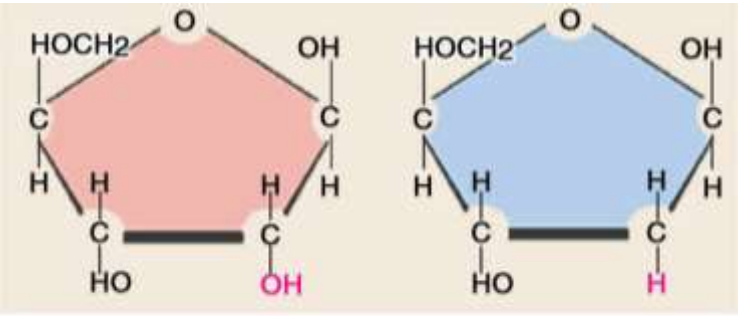
Pentose Phosphate Pathway



Ribose Deoxyribose

Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department

Faculty of Medicine, Mutah University

Pentose Phosphate Pathway

- The predominant pathway for glucose catabolism is glycolysis which yield pyruvate followed by oxidation to CO_2 in the Krebs cycle
- Pentose phosphate pathway (PPP) is an anabolic rather than catabolic pathway. PPP is an alternative pathway for glucose metabolism which draws G6P from the glycolysis cycle
- PPP occurs in the cytosol of the cell. It has two main purposes:

1. To generate the pentose sugar "ribose-5-phosphate" required for nucleotides and nucleic acids biosynthesis
2. To generate NADPH molecules (universal reductant) required for biosynthetic pathways and detoxification reactions

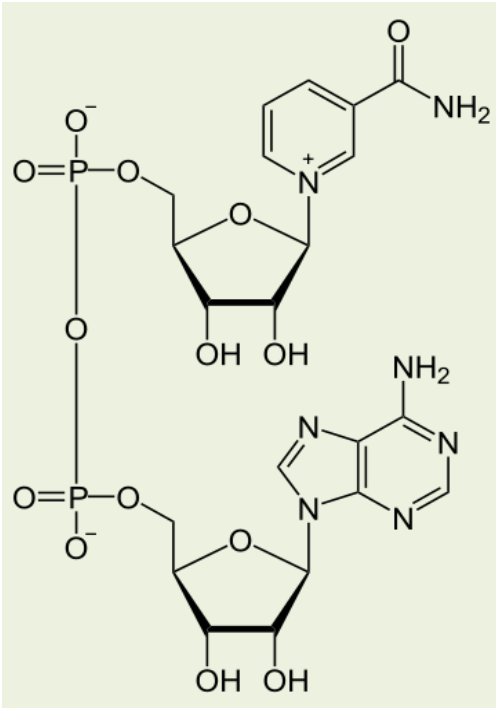
Nicotinamide Adenine Dinucleotide

- Nicotinamide adenine dinucleotide, abbreviated as NAD^+, is a coenzyme found in all living cells
- It is composed of two nucleotides linked through their phosphate groups

- The coenzyme is found in two forms in cells:

1. The oxidized form "NAD^+" is an oxidizing agent which can accept electrons from other molecules and becomes reduced

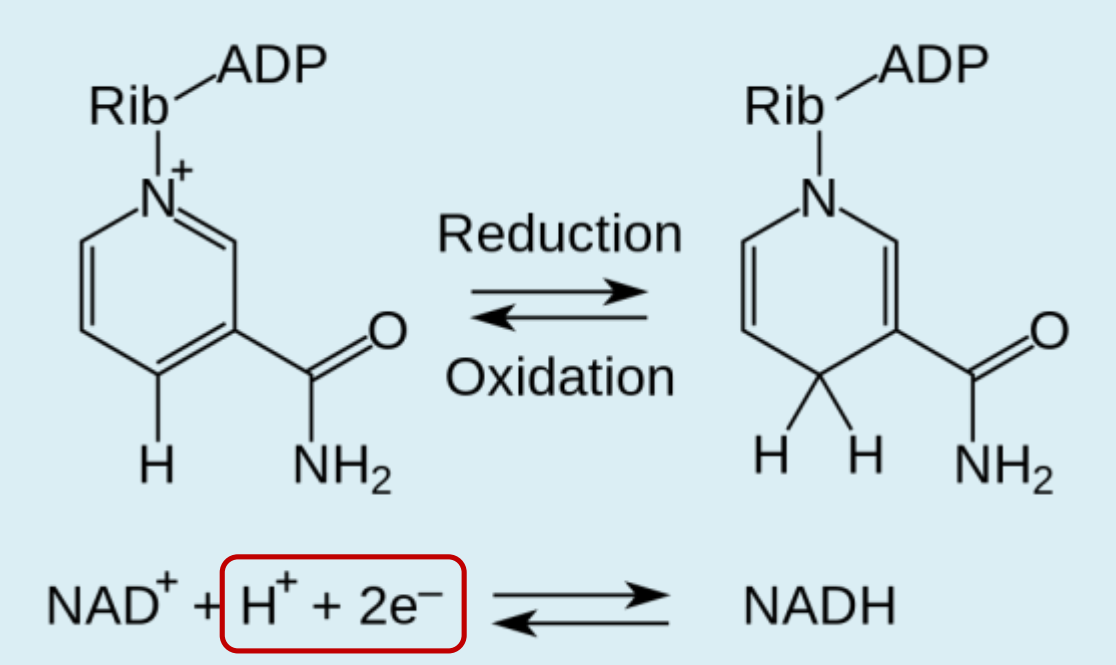
2. The reduced form "NADH" which can be used as a reducing agent (electrons donor)

