-transfer potential.
- One of its phosphoryl groups is transferred to ADP catalyzed by glyceraldehyde 3-phosphate dehydrogenase, in the next step.

- It is really the sum of two processes:
  - The oxidation of the aldehyde to a carboxylic acid by NAD^+ and
  - The joining of the carboxylic acid and orthophosphate to form the acyl-phosphate product.



- Phosphoglycerate kinase catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3-bisphosphoglycerate to ADP.
- ATP and 3-phosphoglycerate are the products.

The Formation of ATP from 1,3- Bisphosphoglycerate.

- *Phosphoglycerate kinase* catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3- bisphosphoglycerate to ADP.
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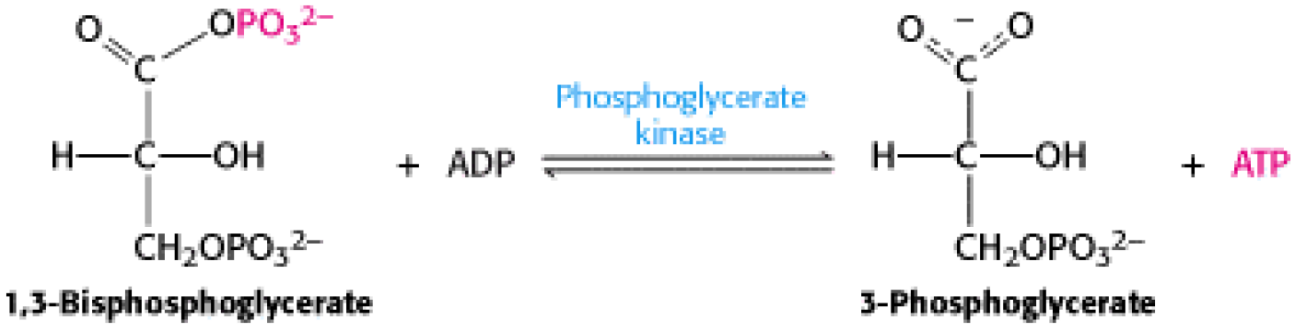
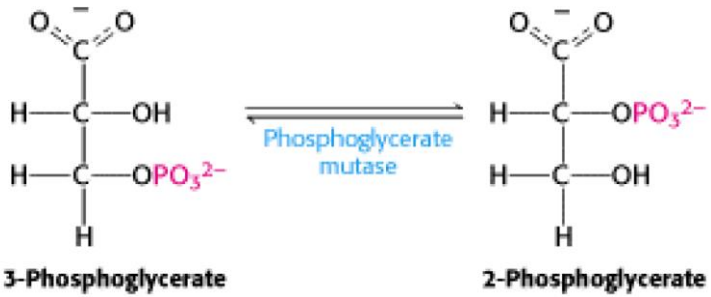


- The phosphate donor, 1,3- BPG, is a substrate with high phosphoryl-transfer potential.
- The formation of ATP in this manner is referred to as *substrate-level phosphorylation*
- The outcomes of the reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase are:
  - Glyceraldehyde 3-phosphate, an aldehyde, is oxidized to 3-phosphoglycerate, a carboxylic acid.
  - NAD^+ is concomitantly reduced to NADH.
  - ATP is formed from Pi and ADP at the expense of carbon oxidation energy.

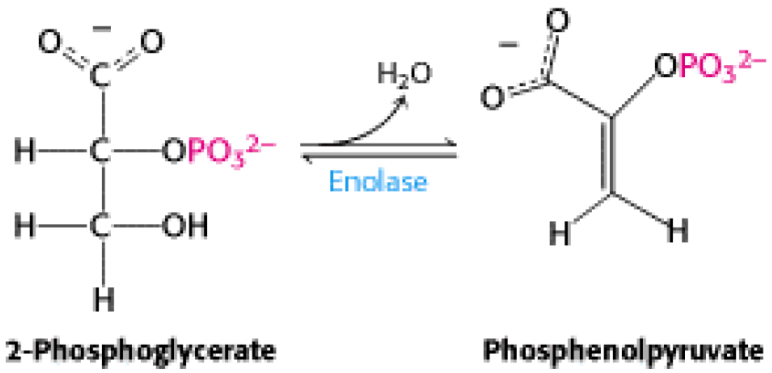
The Generation of Additional ATP and the Formation of Pyruvate

- In the remaining steps of glycolysis:
  - **3-phosphoglycerate** is converted into **pyruvate** with the concomitant conversion of ADP into ATP.
- The first reaction is a rearrangement.
- The position of the phosphoryl group shifts in the conversion of 3-phosphoglycerate into 2-phosphoglycerate, catalyzed by phosphoglycerate mutase.



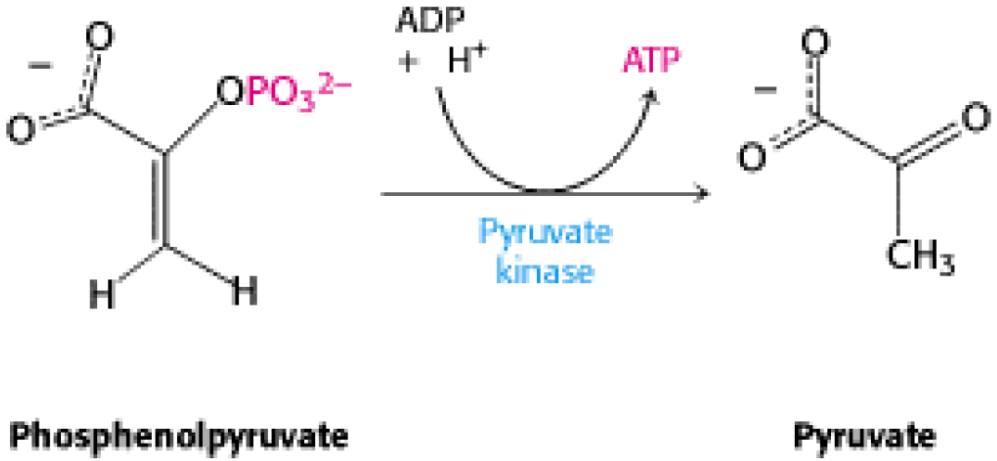
- A mutase is an enzyme that catalyzes the intramolecular shift of a chemical group (phosphoryl group).

- Next, an *enol* is formed by the dehydration of 2-phosphoglycerate.
- *Enolase* catalyzes the formation of *phosphoenolpyruvate* (PEP).



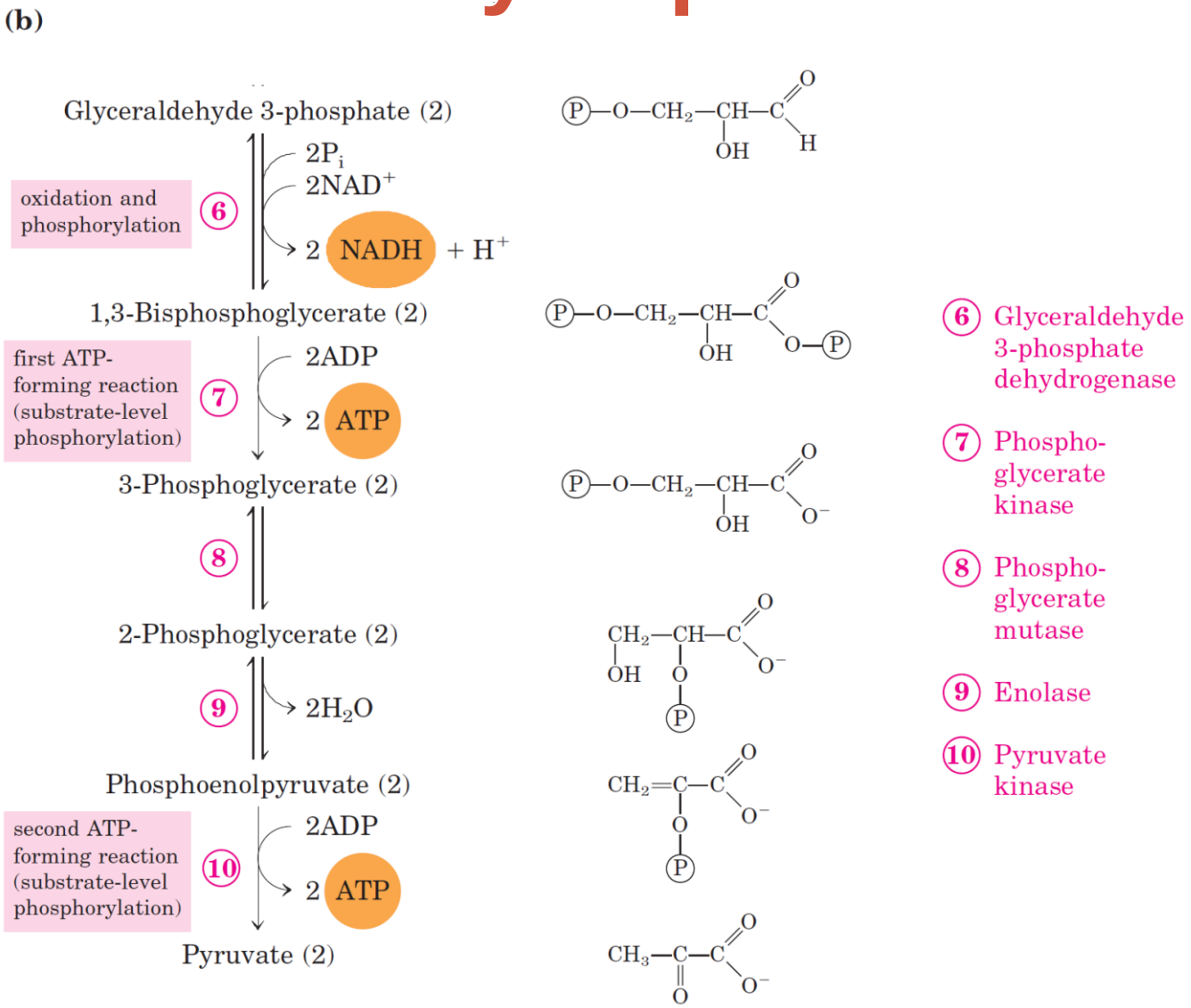
- This dehydration markedly elevates the transfer potential of the phosphoryl group.
- An *enol phosphate* has a high phosphoryl-transfer potential, whereas the phosphate ester, such as 2-phosphoglycerate, of an ordinary alcohol has a low one.

- The high phosphoryl-transfer potential of phosphoenolpyruvate arises primarily from the large driving force of the subsequent enol-ketone conversion.



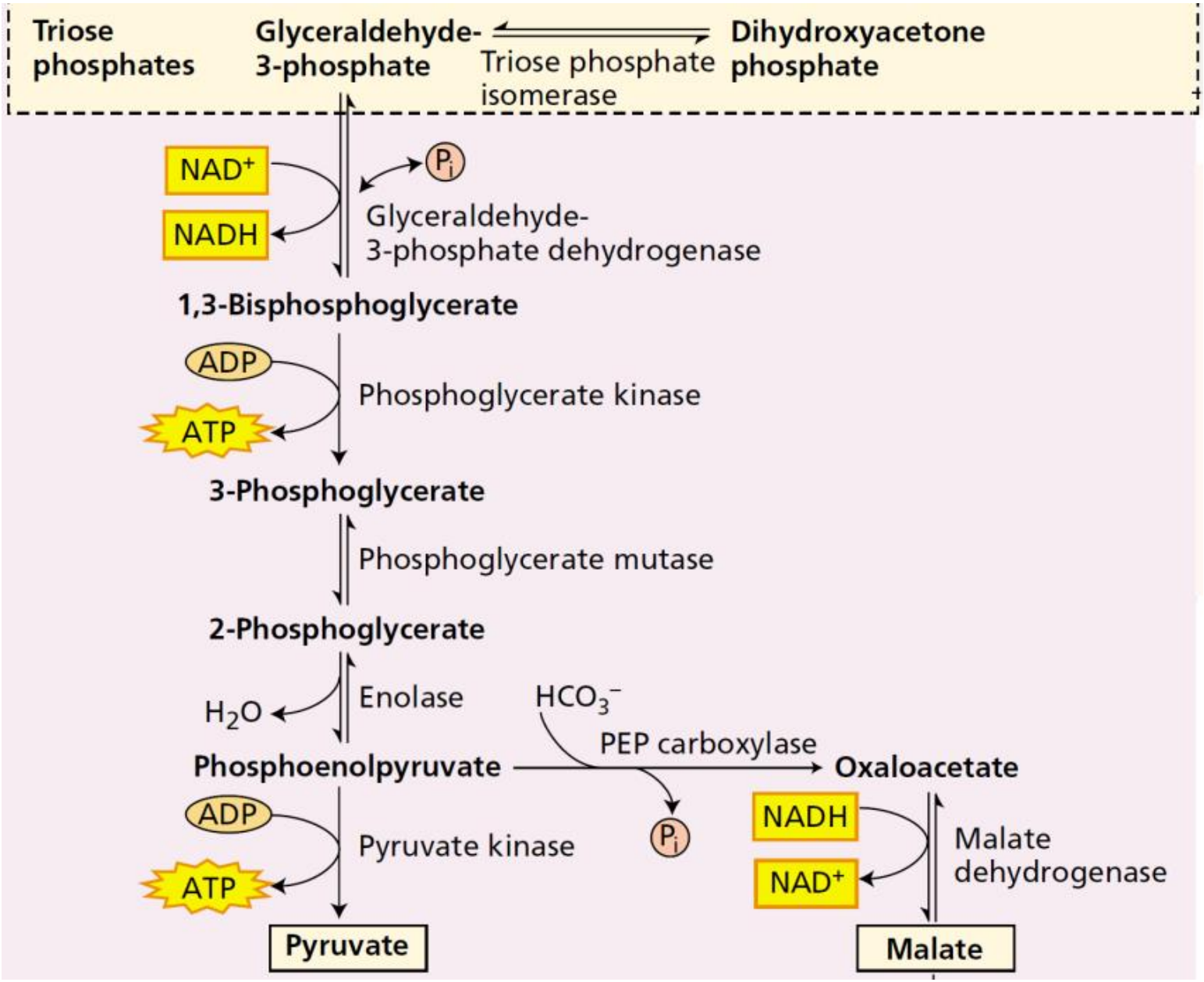
- Pyruvate is formed, and ATP is generated concomitantly.
- The irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by pyruvate kinase.
- Because the molecules of ATP used in forming fructose 1,6-bisphosphate have already been regenerated, the two molecules of ATP generated from phosphoenolpyruvate are "profit."