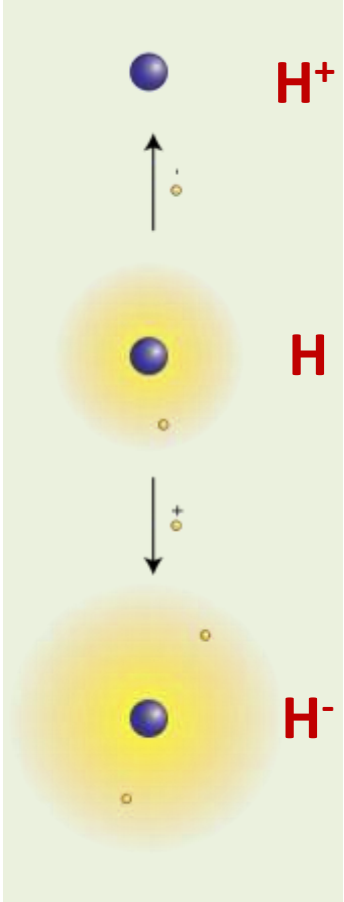


NAD^+

Nicotinamide Adenine Dinucleotide

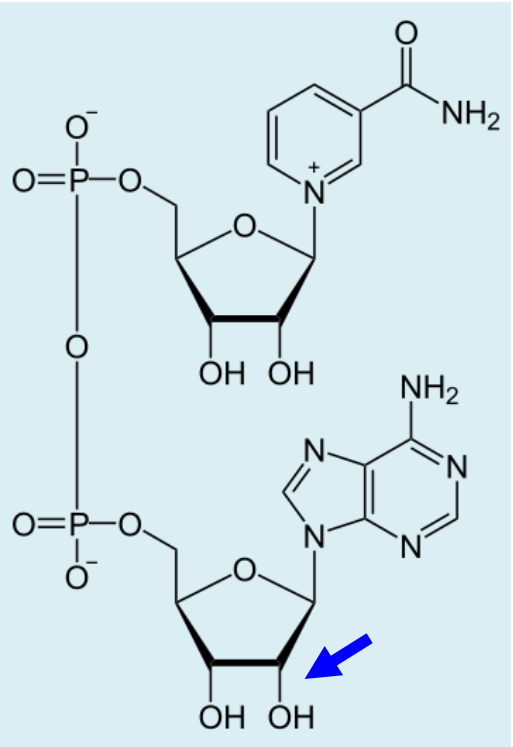
- Therefore, Nicotinamide adenine dinucleotide is used in redox reactions during metabolism carrying electrons from one reaction to another **($RH_2 + NAD^+ \rightarrow NADH + H^+ + R$)**



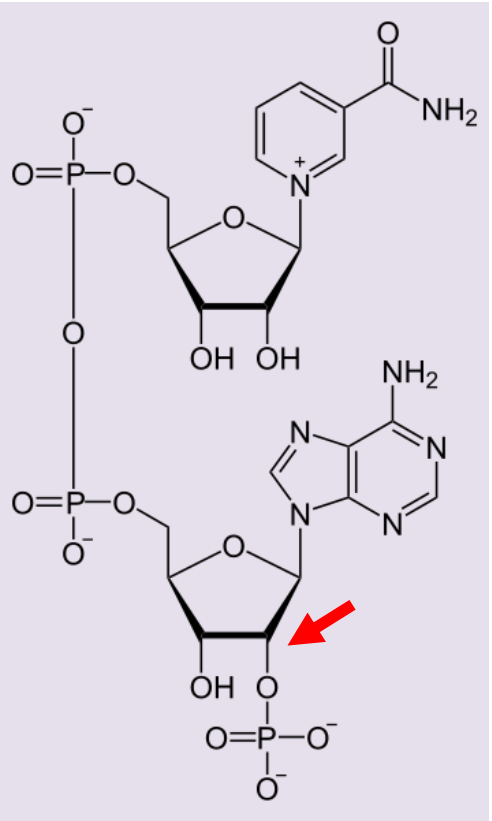


Nicotinamide Adenine Dinucleotide Phosphate

- Nicotinamide adenine dinucleotide phosphate (abbreviated as $NADP^+$) differs from NAD^+ in the presence of additional phosphate group on the C2' of the adenosine ribose ring



NAD^+



$NADP^+$

Nicotinamide Adenine Dinucleotide Phosphate

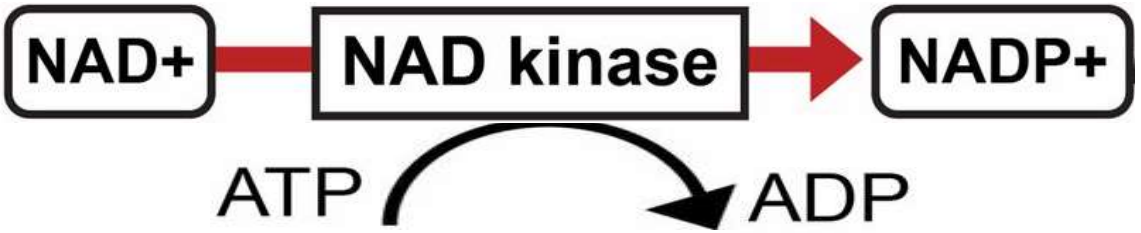
- Nicotinamide adenine dinucleotide phosphate exists in two forms: $NADP^+$ the oxidized form and NADPH the reduced form
- This coenzyme is used in **anabolic** rather than catabolic reactions such as lipid and nucleic acid synthesis which require NADPH as **reducing agent**. Additionally, it has a role in **detoxification** reactions
- Pentose phosphate pathway is the major source of NADPH in animals (continuously regenerated from $NADP^+$)
- Tissues such as liver, adipose tissue, mammary gland and adrenal gland are rich in PPP enzymes because NADPH is used for fatty acids and steroids biosynthesis
- High level of PPP enzymes also seen in rapidly proliferating cells but PPP is nearly absent in other tissues like skeletal muscles

Nicotinamide Adenine Dinucleotide

- Nicotinamide adenine dinucleotide is synthesized by two different metabolic pathways:

1. A *de novo* pathway: most organisms synthesize NAD^+ from simple components like tryptophan in animals and aspartic acid in plants.

- Some NAD^+ is converted to $NADP^+$ via NAD^+ kinase which phosphorylate NAD^+ in an ATP-dependent step



2. A salvage pathways: by recycling preformed components back to NAD^+ such as nicotinic acid and nicotinamide obtained from food (i.e. niacin or vitamin B3)

From Glycolysis to PPP

Glucose

↓ ↑

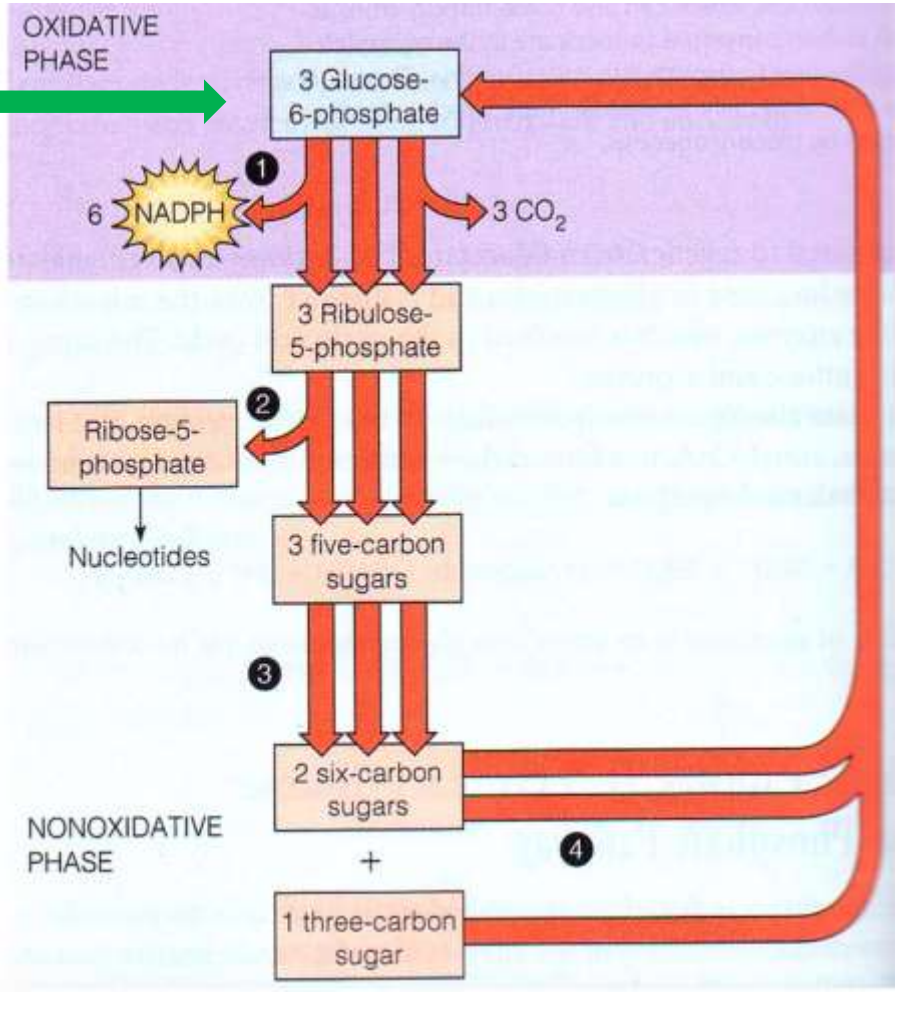
Glucose 6-Phosphate

↓

Glycolysis

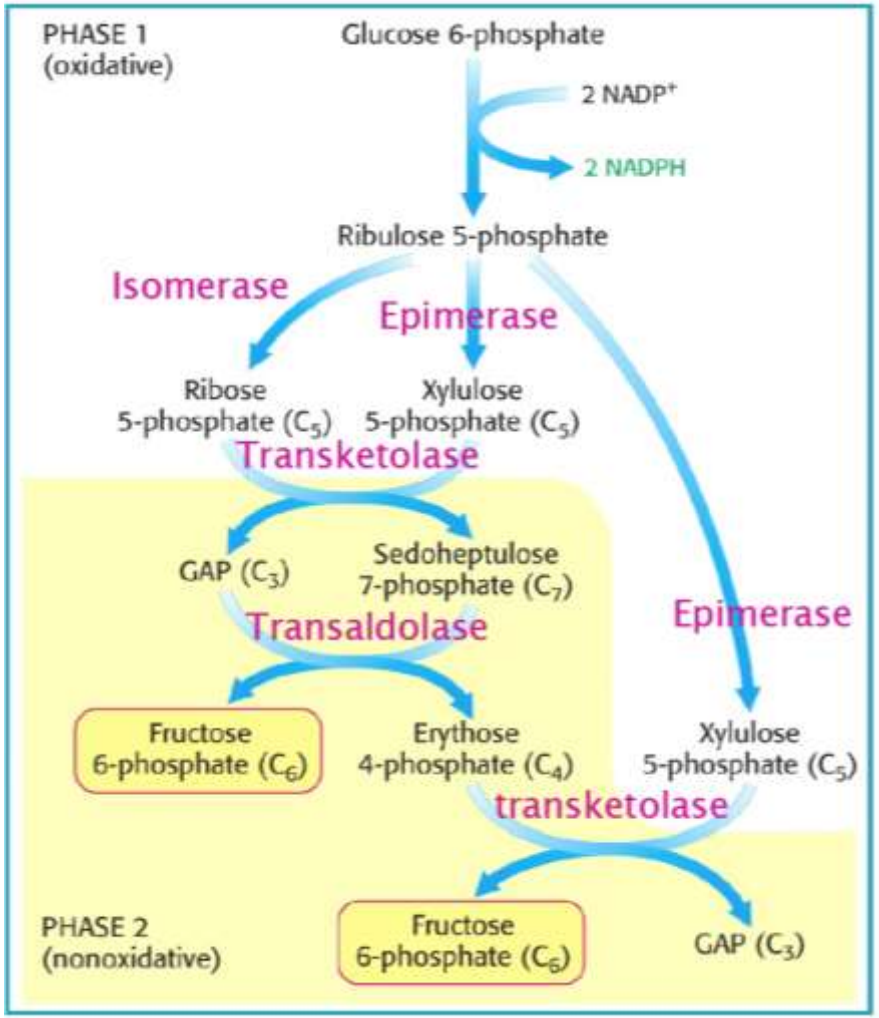
↓

Citric Acid Cycle



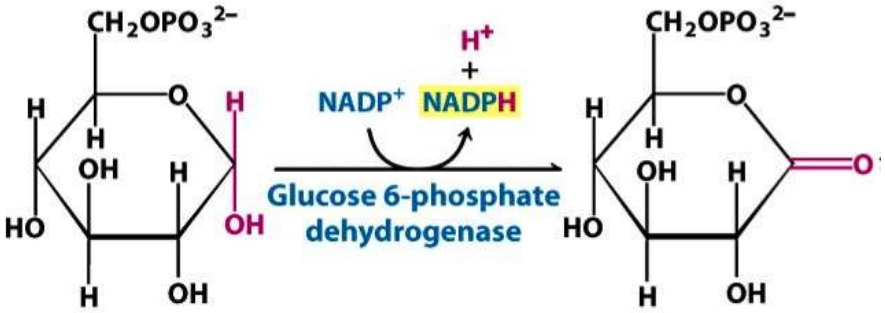
Pentose Phosphate Pathway

- The PPP pathway consists of two phases: the oxidative phase (irreversible reactions) during which NADPH molecules are generated and the non-oxidative phase (reversible reactions) during which different sugars phosphates are synthesized according to cellular need