Payoff phase



Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH

Glycolysis


Triose phosphates
Glyceraldehyde-3-phosphate
Triose phosphate isomerase
Dihydroxyacetone phosphate
NAD+
NADH
Pi
Glyceraldehyde-3-phosphate dehydrogenase
1,3-Bisphosphoglycerate
ADP
ATP
Phosphoglycerate kinase
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
H2O
Enolase
HCO3−
PEP carboxylase
Phosphoenolpyruvate
Oxaloacetate
ADP
ATP
Pyruvate kinase
Pi
NADH
NAD+
Malate dehydrogenase
Pyruvate
Malate

Energy Yield in the Conversion of Glucose into Pyruvate

- The net reaction in the transformation of glucose into pyruvate is:

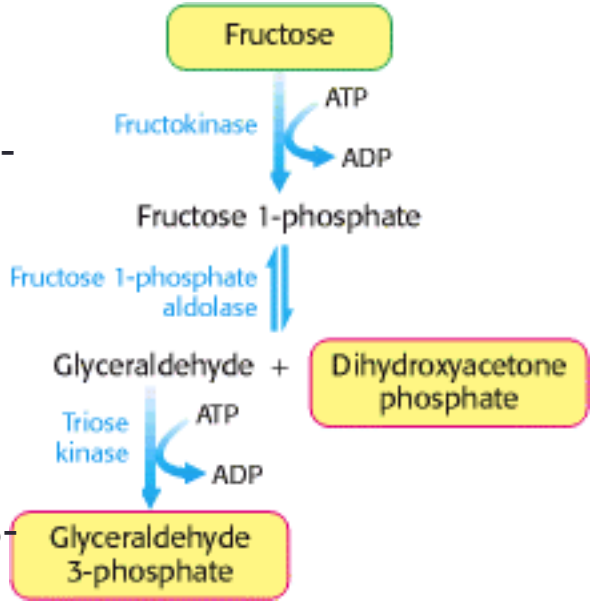
$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} + 2\ \text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H^+ + 2\ H_2O$$

- Two molecules of ATP are generated in the conversion of glucose into two molecules of pyruvate.

Step	Reaction	Enzyme	Reaction type
1	Glucose + ATP $\longrightarrow$ Glucose-6P+ADP+H^+	Hexokinase	Phosphoryl transfer
2	Glucose 6-phosphate $\rightleftharpoons$ Fructose-6-P	Phosphoglucose isomerase	Isomerization
3	Fructose 6-phosphate + ATP $\longrightarrow$ Fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer
4	Fructose 1,6-bisphosphate $\rightleftharpoons$ DHAP + Glyceraldehyde 3-P	Aldolase	Aldol cleavage
5	DHAP $\rightleftharpoons$ Glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization
6	Glyceraldehyde 3-phosphate + Pi + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation
7	1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer
8	3-Phosphoglycerate $\rightleftharpoons$ 2- Phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift
9	2-Phosphoglycerate $\rightleftharpoons$ PEP + H_2O	Enolase	Dehydration
10	PEP + ADP + H^+ $\longrightarrow$ Pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer

The Entry of Fructose and Galactose into Glycolysis

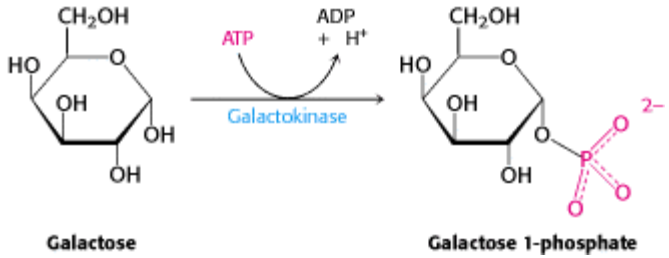
- Glucose is the most widely used monosaccharide.
- Others also are important fuels: Two abundant sugars
  - Fructose and
  - Galactose can be funneled into the glycolytic pathway.
- Fructose is metabolized by the liver, using the fructose 1-phosphate pathway.
- The first step is the phosphorylation of fructose to fructose 1-phosphate by fructokinase.
- Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate, an intermediate in glycolysis.
- This aldol cleavage is catalyzed by a specific fructose 1-phosphate aldolase.
- Glyceraldehyde is then phosphorylated to glyceralde-hyde 3-phosphate, a glycolytic intermediate, by triose kinase.
- Alternatively, fructose can be phosphorylated to fructose 6-phosphate by hexokinase.
- However, the affinity of hexokinase for glucose is 20 times as great as it is for fructose.



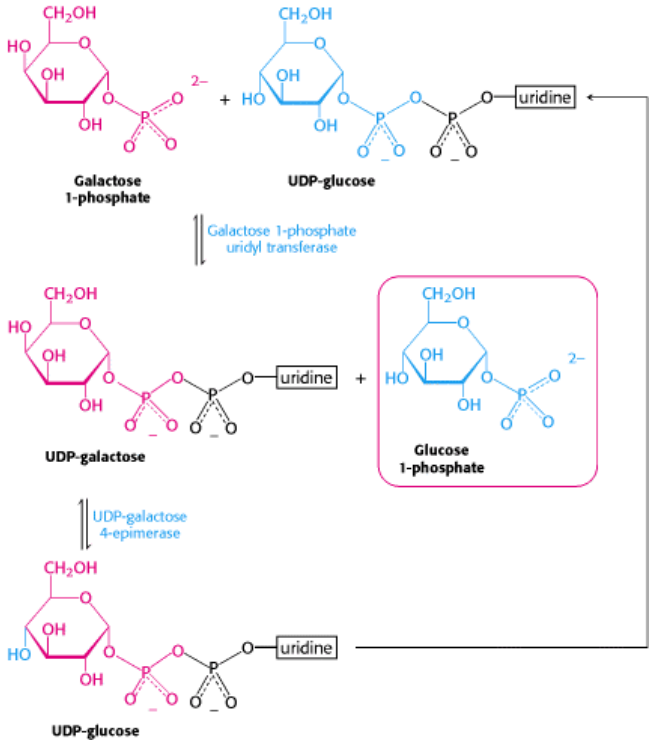
- Little fructose 6-phosphate is formed in the liver because glucose is so much more abundant in this organ.
- Moreover, glucose, as the preferred fuel, is also trapped in the muscle by the hexokinase reaction.
- Because liver and muscle phosphorylate glucose rather than fructose, adipose tissue is exposed to more fructose than glucose.
- Most of the fructose in adipose tissue is metabolized through fructose 6-phosphate.

Conversion of galactose into glucose

- Galactose is converted into glucose 6-phosphate in four steps.
  1. The first reaction: The phosphorylation of galactose to galactose 1-phosphate by galactokinase.



  2. Galactose 1-phosphate then acquires a uridyl group from uridine diphosphate glucose (UDP-glucose).



- UDP-glucose is catalyzed by *galactose 1-phosphate uridyl transferase* to UDP-galactose and glucose 1-phosphate.
- The galactose moiety of UDP-galactose is then epimerized to glucose.
- The configuration of the hydroxyl group at carbon 4 is inverted by UDP-galactose 4-epimerase.
- The sum of the reactions catalyzed by galactokinase, the transferase, and the epimerase is:

$$\text{Galactose} + \text{ATP} \longrightarrow \text{glucose 1-phosphate} + \text{ADP} + \text{H}^+$$

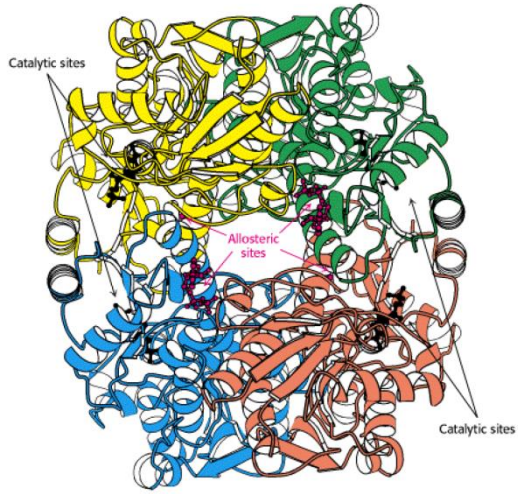
- Finally, glucose 1-phosphate, is isomerized to glucose 6-phosphate by phosphoglucomutase.

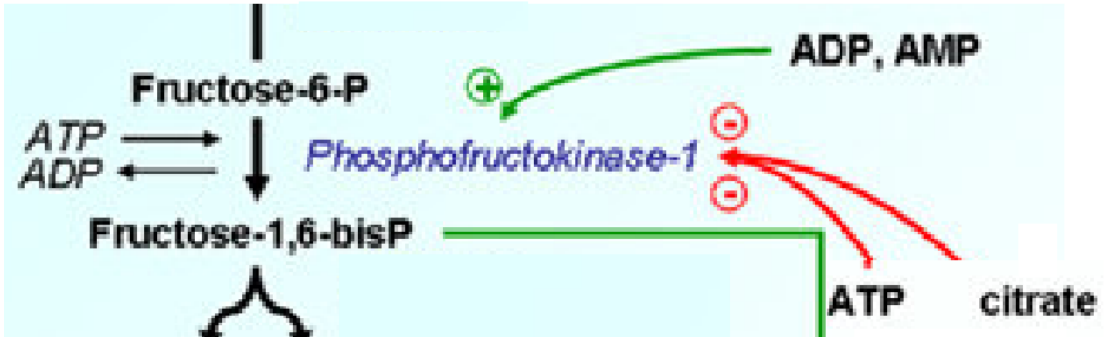
The Glycolytic Pathway Is Tightly Controlled

- The rate of conversion of glucose into pyruvate is regulated to meet two major cellular needs:
  - The **production of ATP**, generated by the degradation of glucose, and
  - The provision of **building blocks** for synthetic reactions, such as the formation of fatty acids.
- In metabolic pathways, enzymes catalyzing essentially **irreversible reactions** are potential sites of control.
- In glycolysis, the reactions catalyzed by
  - *Hexokinase*,
  - *Phosphofructokinase*, and
  - *Pyruvate kinase* are **irreversible**.
- These enzymes have regulatory as well as catalytic roles.
- Their activities are regulated by
  - The reversible binding of allosteric effectors or
  - By covalent modification.
- In addition, the **amounts** of these important enzymes are varied by the **regulation of transcription** to meet changing metabolic needs.

Phosphofructokinase Is the Key Enzyme in the Control of Glycolysis

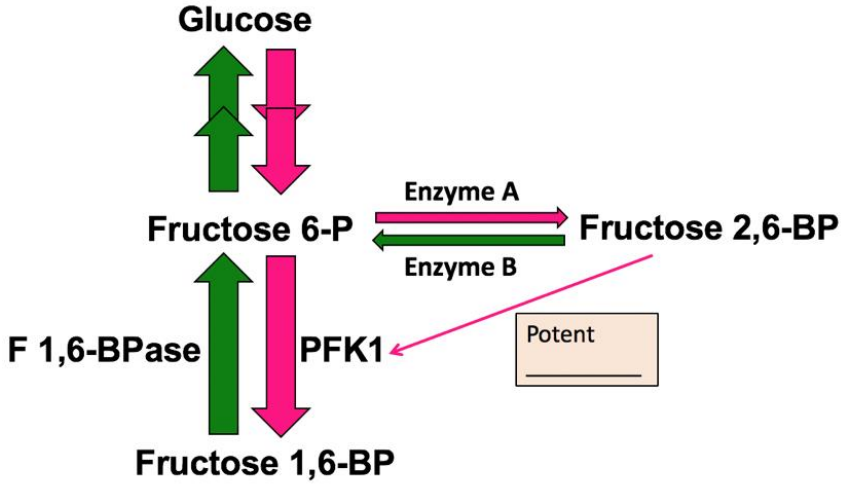
- Phosphofructokinase is the most important control element in the glycolytic pathway.
- Phosphofructokinase in the liver is a tetramer of four identical subunits.

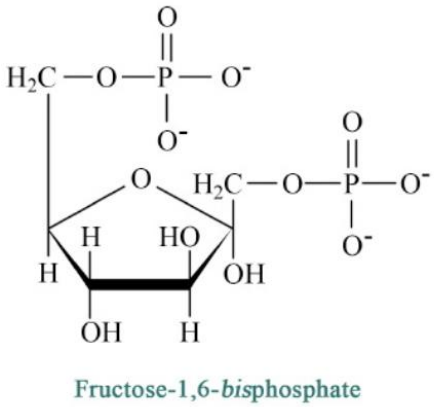


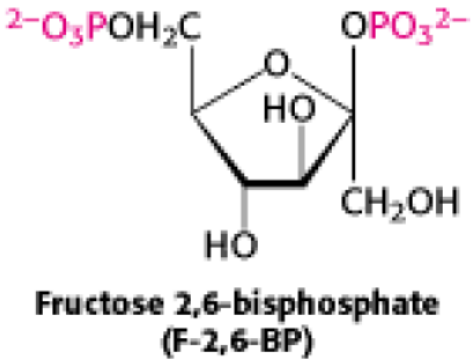


1. High levels of ATP allosterically inhibit the enzyme, lowering its affinity for fructose 6-phosphate.
   a) ATP elicits this effect by binding to a specific regulatory site that is distinct from the catalytic site.
   b) AMP reverses the inhibitory action of ATP.
   c) The activity of the enzyme increases when the ATP/AMP ratio is lowered.
   d) In other words, glycolysis is stimulated as the energy charge falls.
2. A fall in pH also inhibits phosphofructokinase activity.
   a) The inhibition of phosphofructokinase by H^+ prevents excessive formation of lactic acid and a precipitous drop in blood pH (acidosis).

3) Phosphofructokinase is inhibited by citrate, an early intermediate in the citric acid cycle.
   a) A high level of citrate means that biosynthetic precursors are abundant.
   b) Additional glucose should not be degraded for this purpose.
   c) Citrate inhibits phosphofructokinase by enhancing the inhibitory effect of ATP.
4) In 1980, ***fructose 2,6-bisphosphate*** (F-2,6-BP) was identified as a ***potent activator*** of phosphofructokinase.
   a) Fructose 2,6-bisphosphate:
      i. Activates phosphofructokinase: by increasing its affinity for fructose 6-phosphate and
      ii. Diminishing the inhibitory effect of ATP.
   b) Fructose 2,6-bisphosphate is an ***allosteric activator:*** It shifts the conformational equilibrium of this tetrameric enzyme from the T state (a low-activity, low-affinity "tense“) to the R state (a high-activity, high-affinity"relaxed“).