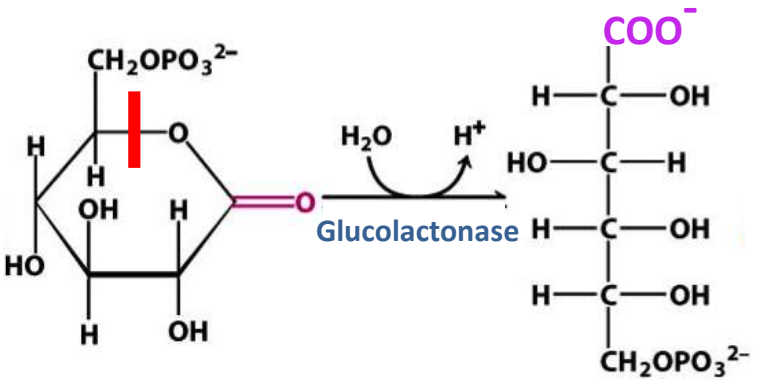
The Oxidative Pathway

- **Step 1:** Glucose-6-phosphate (G6P) is oxidized by G6P dehydrogenase **(G6PD)** generating 6-phosphogluconolactone. One $NADP^+$ is reduced to NADPH



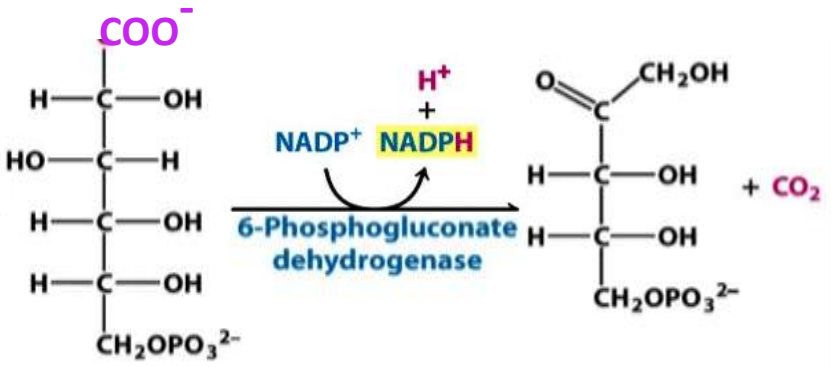
- **Step 2:** 6-phosphogluconolactone is hydrolyzed in presence of H_2O by glucolactonase to 6-phosphogluconate

The Oxidative Pathway



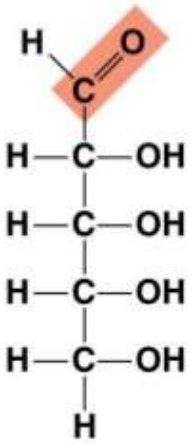
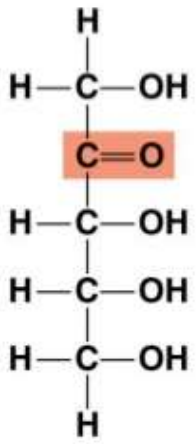
6-Phosphogluconolactone **6-Phosphogluconate**

- **Step 3:** 6-phosphogluconate undergoes oxidative decarboxylation to yield ribulose-5-phosphate, CO_2 and another NADPH. Initially, OH at C3 is oxidized to carbonyl group and subsequently carboxyl group at C1 is eliminated as CO_2



6-Phosphogluconate **Ribulose-5-phosphate**

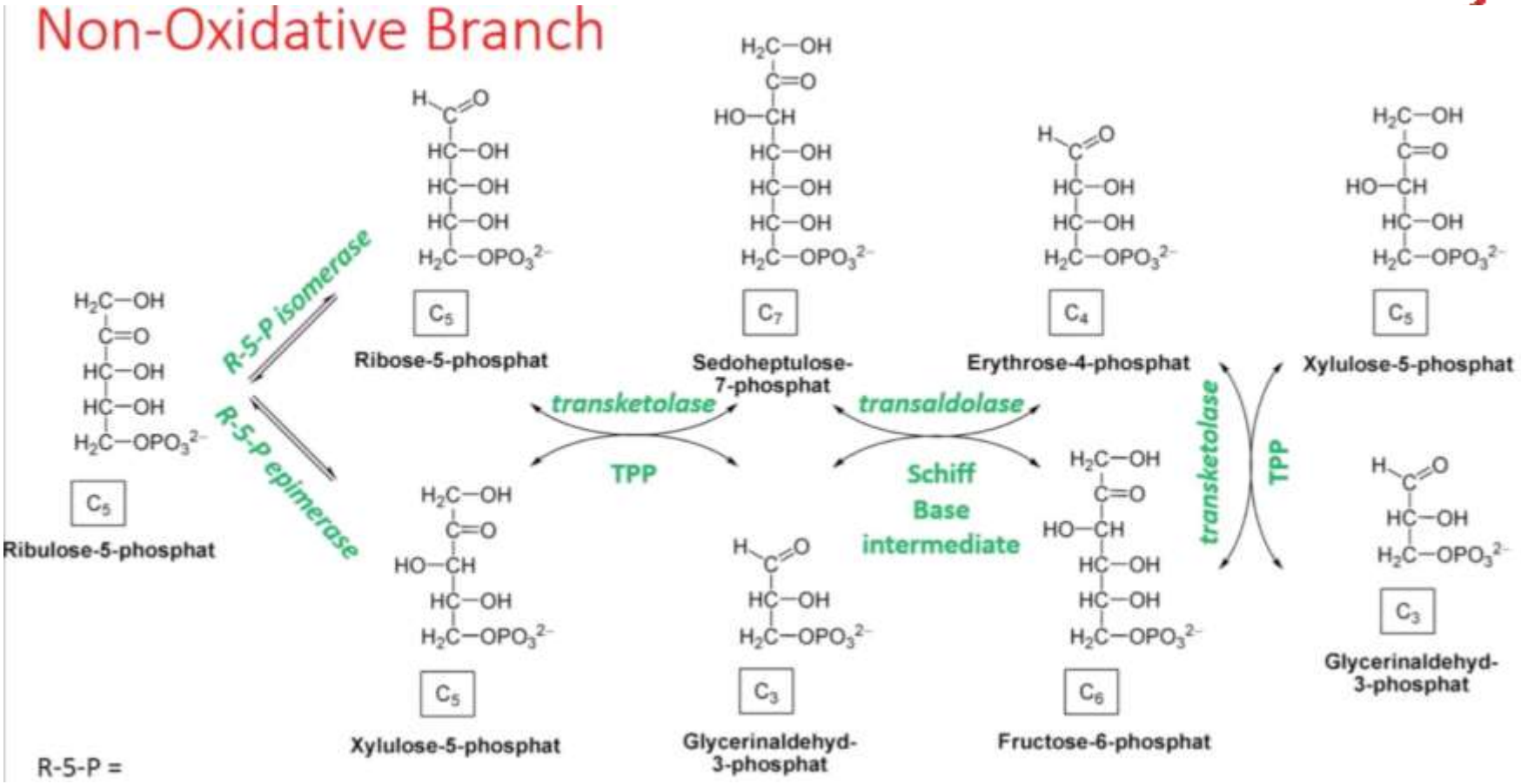
The Oxidative Pathway

Aldose (Aldehyde Sugar)	Ketose (Ketone Sugar)
Pentoses: 5-carbon sugars ($C_5H_{10}O_5$)	
 Ribose	 Ribulose

- **The net result of this process is:**

G6P + 2NADP$^+$ + H_2O → ribulose-5-phosphate + 2NADPH + 2H^+ + CO_2

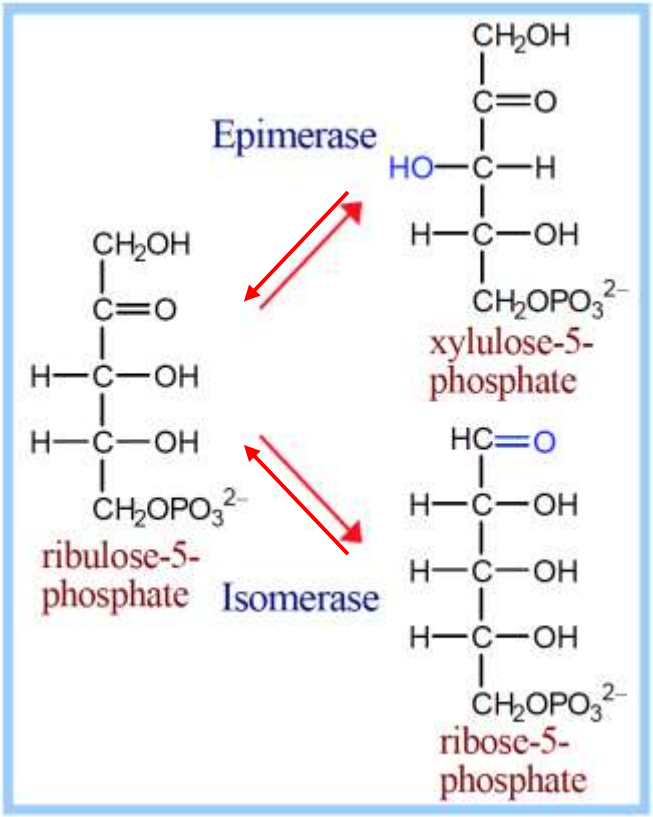
The Non-oxidative Pathway



The non-oxidative pathway is the alternative fates of pentose phosphates

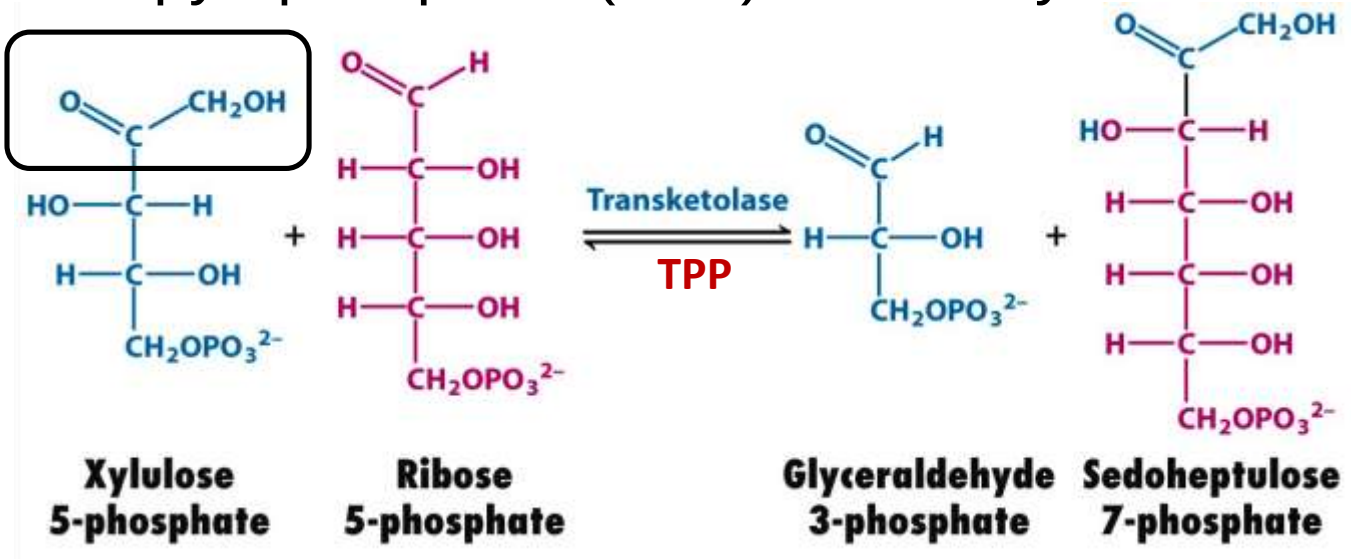
The Non-oxidative Pathway

- **Step 1**: is the beginning of the non-oxidative phase. Some of the ribulose molecules are converted to ribose-5-phosphate by phosphopentose isomerase and some are converted to xylulose-5-phosphate by phosphopentose 3-epimerase