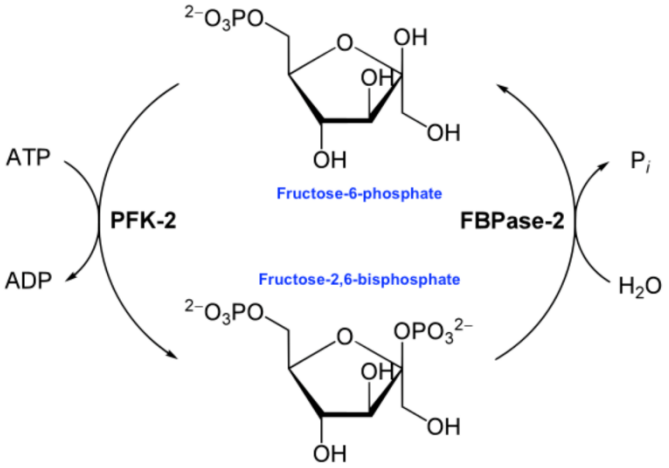


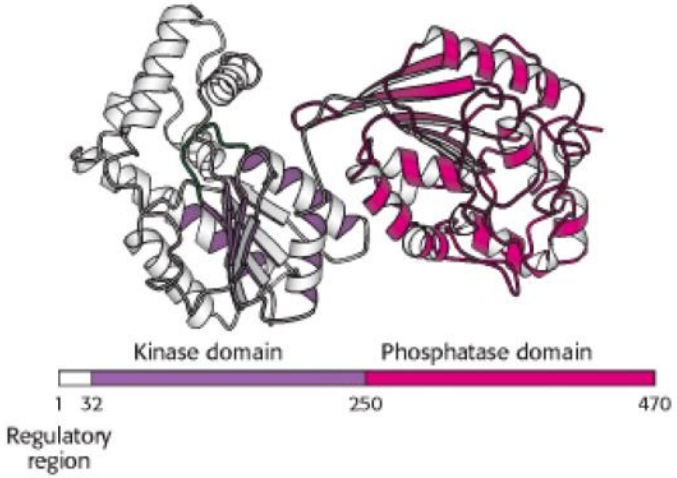




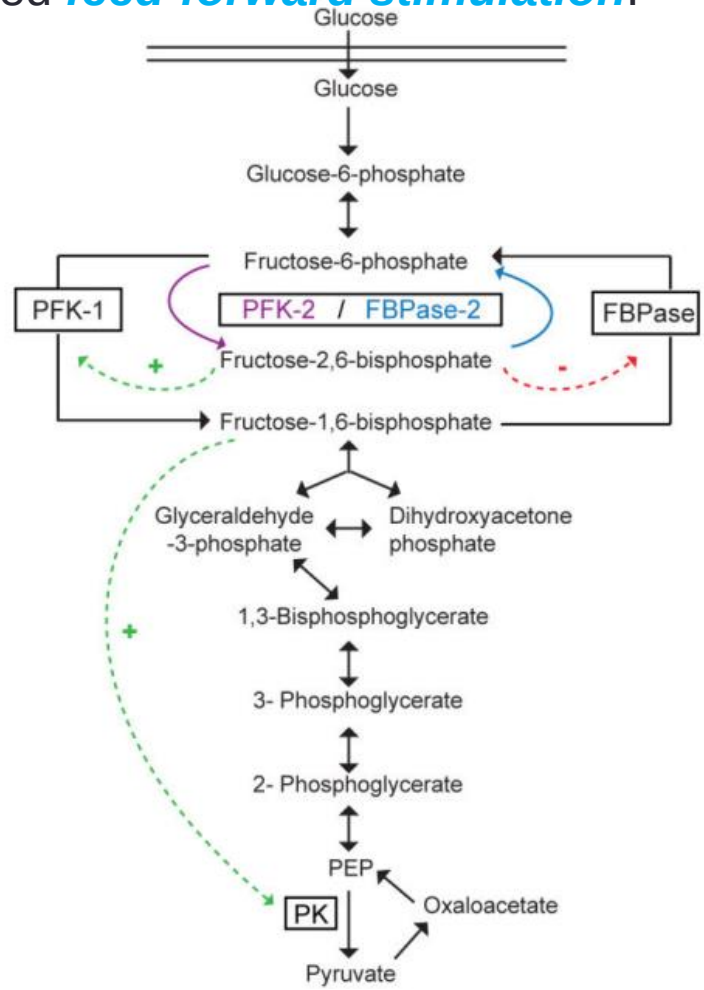
A Regulated Bifunctional Enzyme Synthesizes and Degrades Fructose 2,6 - bisphosphate

- **Two enzymes** regulate the concentration of this important **regulator** of glycolysis by:
  - Phosphorylating fructose 6-phosphate and
  - Dephosphorylating fructose 2,6-bisphosphate.
- ***Phosphofructokinase 2*** (***PFK2***):
  - A different enzyme from phosphofructokinase.
  - Forms Fructose 2,6-bisphosphate .
- ***Fructose bisphosphatase 2*** (***FBPase2***):
  - A specific phosphatase.
  - Hydrolyzes the Fructose 2,6-bisphosphate to fructose 6-phosphate.
- Both PFK2 and FBPase2 are present in a single 55-kd polypeptide chain
- This bifunctional enzyme contains
  - An N-terminal **regulatory domain**,
  - Followed by a kinase domain and
  - A phosphatase domain.

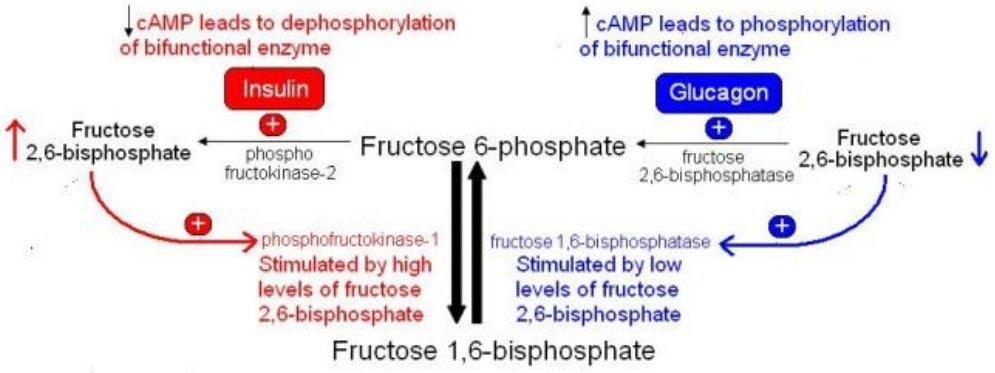




- In the liver, the concentration of fructose 6-phosphate rises when blood-glucose concentration is high.
- The abundance of fructose 6-phosphate accelerates the synthesis of F-2,6-BP.
- An abundance of fructose 6-phosphate leads to a higher concentration of F-2,6-BP.
- This in turn stimulates phosphofructokinase.
- Such a process is called ***feed-forward stimulation***.

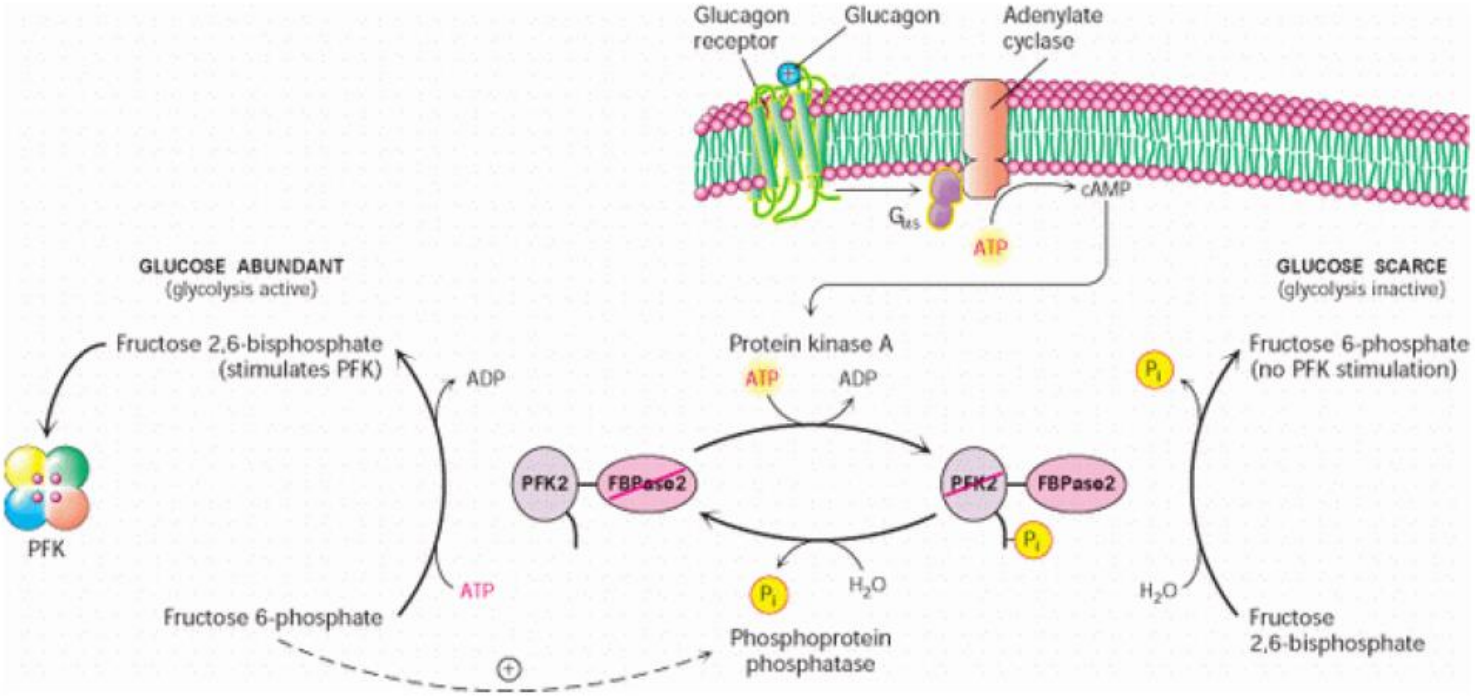


- The activities of PFK2 and FBPase2 are ***reciprocally controlled*** by phosphorylation of a single serine residue.
- When glucose is scarce:
  - A rise in the blood level of the hormone glucagon triggers a cyclic AMP cascade.
  - This leads the phosphorylation of this bifunctional enzyme by protein kinase A.
  - This covalent modification activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP.
  - Glucose metabolism by the liver is curtailed.
- When glucose is abundant:
  - The enzyme loses its attached phosphate group.
  - This covalent modification activates PFK2 and inhibits FBPase2.
  - This raises the level of F-2,6-BP and accelerating glycolysis.
- This coordinated control is facilitated by the location of the kinase and phosphatase domains on the same polypeptide chain as the regulatory domain.



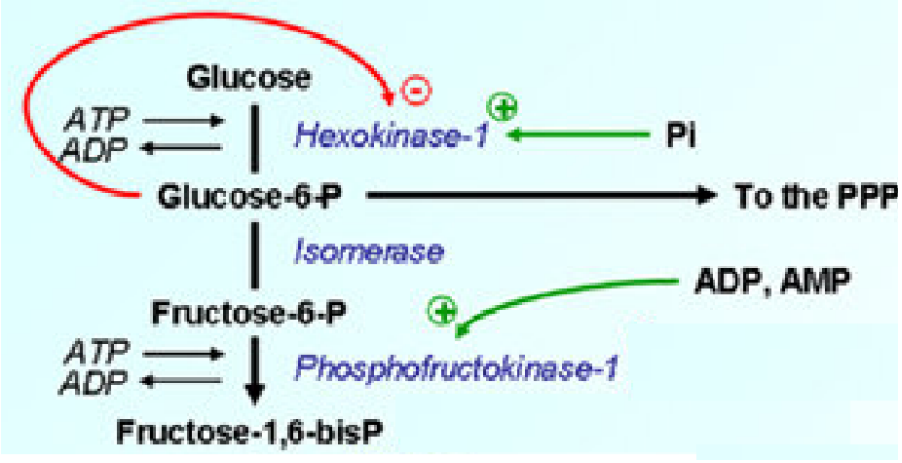
Control of the Synthesis and Degradation of Fructose 2,6-Bisphosphate

- A low blood-glucose level
  - Is signaled by glucagon
  - This leads to the phosphorylation of the bifunctional enzyme and
  - Leads to a lower level of fructose 2,6- bisphosphate.
  - Slowing glycolysis.
- High levels of fructose 6-phosphate
  - Facilitate the dephosphorylation of the bifunctional enzyme
  - Accelerate the formation of fructose 2,6-bisphosphate.

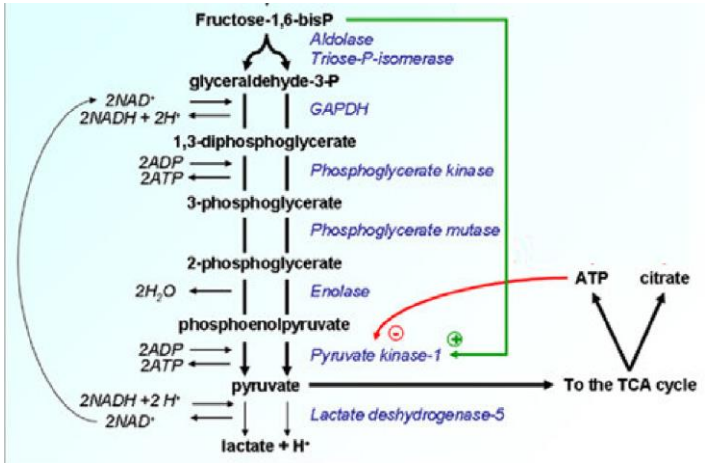


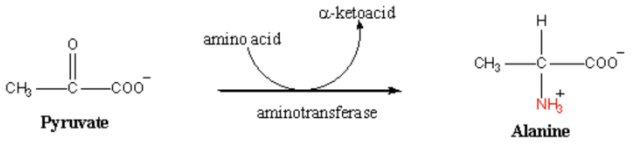
Hexokinase and Pyruvate kinase Also Set the Pace of Glycolysis

- Hexokinase:
  - The enzyme catalyzing the first step of glycolysis.
  - It is inhibited by its product: Glucose 6-phosphate.
  - High concentrations of Glucose 6-phosphate signal that the cell no longer requires glucose for energy
  - But it is needed for storage in the form of glycogen, or as a source of biosynthetic precursors.
  - The glucose will be left in the blood.
  - When phosphofructokinase is inactive:
    - The concentration of fructose 6-phosphate rises.
    - In turn, the level of glucose 6-phosphate rises because it is in equilibrium with fructose 6-phosphate.
    - Hence, the inhibition of phosphofructokinase leads to the inhibition of hexokinase.



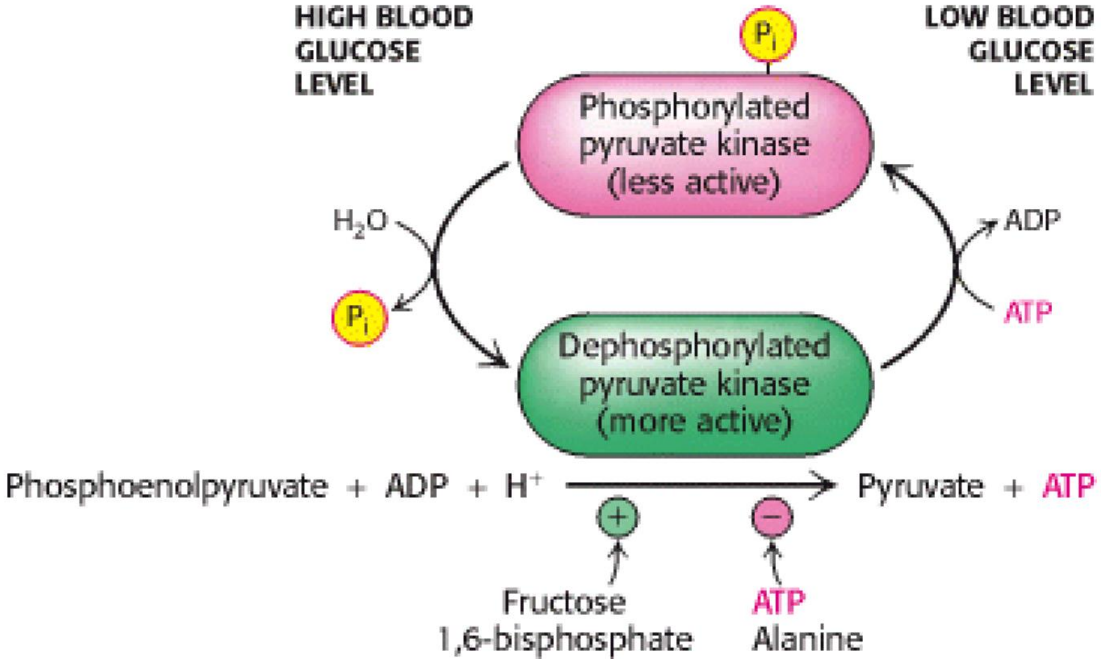
- Pyruvate kinase:
  - The enzyme catalyzing the third irreversible step in glycolysis.
  - It controls the outflow from this pathway.
  - This final step yields ATP and pyruvate:
    - It can be oxidized further or
    - It can be used as a building block.
  - Fructose 1,6-bisphosphate:
    - The product of the preceding irreversible step in glycolysis.
    - Activates Pyruvate kinase.
    - It enables them to keep pace with the oncoming high flux of intermediates.
  - ATP allosterically inhibits the pyruvate kinase to slow glycolysis when the energy charge is high.
  - Alanine (synthesized in one step from pyruvate) also allosterically inhibits the pyruvate kinases
    - It signals that building blocks are abundant.

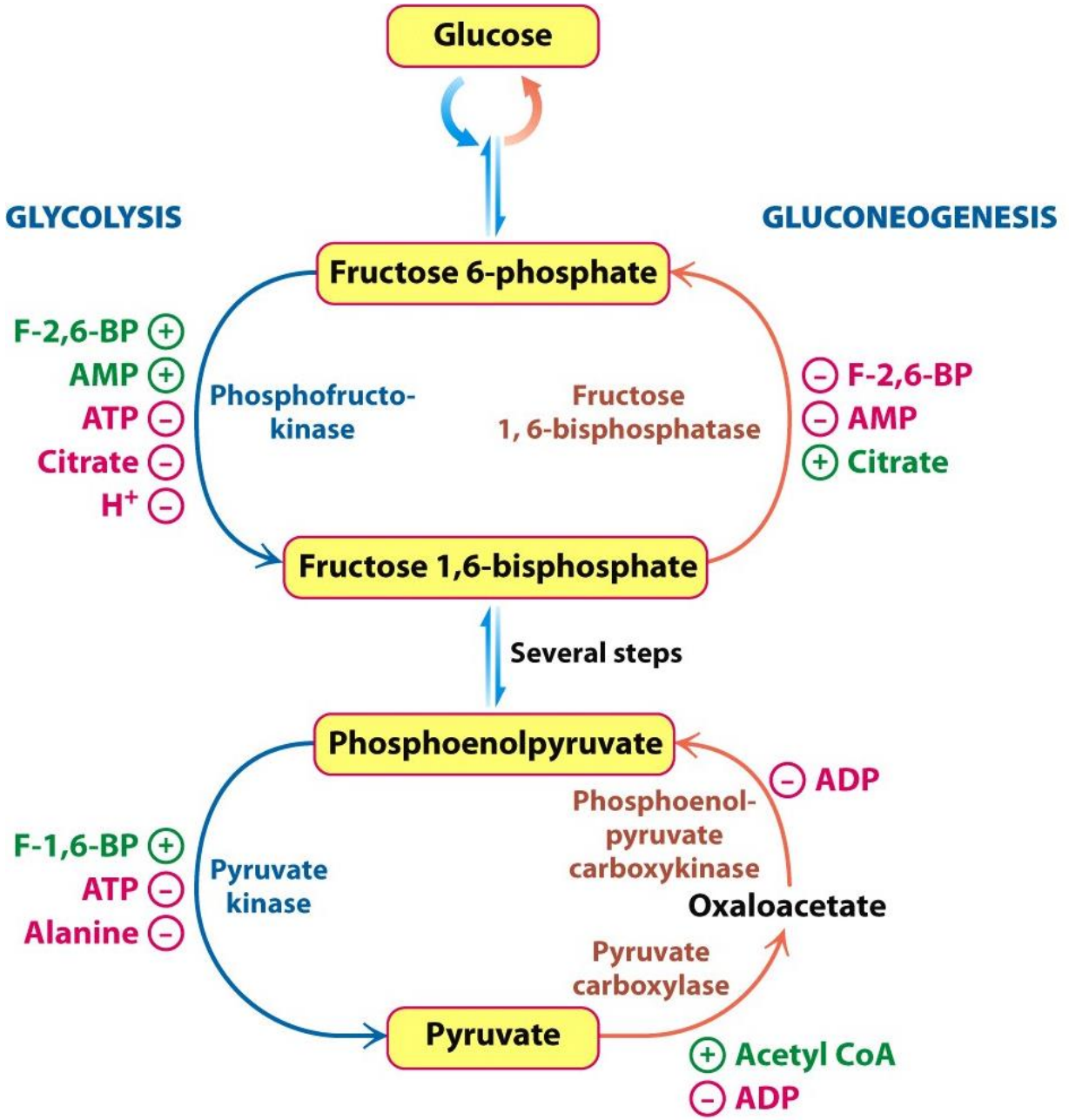




Control of the Catalytic Activity of Pyruvate Kinase

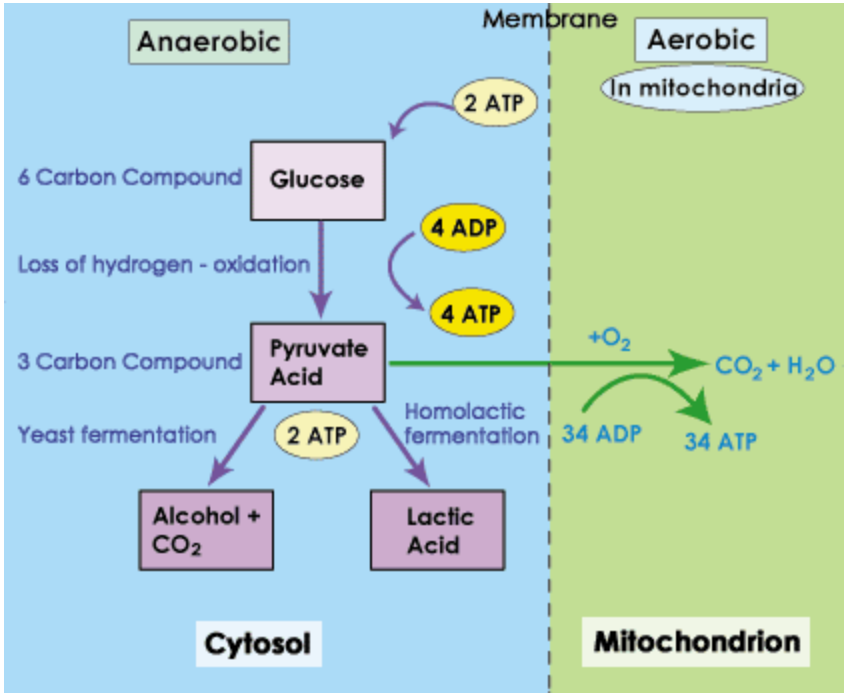
- Pyruvate kinase is regulated by ***allosteric effectors*** and ***covalent modification***.
- When the blood-glucose level is **low**:
  - The glucagon-triggered cyclic ***AMP cascade***
  - This leads to the ***phosphorylation*** of pyruvate kinase.
  - This ***diminishes*** its activity.
  - These hormone-triggered phosphorylations, prevent the liver from consuming glucose when it is more urgently needed by brain and muscle.

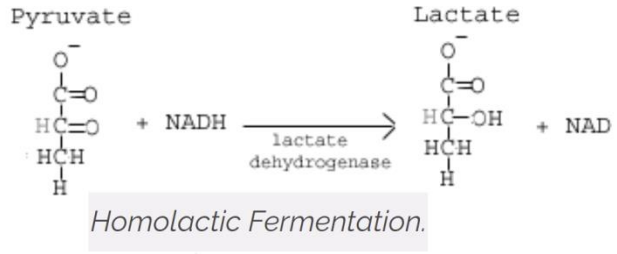



Glucose
GLYCOLYSIS
GLUCONEOGENESIS
Fructose 6-phosphate
F-2,6-BP +
AMP +
ATP −
Citrate −
H+ −
Phosphofructo-kinase
Fructose 1, 6-bisphosphatase
− F-2,6-BP
− AMP
+ Citrate
Fructose 1,6-bisphosphate
Several steps
Phosphoenolpyruvate
− ADP
Phosphoenol-pyruvate carboxykinase
Oxaloacetate
F-1,6-BP +
ATP −
Alanine −
Pyruvate kinase
Pyruvate carboxylase
Pyruvate
+ Acetyl CoA
− ADP

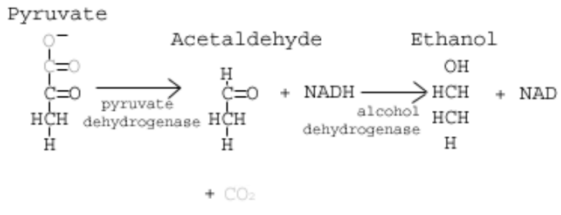
The Citric Acid Cycle

- Glycolytic pathway: Glucose is metabolized to pyruvate.
- Immediately upon finishing glycolysis:
  - The cell must continue respiration in either an aerobic or anaerobic direction.
    - Aerobic respiration:
      - In the presence of oxygen
      - Continue on to the aerobic citric acid cycle in the mitochondria.
      - Most of the ATP generated in metabolism.
    - Situation where there is no oxygen:
      - Muscles (under extreme exertion): Anaerobic respiration called homolactic fermentation.
      - Yeast cells: Anaerobic respiration called alcoholic fermentation.
      - Synthesize ATP though.
      - But a fraction of the ATP available from glucose.





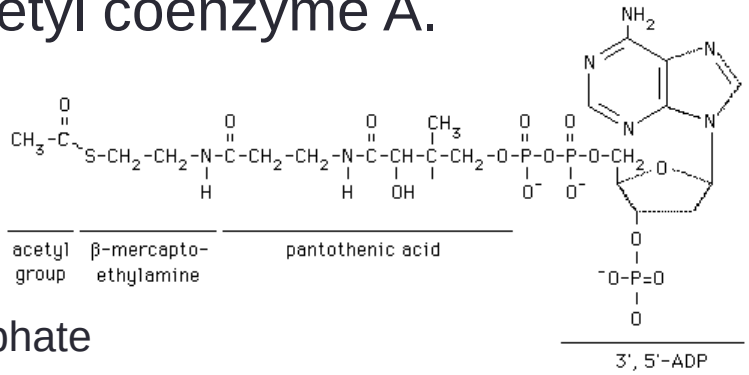
Homolactic Fermentation.



Alcoholic Fermentation.

Aerobic processing of glucose

- Complete oxidation of glucose derivatives to CO_2.
- A series of reactions also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle.
- The citric acid cycle is the final common pathway for the oxidation of fuel molecules:
  - Fatty acids and
  - Carbohydrates.
- Most fuel molecules enter the cycle as acetyl coenzyme A.
- Acetyl CoA consists of two parts.
  - Acetyl group
  - Coenzyme A:
    - Beta-mercaptoethylamine
    - Pantothenic acid (not synthesized in human) Phosphate
    - 3', 5'-adenosine diphosphate.

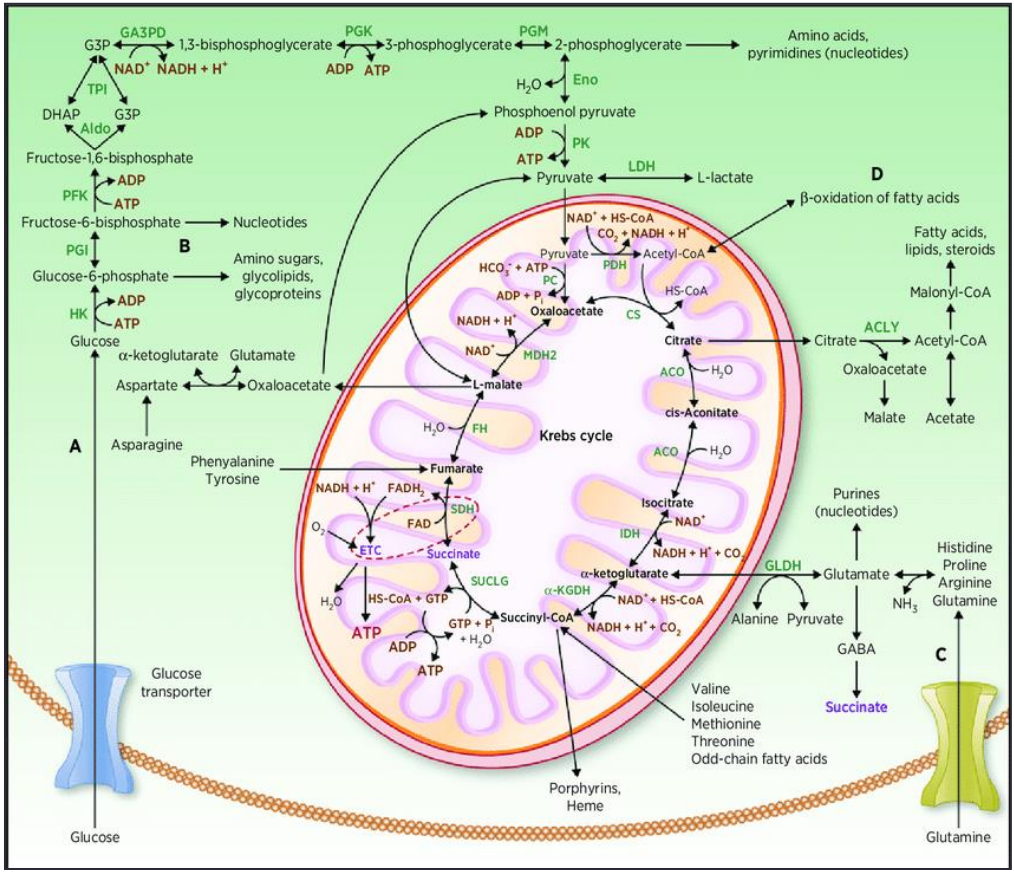


Acetyl coenzyme A, showing its constituents

- The pyruvate is oxidatively decarboxylated to form acetyl CoA.
- In eukaryotes:
  - Glycolysis: takes place in the cytosol
  - The citric acid cycle: takes place inside mitochondria,

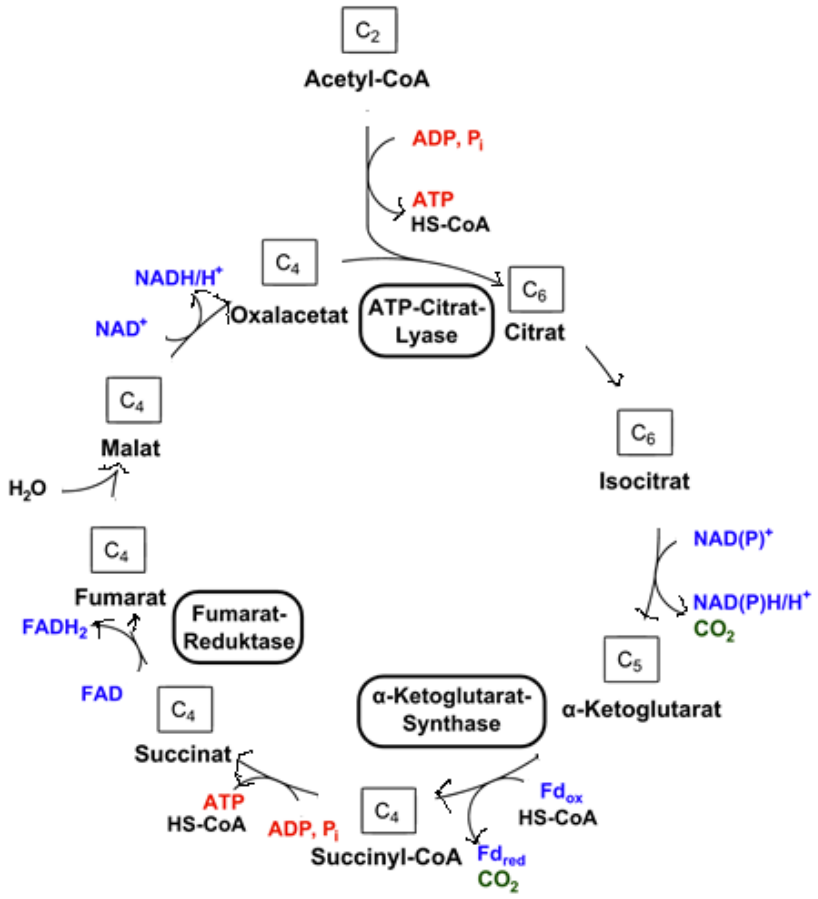
An Overview

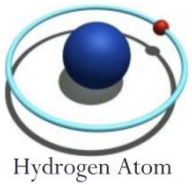
- TCA cycle is the central metabolic hub of the cell.
- It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group.
- The cycle is also an important source of precursors:
  - The storage forms of fuels
  - The building blocks of many other molecules
    - Amino acids,
    - Nucleotide bases,
    - Cholesterol, and
    - Porphyrin (the organic component of heme).