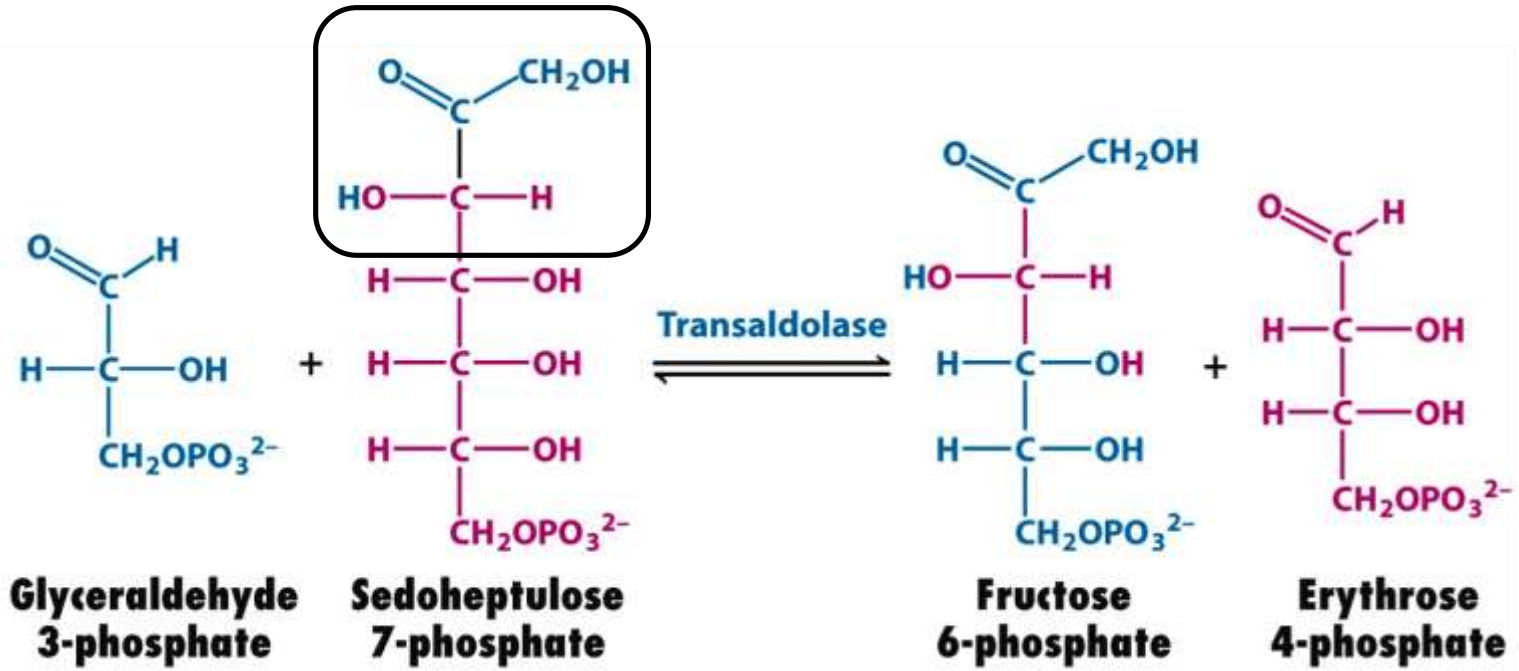
The Non-oxidative Pathway

- **Step 2:** the produced two pentoses: ribose-5-phosphate and xylulose-5-phosphate can react together in a reaction catalyzed by transketolase which transfers a two carbon fragment from xylulose-5-phosphate to ribose-5-phosphate to generate sedoheptulose-7-phosphate (7C) and glyceraldehyde-3-phosphate (3C)
- An activated glycolaldehyde fragment is transferred using thiamine pyrophosphate (TPP) as coenzyme



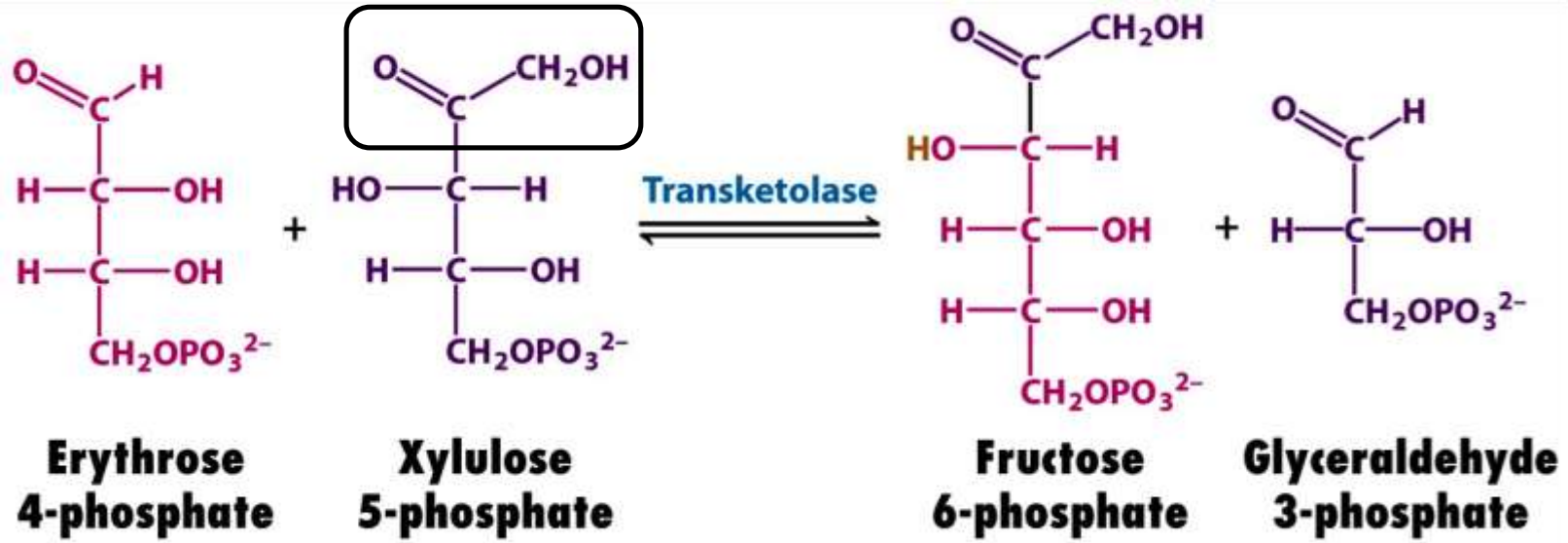
The Non-oxidative Pathway

- **Step 3:** transaldolase acts on the transketolase two products with the transfer of dihydroxyacetone fragment (3C) from 7C substrate to 3C substrate. This reaction generates erythrose-4-phospahte and fructose-6-phoshate



The Non-oxidative Pathway

- **Step 4:** transketolase acts on another molecule of xylulose-5-phosphate by transferring glycolaldehyde fragment (2C) to erythrose-4-phospahte (4C). This produces glyceraldehyde-3-phosphate and fructose-6-phosphate