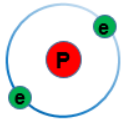


Overall Pattern of the citric acid cycle

- A series of ***oxidation-reduction*** reactions: Oxidation of an acetyl group to 2 molecules of CO_2.
- A ***four- carbon*** compound (oxaloacetate) condenses with a ***two-carbon*** acetyl unit to yield a ***six-carbon*** tricarboxylic acid (citrate).
- An isomer of citrate is then oxidatively decarboxylated.
- The resulting ***five-carbon compound*** (a-ketoglutarate) also is oxidatively decarboxylated to yield a ***four carbon compound*** (succinate).
- Oxaloacetate is then regenerated from succinate.
- ***Two carbon atoms*** enter the cycle as an acetyl unit and ***two carbon atoms*** leave the cycle in the form of two molecules of carbon dioxide.

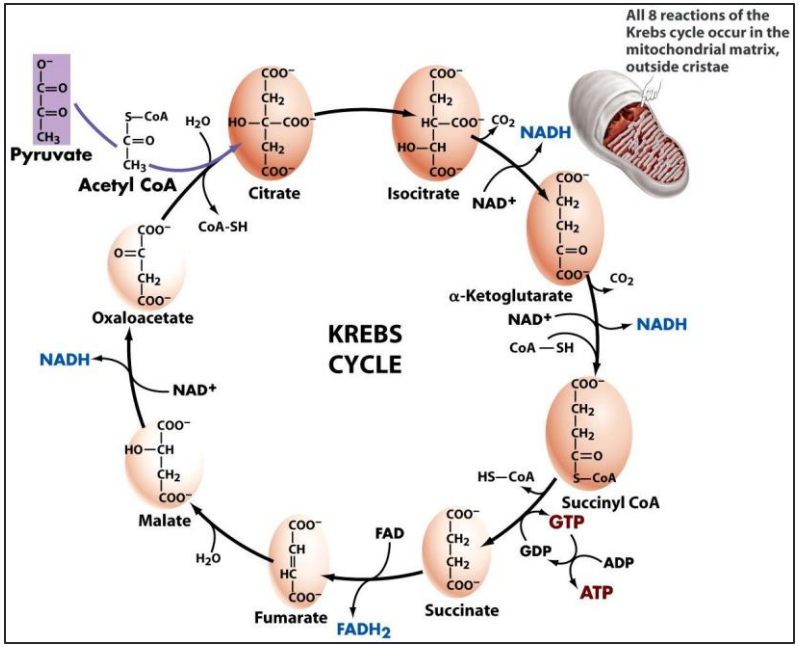




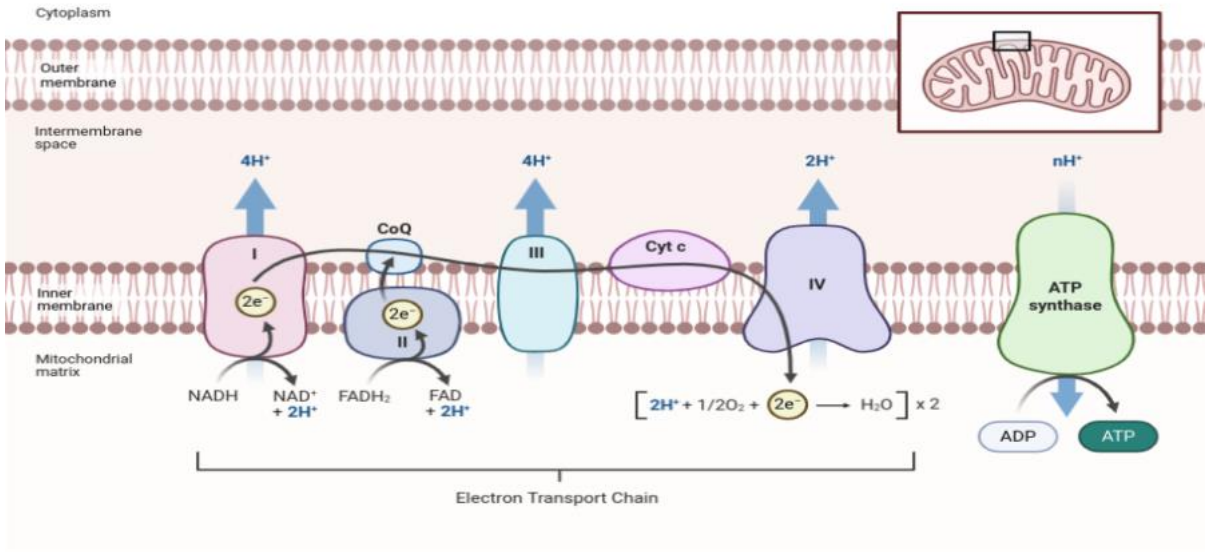


Hydrogen Anion

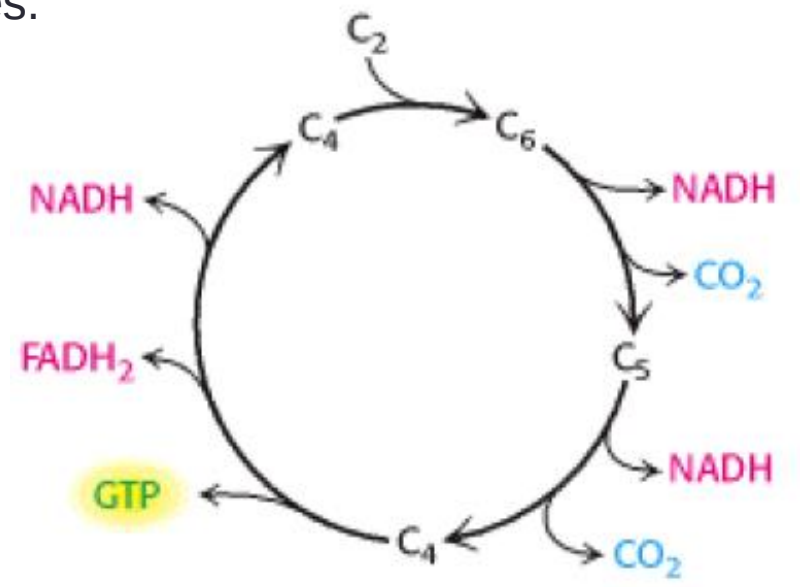
- Three hydride ions (the anion of hydrogen, H^-) (six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD^+) and
- One pair of hydrogen atoms (two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD).
- The function of the citric acid cycle: Harvesting of high-energy electrons from carbon fuels.
- TCA cycle: Neither generates a large amount of ATP nor includes oxygen as a reactant.
- It removes electrons from acetyl CoA and uses these electrons to form NADH and $FADH_2$.



- ***In oxidative phosphorylation***
  - Electrons released in the reoxidation of NADH and $FADH_2$ flow through a series of membrane proteins (the electron-transport chain)
  - This generates a proton gradient across the membrane.
  - These protons then flow through ATP synthase to generate ATP from ADP and inorganic phosphate.
  - Oxygen is required for the citric acid cycle to regenerate NAD^+ and FAD.

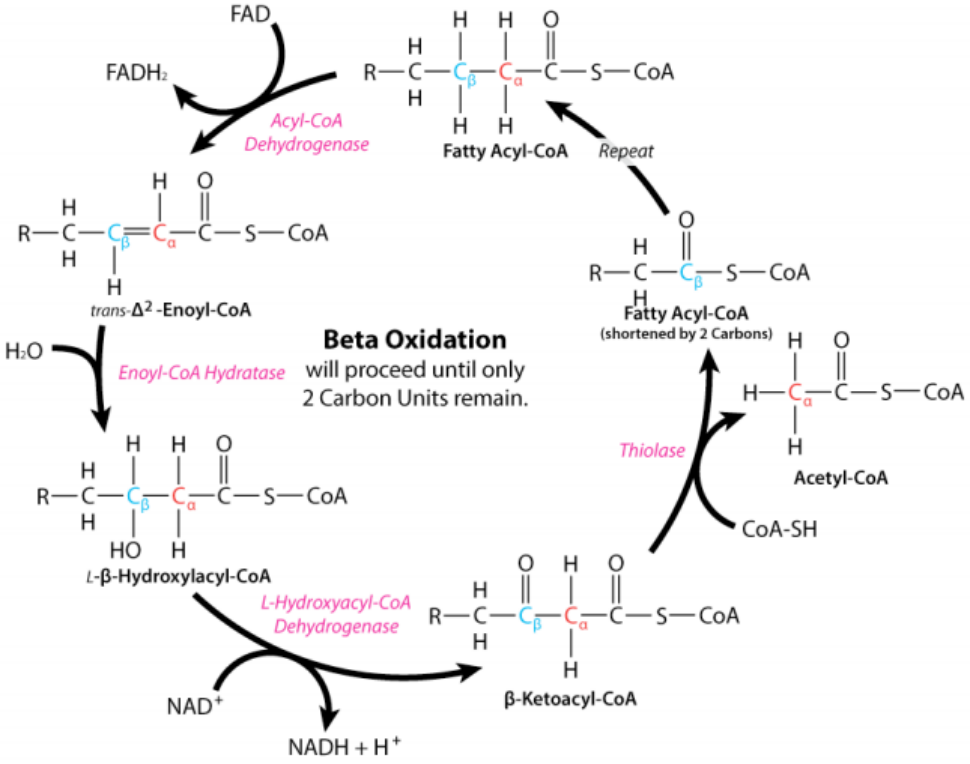


- The citric acid cycle with oxidative phosphorylation:
  - Provides the vast majority of energy used by aerobic cells in human beings (greater than 95%).
  - It is highly efficient because a limited number of molecules can generate large amounts of NADH and $FADH_2$.
  - The four-carbon molecule (oxaloacetate) initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle.
  - The oxaloacetate acts catalytically: it participates in the oxidation of the acetyl group but is itself regenerated.
  - Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.



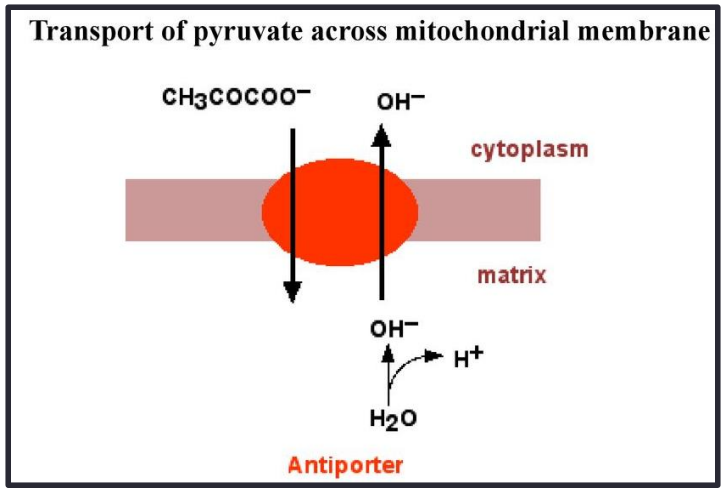
The Citric Acid Cycle Oxidizes Two-Carbon Units

- Acetyl CoA is the fuel for the citric acid cycle.
- This important molecule is formed from: The breakdown of
  - Glycogen (the storage form of glucose),
  - Fats
  - Many amino acids.
- Fats contain strings of reduced two-carbon units
  - These are first oxidized to acetyl CoA and
  - Then completely oxidized to CO_2 by the citric acid cycle.



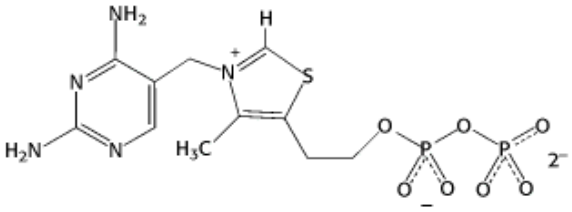
The Formation of Acetyl Coenzyme A from Pyruvate

- The formation of acetyl CoA from carbohydrates is less direct than from fat.
- Glucose are processed by glycolysis into pyruvate.
- Aerobic conditions: the pyruvate is transported into mitochondria in exchange for OH- by the pyruvate carrier, an ***antiporter***.
- In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the ***pyruvate dehydrogenase complex*** to form acetyl CoA.

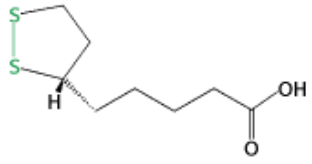


$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

- This irreversible reaction: The link between glycolysis and the citric acid cycle.
- The pyruvate dehydrogenase complex: Large, highly integrated complex of ***three kinds of enzymes***.
- These complexes are giant (molecular masses: 4-10 million daltons).
- Their elaborate structures allow groups to travel from one active site to another.
- The reaction requires the participation of:
  - ***Three enzymes*** of the pyruvate dehydrogenase complex (each composed of several polypeptide chains)
  - ***Five coenzymes***:
    - Thiamine pyrophosphate (TPP),
    - Lipoic acid, and
    - FAD serve as catalytic cofactors, and
    - CoA and
    - NAD^+ are stoichiometric cofactors



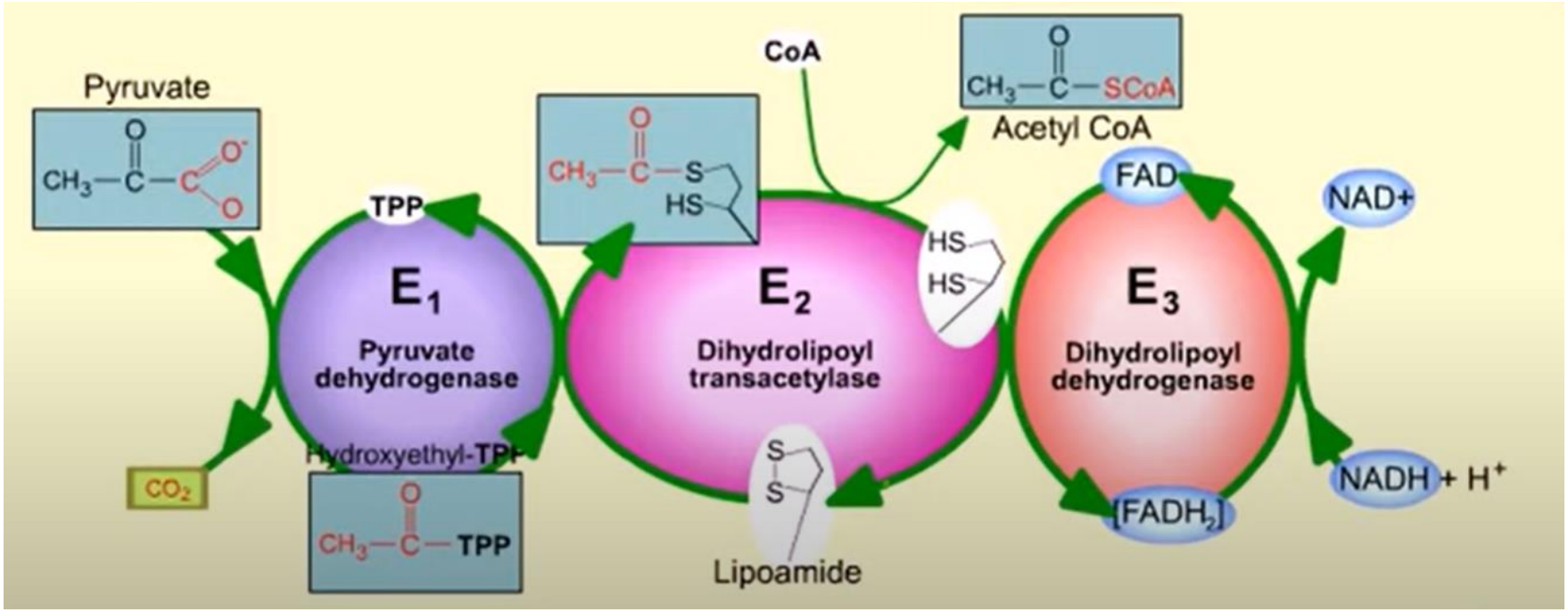
Thiamine pyrophosphate (TPP)



Lipoic acid

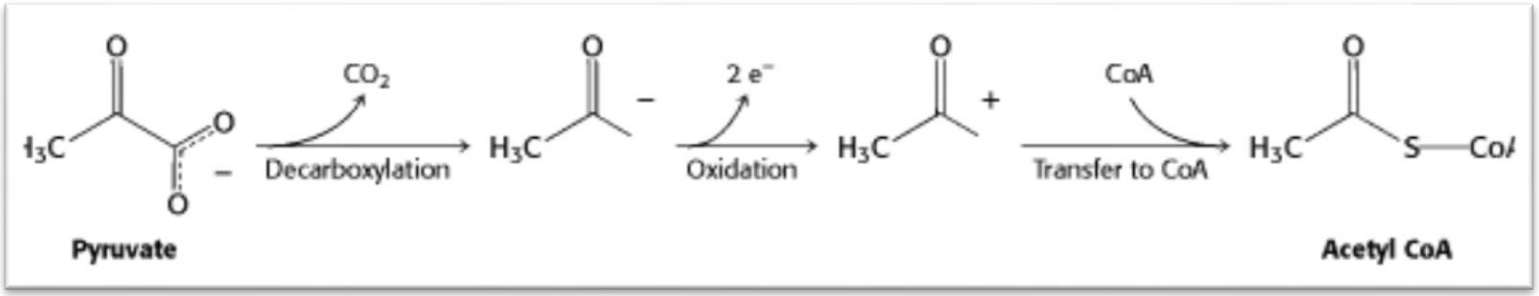
Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E3	12	FAD	Regeneration of the oxidized form of lipoamide



The conversion of pyruvate into acetyl CoA

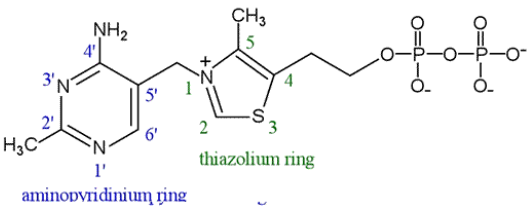
- It consists of ***three main steps***:
  - Decarboxylation,
  - Oxidation, and
  - Transfer of the resultant acetyl group to CoA.



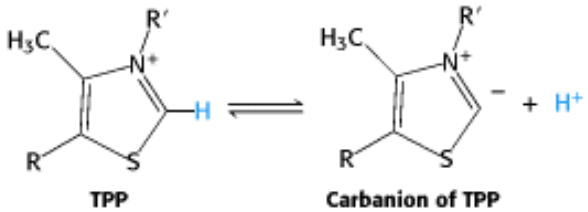
- These steps must be coupled to preserve the free energy derived from the decarboxylation step to drive the formation of NADH and acetyl CoA

The Steps

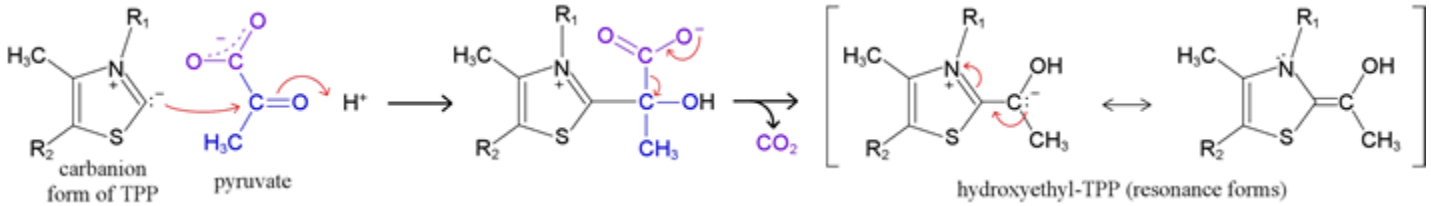
- **First step: *Decarboxylation***
  - Pyruvate combines with TPP and is then decarboxylated.
  - Catalyzed by the ***pyruvate dehydrogenase component*** (E1) of the multienzyme complex.
  - ***TPP*** is the ***prosthetic*** group of the pyruvate dehydrogenase component,
  - TPP: The carbon atom between the ***nitrogen*** and ***sulfur*** atoms in the thiazole ring is much more ***acidic*** than most =CH- groups.
  - This center ionizes to form a ***carbanion***, which readily ***adds to the carbonyl*** group of pyruvate.



Thiamine pyrophosphate

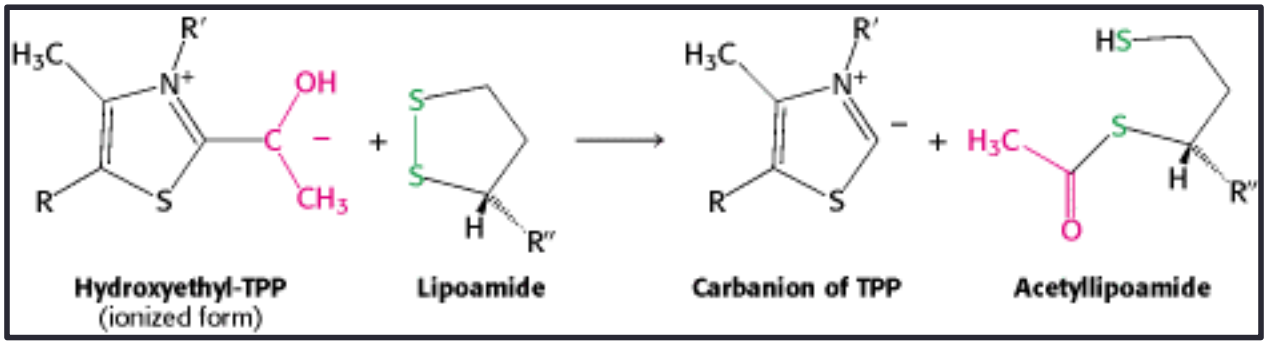


  - Protonation yields hydroxyethyl-TPP.

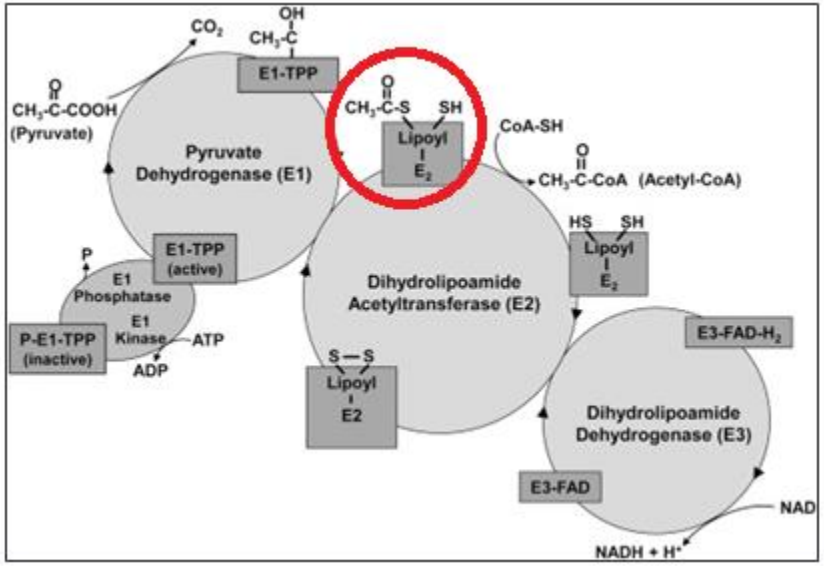


  - This addition is followed by the decarboxylation of pyruvate.

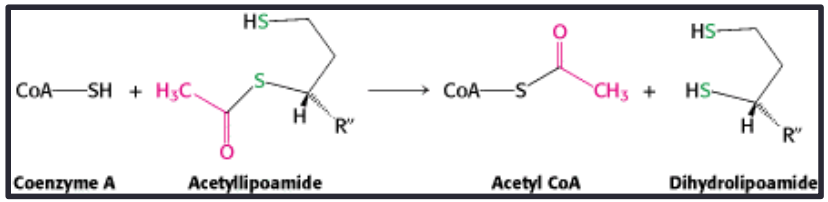
- **Second step : *Oxidation***
  - The hydroxyethyl group is oxidized to form an acetyl group.
  - Then concomitantly transferred to lipoamide
  - Lipoamide: a derivative of lipoic acid that is linked to the side chain of a lysine residue by an amide linkage.



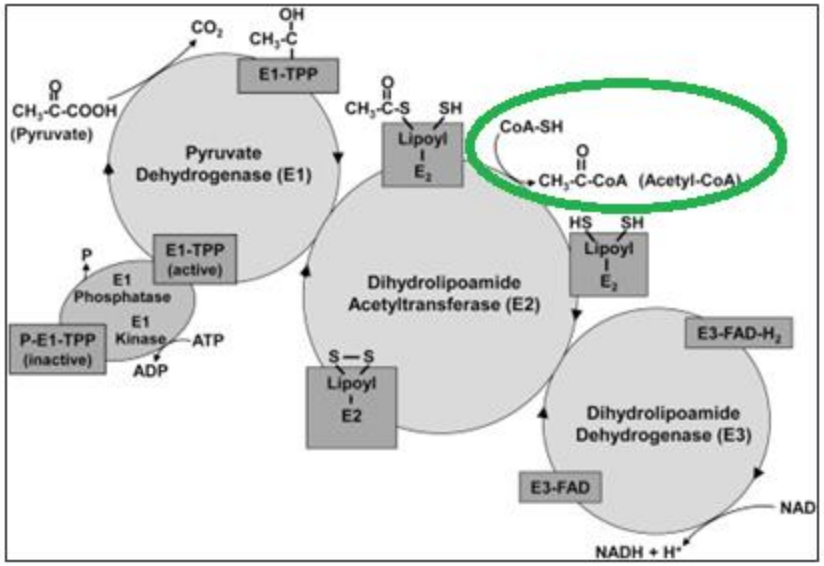
  - The **oxidant** in this reaction is the **disulfide group** of lipoamide
  - **Disulfide** is reduced to its disulfhydryl form.
  - This reaction, also catalyzed by the pyruvate dehydrogenase component E1
  - It yields ***acetyllipoamide***.



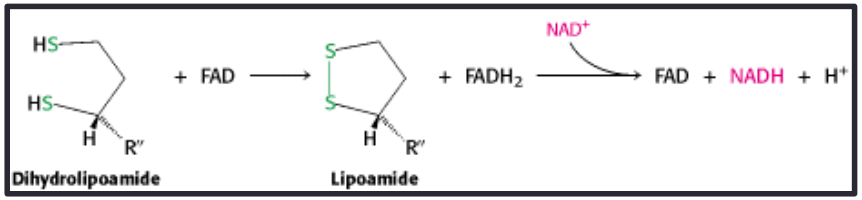
- **Third step : *Transfer of the acetyl group to CoA***
  - The acetyl group is transferred from acetyllipoamide to CoA to form acetyl CoA.



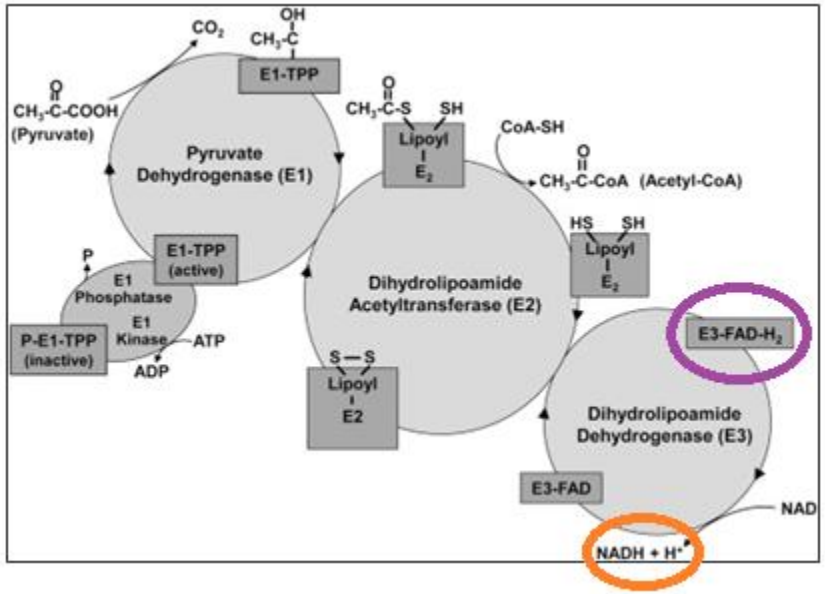
  - ***Dihydrolipoyl transacetylase*** (E2) catalyzes this reaction.
  - The energy-rich thioester bond is preserved as the acetyl group is transferred to CoA.
  - CoA serves as a carrier of activated acyl groups.
  - ***Acetyl CoA*** is generated from pyruvate.
  - The pyruvate dehydrogenase complex cannot complete another catalytic cycle: until the ***dihydrolipoamide is oxidized to lipoamide***.



- **Fourth step:**
  - The **oxidized** form of **lipoamide** is regenerated by ***dihydrolipoyl dehydrogenase*** (E3).
  - Two electrons are first transferred to an **FAD prosthetic** group of the enzyme
  - Finally to NAD^+.