Pentose Phosphate Pathway

- **Oxidative phase:**

3 G6P + 6 $NADP^+$ → 3 ribulose-5-phosphate +

6 H^+ + 6 NADPH

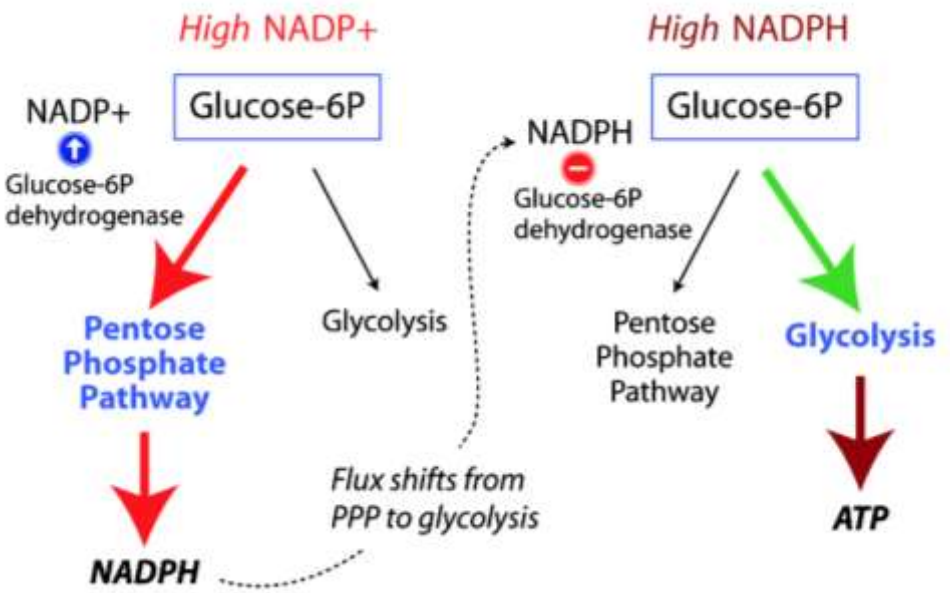
- **Non-oxidative phase:**

2 xylulose-5-phosphate + 1 ribose-5-phosphate →

2 fructose-6-phosphate + 1 glyceraldehyde-3-phosphate

Pentose Phosphate Pathway Regulation

- The activity of glucose-6-phosphate dehydrogenase (catalyzing the rate limiting reaction) is controlled by the ratio of NADPH/$NADP^+$
- It is allosterically stimulated by $NADP^+$ and strongly inhibited by NADPH



Regulation of the G6PD activity controls flux through the glycolytic pathway and pentose phosphate pathways

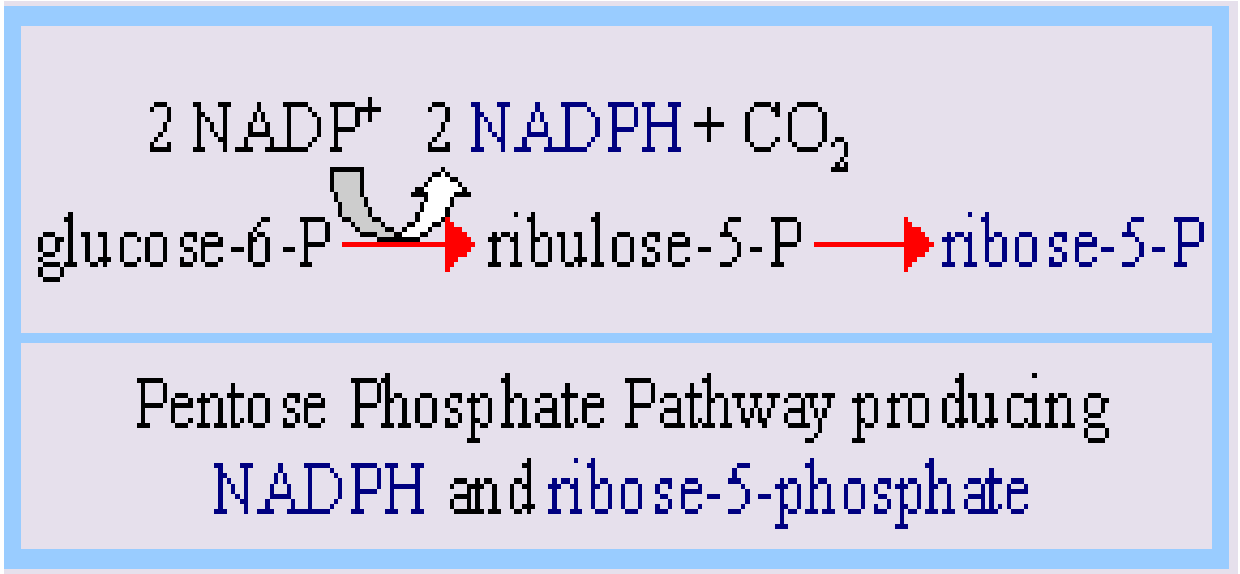
Metabolic Needs of the Cell Direct the Fates of PPP products

- Although PPP is not primarily an energy-generating pathway but in certain modes it can operate to oxidize glucose completely to CO_2 and H_2O
- The actual fates of PPP sugar phosphates depend on the metabolic needs of the cell in which the pathway is functioning
- Therefore, PPP can operate in various modes/scenarios to maximize the level of its different products (i.e. NADPH, ribose-5-phosphate and ATP)
- Because of the multiple metabolic needs of a particular cell, more than one model operates in that cell in temporal fashion (time based)