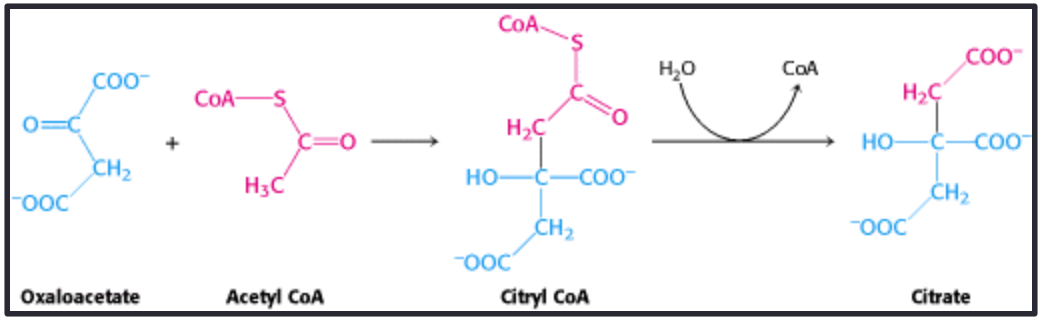


  - This electron transfer to FAD is unusual.
    - The common role for FAD is to receive electrons from NADH.
    - The electron transfer potential of FAD is altered by its association with the enzyme and enables it to transfer electrons to NAD^+.
    - Proteins tightly associated with FAD or flavin mononucleotide (FMN) are called flavoproteins.



The citric acid cycle begins

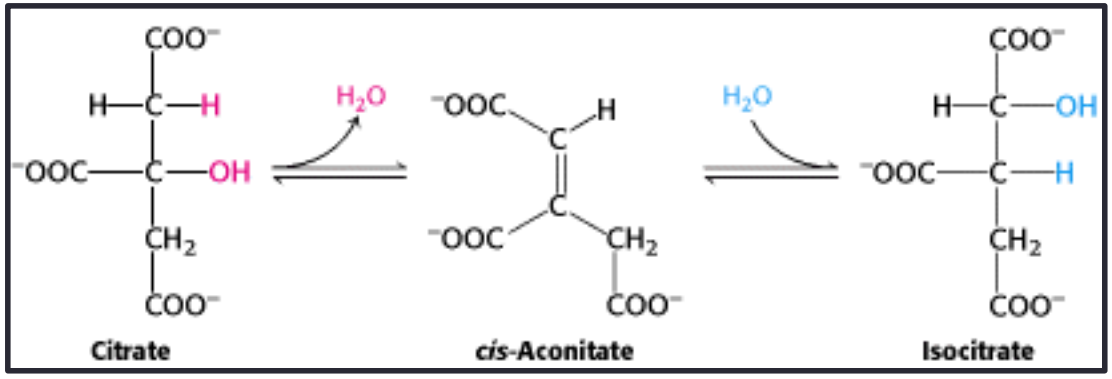
- It begins with the condensation of oxaloacetate (4C) and the acetyl group (2C) of acetylCoA
- Oxaloacetate reacts with acetyl CoA and H_2O to yield ***citrate*** and ***CoA***.
- ***Citrate Synthase*** : Catalyzes a synthetic reaction in which two units are joined **without** the direct participation of ATP (or any NTP).



- This reaction is an ***aldol condensation*** followed by a ***hydrolysis***.
- Oxaloacetate first condenses with acetyl CoA to form citryl CoA.
- Citryl CoA is hydrolyzed to citrate and CoA.
- The hydrolysis of citryl CoA, a high-energy thioester intermediate.
- The hydrolysis of the thioester powers the synthesis of a new molecule from two precursors.

Citrate Is Isomerized into Isocitrate

- The tertiary hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylations.
- So, citrate is ***isomerized*** into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation.
- The isomerization of citrate: A ***dehydration*** step followed by a ***hydration*** step.
- The result is an interchange of a hydrogen atom and a hydroxyl group.
- The enzyme catalyzing both steps is called ***aconitase*** because cis-aconitate is an intermediate.

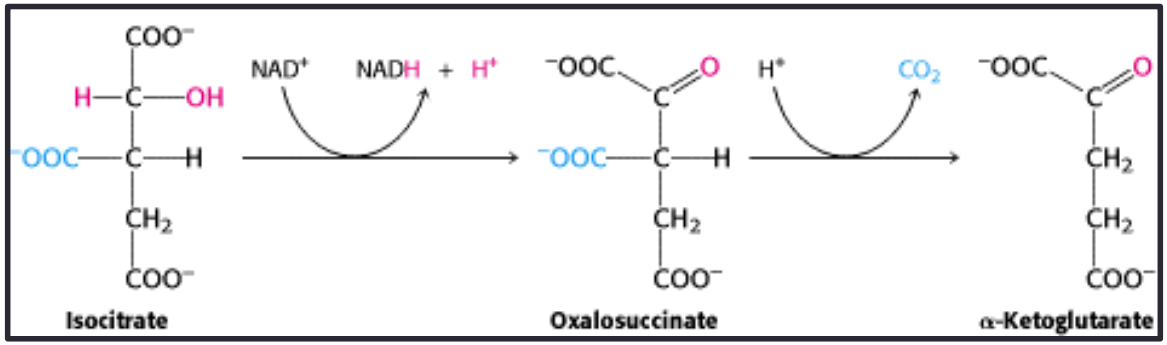


Isocitrate Is Oxidized and Decarboxylated to a-Ketoglutarate

- Now the ***first*** of ***four oxidation-reduction*** reactions in the citric acid cycle.
- The oxidative decarboxylation of isocitrate is catalyzed by ***isocitrate dehydrogenase***.

$$\text{Isocitrate} + \text{NAD}^+ \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + \text{NADH}$$

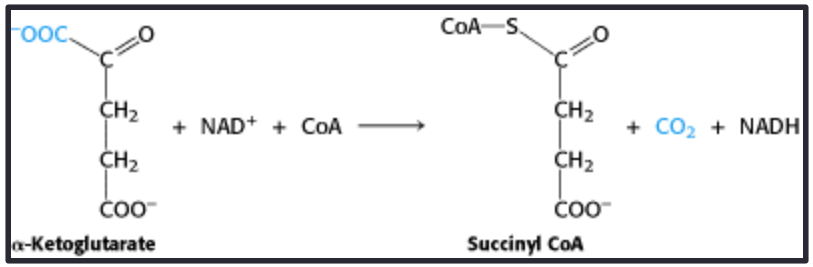
- The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid.
- While bound to the enzyme, it loses CO_2 to form a-ketoglutarate.



- This oxidation generates the first high-transfer-potential electron carrier NADH in the cycle.

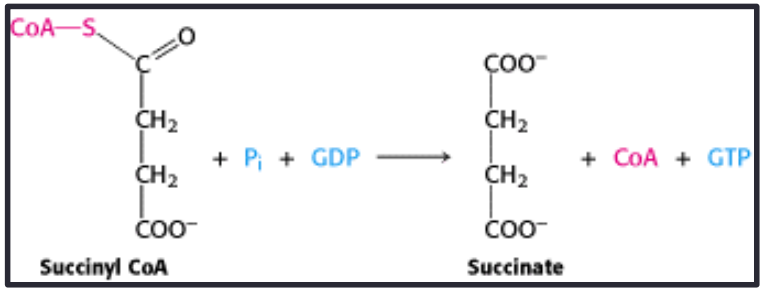
Succinyl Coenzyme A Is Formed: Oxidative Decarboxylation of a-Ketoglutarate

- ***Second oxidative decarboxylation*** reaction,
- Succinyl Co A is formed from a-ketoglutarate.



A High Phosphoryl-Transfer Potential Compound Is Generated from Succinyl Coenzyme A

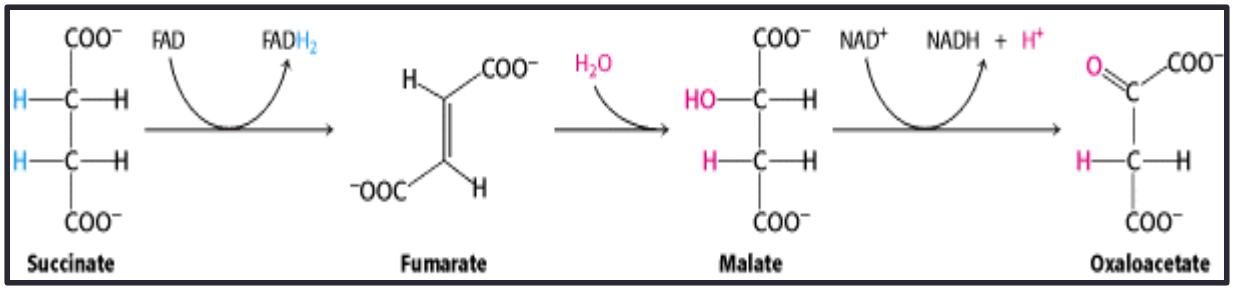
- Succinyl CoA is an energy-rich thioester compound.
- The Δ G° for the hydrolysis of succinyl CoA is about -8 kcal mol^{-1} (Δ G° for ATP = -7.3 kcal mol^{-1}).
- The cleavage of the thioester bond of succinyl CoA is coupled to the ***phosphorylation*** of a purine nucleoside diphosphate, GDP.
- This reaction is catalyzed by ***succinyl CoA synthetase*** (succinate thiokinase).



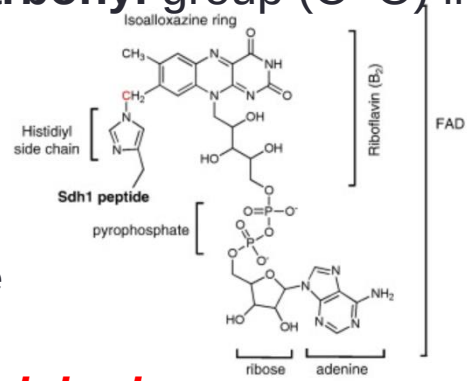
$$GTP + ADP \rightleftharpoons GDP + ATP$$

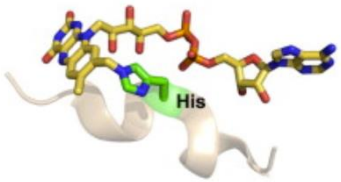
Oxaloacetate Is Regenerated by the Oxidation of Succinate

- The final stage of the citric acid cycle: the regeneration of oxaloacetate.



- A **methylene** group (CH_2) is converted into a **carbonyl** group (C=O) in **three** steps:
  a) An **oxidation**,
  b) A **hydration**, and
  c) A **second oxidation** reaction.
- Outcome:
  - Oxaloacetate is regenerated for another round of the cycle
  - More energy is extracted in the form of $FADH_2$ and NADH.



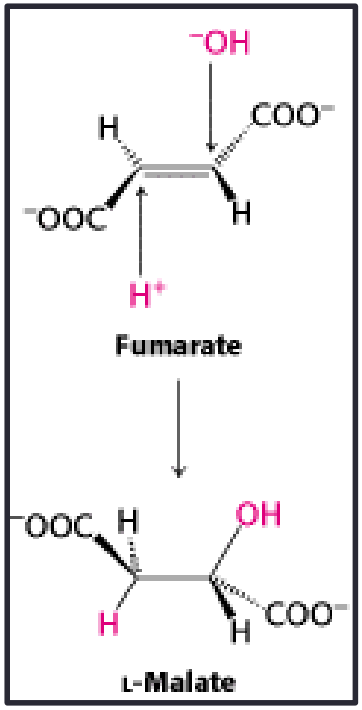


- Succinate is oxidized to fumarate by ***succinate dehydrogenase***.
- The hydrogen acceptor is FAD rather than NAD+: ***Third oxidation*** reaction.
- In succinate dehydrogenase, the iso-alloxazine ring of FAD is covalently attached to a histidine side chain of the enzyme (denoted E-FAD).
- FAD is the hydrogen acceptor in this reaction because the free-energy change is insufficient to reduce NAD^+.

$$\text{E-FAD} + \text{succinate} \rightleftharpoons \text{E-FADH}_2 + \text{fumarate}$$

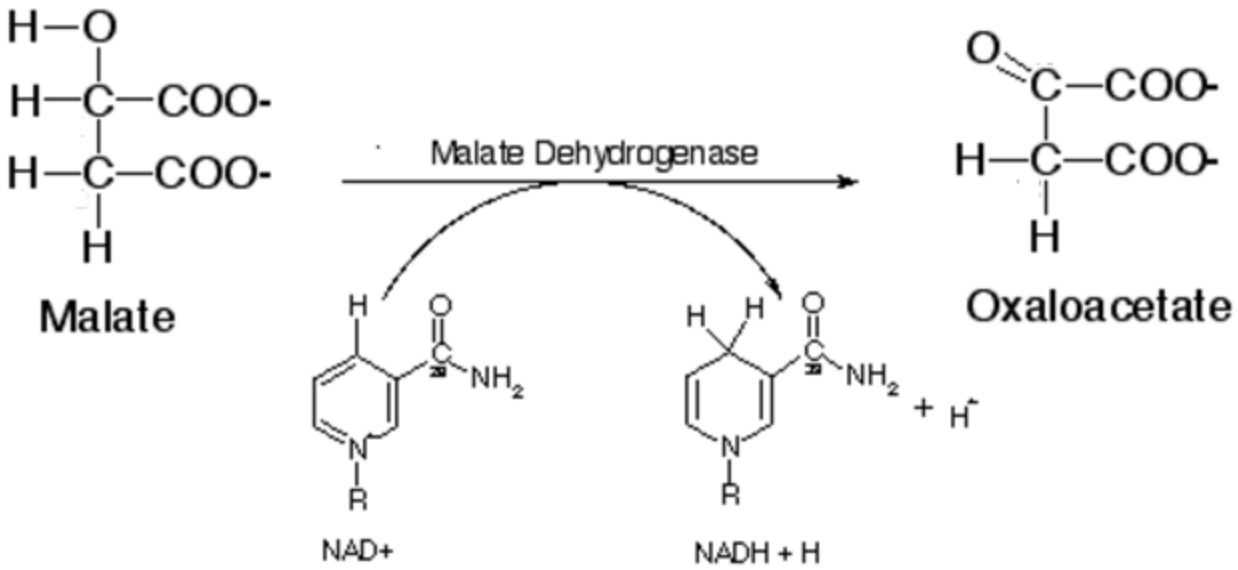
Hydration of fumarate to form l-malate

- ***Fumarase*** catalyzes a stereospecific **trans addition** of a **hydrogen** atom and a **hydroxyl** group.
- The hydroxyl group adds to only one side of the double bond of fumarate.
- Only the L isomer of malate is formed.



Malate to Oxaloaceticacid

- Finally, malate is oxidized to form oxaloacetate.
- Catalyzed by ***malate dehydrogenase***
- NAD^+ is again the hydrogen acceptor.
- The oxidation of malate is driven by the utilization of the products
  - Oxaloacetate by citrate synthase and
  - NADH by the electron-transport chain.



$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$

Stoichiometry of the Citric Acid Cycle

- The net reaction of the citric acid cycle is:

$$\text{Acetyl CoA} + 3\,\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2\,\text{H}_2\text{O} \longrightarrow 2\,\text{CO}_2 + 3\,\text{NADH} + \text{FADH}_2 + \text{GTP} + 2\,\text{H}^+ + \text{CoA}$$

- Recapitulations:
  i. Two carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl CoA) with oxaloacetate.
     a) Two carbon atoms leave the cycle in the form of CO_2 in the successive decarboxylations catalyzed by
        a) Isocitrate dehydrogenase and
        b) α-ketoglutarate dehydrogenase.
     b) Isotope-labeling studies: The two carbon atoms that enter each cycle are **not** the ones that leave.
  ii. **Four pairs** of **hydrogen** atoms leave the cycle in **four oxidation** reactions.
     a) **Two** molecules of **NAD^+** are reduced in the oxidative decarboxylations of isocitrate and a -ketoglutarate,
     b) **One** molecule of **FAD** is reduced in the oxidation of succinate, and
     c) **One** molecule of **NAD^+** is reduced in the oxidation of malate.
  iii. **One** compound with high phosphoryl transfer potential, usually **GTP**, is generated from the cleavage of the thioester linkage in succinyl CoA.

- **Two** molecules of **water** are consumed:
  - One in the synthesis of citrate by the hydrolysis of citryl CoA and
  - The other in the hydration of fumarate.
- Recall: NADH is generated in the formation of acetyl CoA from pyruvate by the pyruvate dehydrogenase reaction.
- The electron-transport chain oxidizes the NADH and $FADH_2$ formed in the citric acid cycle.
- The transfer of electrons from these carriers to O_2, the ultimate electron acceptor, leads to the generation of a proton gradient across the inner mitochondrial membrane.
- This proton-motive force then powers the generation of ATP; the net stoichiometry is
  - About 2.5 ATP per NADH, and
  - 1.5 ATP per $FADH_2$.
- The electron-transport chain:
  - 9 high-transfer-potential phosphoryl groups are generated when oxidizes 3 molecules of NADH (7.5) and 1 molecule of $FADH_2$ (1.5).
  - 1 high-transfer-potential phosphoryl group per acetyl unit is directly formed in the TCA cycle.
- So, ***1 acetate unit generates approximately 10 molecules of ATP***.
- Only 2 molecules of ATP are generated per molecule of glucose (which generates 2 molecules of acetyl CoA) by anaerobic glycolysis.

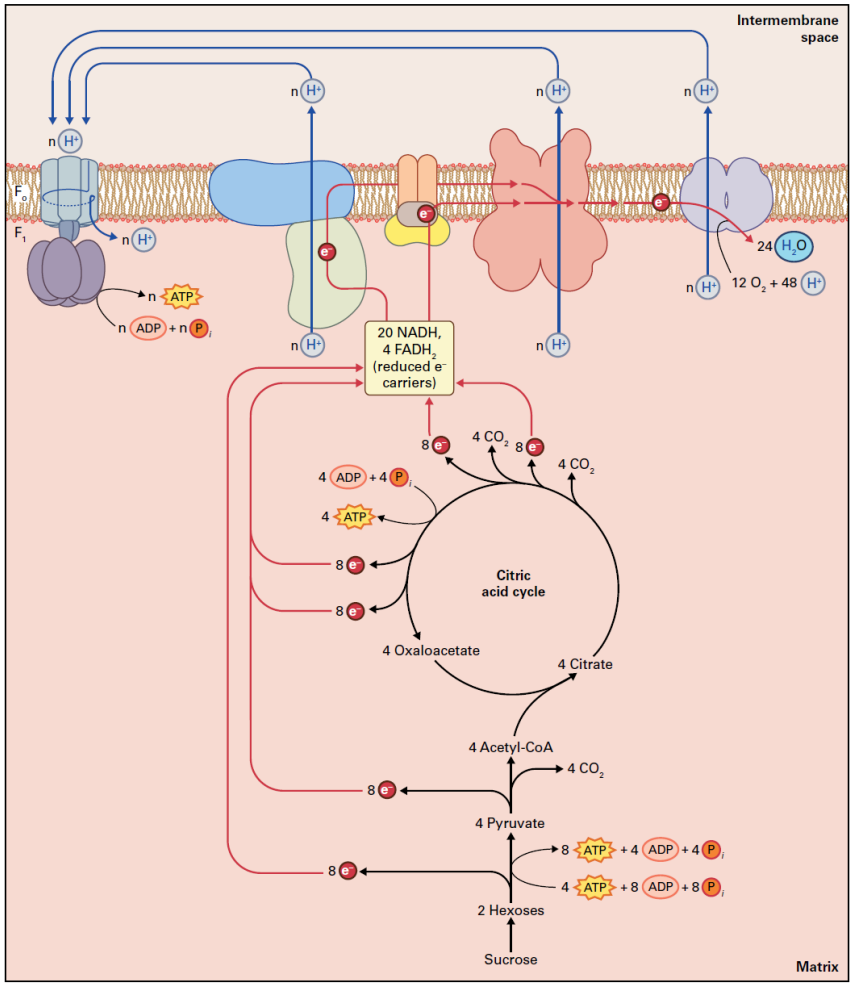
Stoichiometry of Coenzyme Reduction and ATP Formation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed *
Glucose → Glucose 6-phosphate	1 ATP	-1
Fructose 6-P → Fructose 1,6-bisphosphate	1 ATP	-1
2 Glyceraldehyde3-P → 2 1,3-Bis Phosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate → 2 3-Phosphoglycerate	2 ATP	-2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
Succinate → 2 fumarate	2 FADH2	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.
† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix;

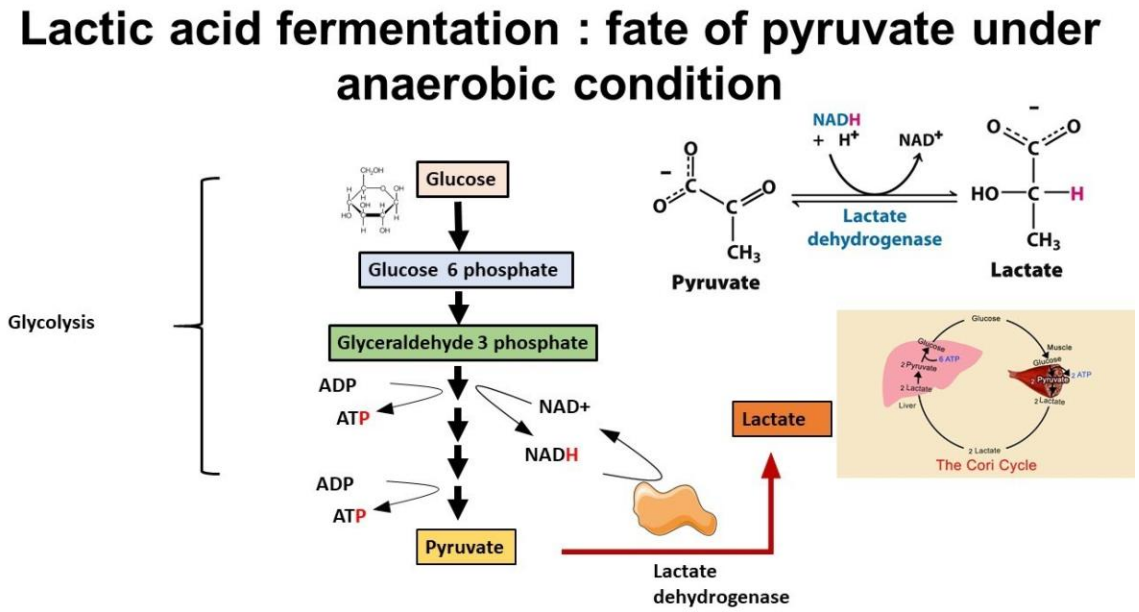
General mechanism of oxidative phosphorylation in mitochondria

- Electrons released during the oxidative steps:
  - Glycolysis and
  - The citric acid cycle
- They produce 20 molecules of NADH and 4 molecules of $FADH_2$.
- These reduced coenzymes are subsequently oxidized by the mitochondrial electron transport chain.
- The free energy released during mitochondrial electron transfer is coupled to the translocation of protons across the inner mitochondrial membrane (from the matrix into the intermembrane space), which generates a proton electrochemical gradient across the inner membrane.
- As protons move back across the inner membrane through the Fo proton channel of the ATP synthase complex, the free energy released is used by the complex's F1 component to catalyze conversion of ADP and Pi to ATP in the mitochondrial matrix.



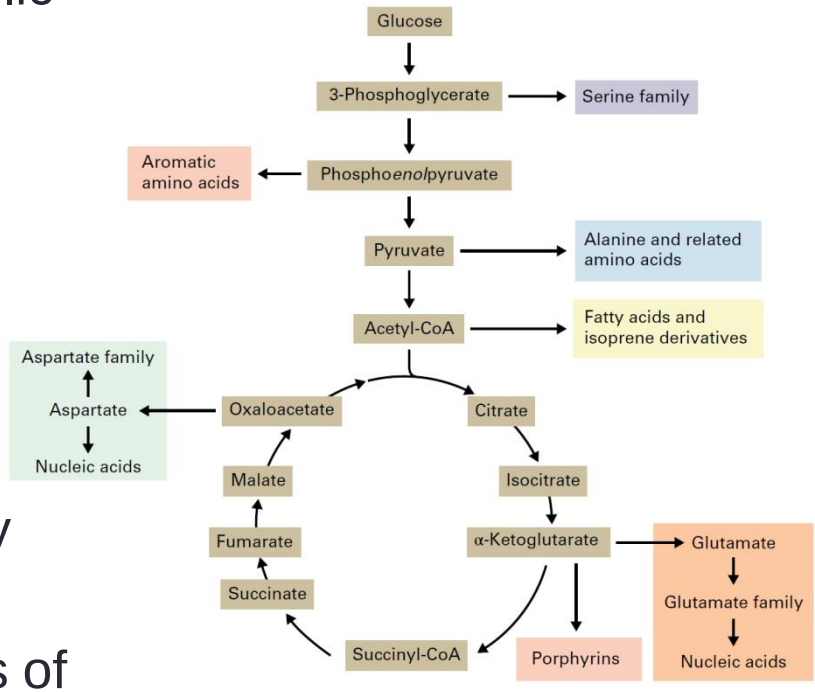
Glycolysis, TCA cycle and Oxygen

- Molecular oxygen does not participate directly in the citric acid cycle.
- But, the cycle operates only under aerobic conditions because
  - NAD^+ and FAD can be ***regenerated*** in the mitochondrion only by the transfer of electrons to molecular ***oxygen***.
- Glycolysis has both an aerobic and an anaerobic mode, whereas the citric acid cycle is strictly aerobic.
- Glycolysis can proceed under anaerobic conditions because NAD^+ is regenerated in the conversion of pyruvate into lactate.

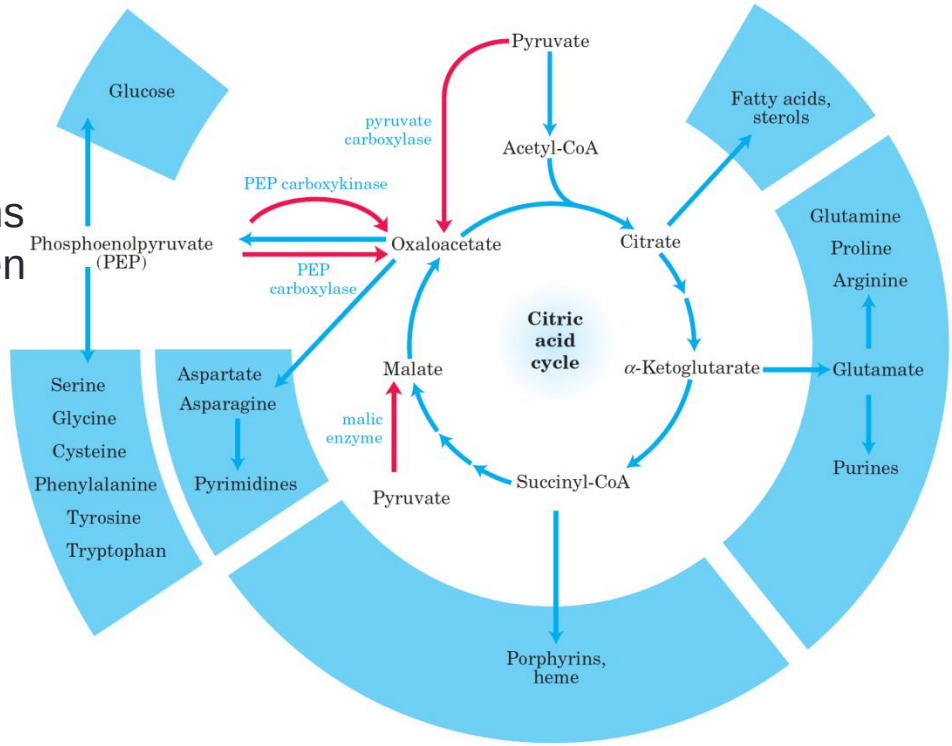


Amphibolic role

- Amphibolic pathways: serves in both catabolic and anabolic processes.
- Citric Acid Cycle Components: Important biosynthetic intermediates.
- Its role in the oxidative catabolism of
  - Carbohydrates,
  - Fatty acids,
  - Amino acids.
- The cycle also provides precursors for many biosynthetic pathways.
- Intermediates produced during the reactions of glycolysis and the citric acid cycle serve as substrates for numerous plant biosynthetic pathways.
- The primary intermediates for the production of:
  - Amino acids,
  - Lipids,
  - Nucleic acids,
  - Cell wall sugars etc.



- Anaerobic ancestors.
  - α-Ketoglutarate and oxaloacetate can, serve as precursors of the amino acids glutamate and aspartate by simple transamination.
  - Through aspartate and glutamate, the carbons of oxaloacetate and α—ketoglutarate are then used to build other amino acids, as well as purine and pyrimidine nucleotides.
  - Oxaloacetate is converted to glucose in gluconeogenesis.
  - Succinyl- CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin) and electron carriers (in cytochromes).
  - The citrate produced in some organisms is used commercially for a variety of purposes.
    - Ingredients in most soft drinks
    - To provide a tart or fruity flavor.
    - As a mordant to brighten colors,
    - As an antioxidant to preserve the flavors of foods.
    - Citric acid is produced industrially by growing the fungus *Aspergillus niger* in the presence of an inexpensive sugar source



Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways.
In red are four anaplerotic reactions that replenish depleted cycle intermediates

Anaplerotic Reactions

- Chemical reactions that form intermediates of a metabolic pathway.
- In normal function of this cycle for respiration, concentrations of TCA intermediates remain constant.
- However, many biosynthetic reactions also use these molecules as a substrate.
- ***Anaplerosis*** is the act of replenishing TCA cycle intermediates that have been extracted for biosynthesis (Anaplerotic reactions).

Reaction	Tissue(s)/organism(s)
1. $Pyruvate + HCO_3 + ATP \xrightarrow{\text{pyruvate carboxylase}} Oxaloacetate + ADP + Pi$	Liver, kidney
2. $PEP + CO_2 + GDP \xrightarrow{\text{PEP carboxykinase}} Oxaloacetate + GTP$	Heart, skeletal muscle
3. $PEP + HCO_3 \xrightarrow{\text{PEP carboxylase}} Oxaloacetate + Pi$	Higher plants, yeast, bacteria
4. $Pyruvate + HCO_3 + NAD(P)H \xrightarrow{\text{Malic enzyme}} Malate + NAD(P)^+$	Widely distributed in eukaryotes and prokaryotes

Thank You