Metabolic Needs of the Cell Direct the Fates of PPP products

1. First Metabolic Mode **"nucleic acids biosynthesis"**

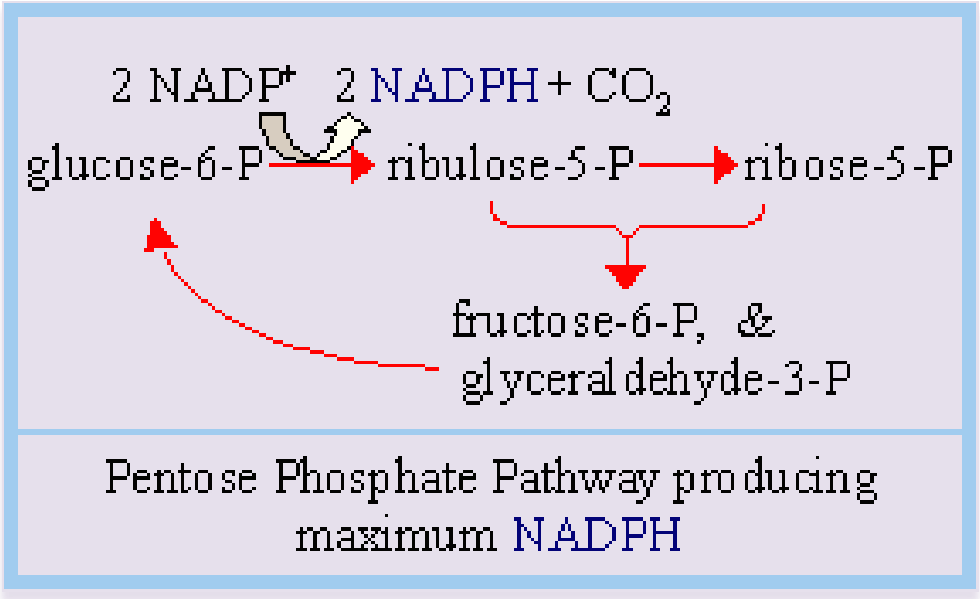
- If the primary need is for nucleotide and nucleic acid synthesis (as in rapidly proliferating cells), the major product is ribose-5-phosphate and most of the non-oxidative phase does not take place. Some NADPH are also produced



Metabolic Needs of the Cell Direct the Fates of PPP products

2. Second Metabolic Mode **"NADPH Synthesis"**

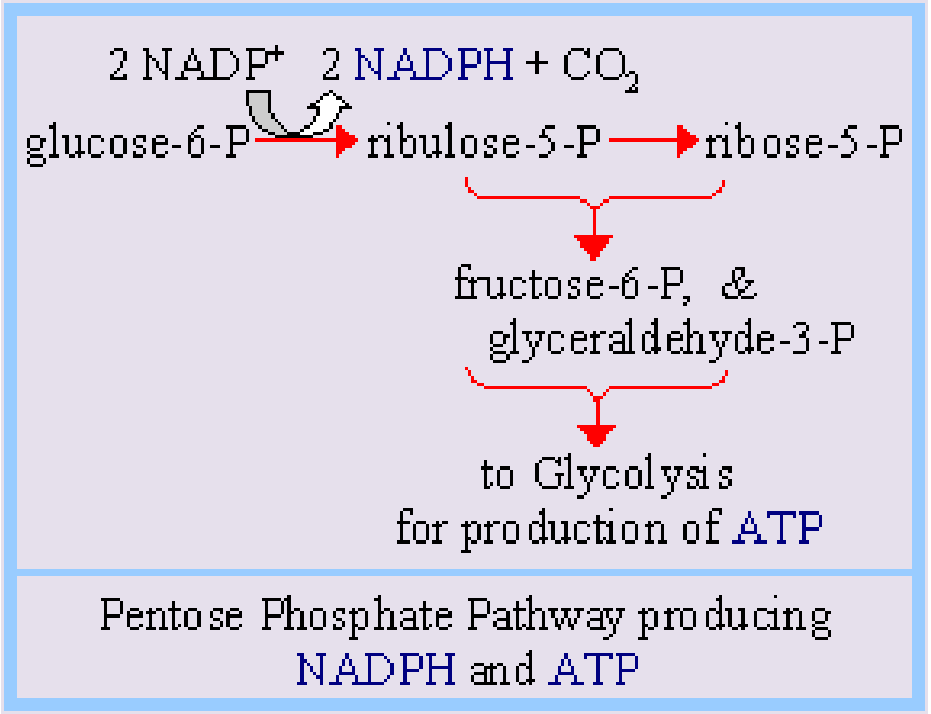
- If the primary need is for NADPH (i.e. for fatty acids or steroids synthesis), the non-oxidative phase generates compounds that can be easily reconverted to G6P for subsequent passage through the oxidative phase maximizing the NADPH production



Metabolic Needs of the Cell Direct the Fates of PPP products

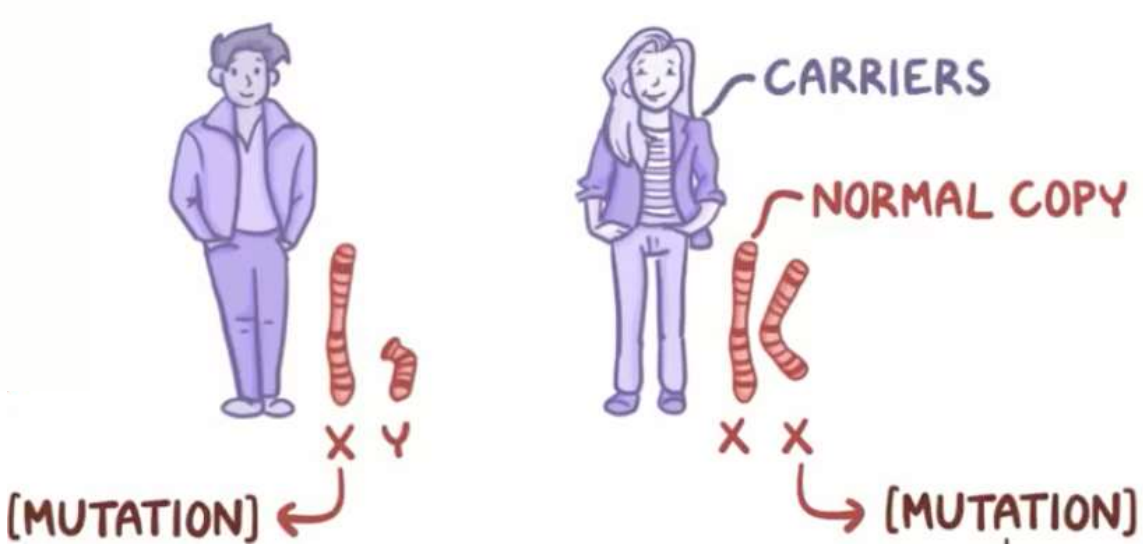
3. Third Metabolic Mode **"Energy Generation"**

- If the cell in moderate need for both NADPH and ribose-5-phosphate, the end products of non-oxidative phase F6P and G3P can be further catabolized by glycolysis and TCA cycle to produce ATP. This pathway also produce some NADPH



G6P Dehydrogenase Deficiency

- One of well known disorder is the deficiency in G6P dehydrogenase also known as “favism” consequently, reduced intracellular NADPH level. It is an X-linked recessive genetic condition

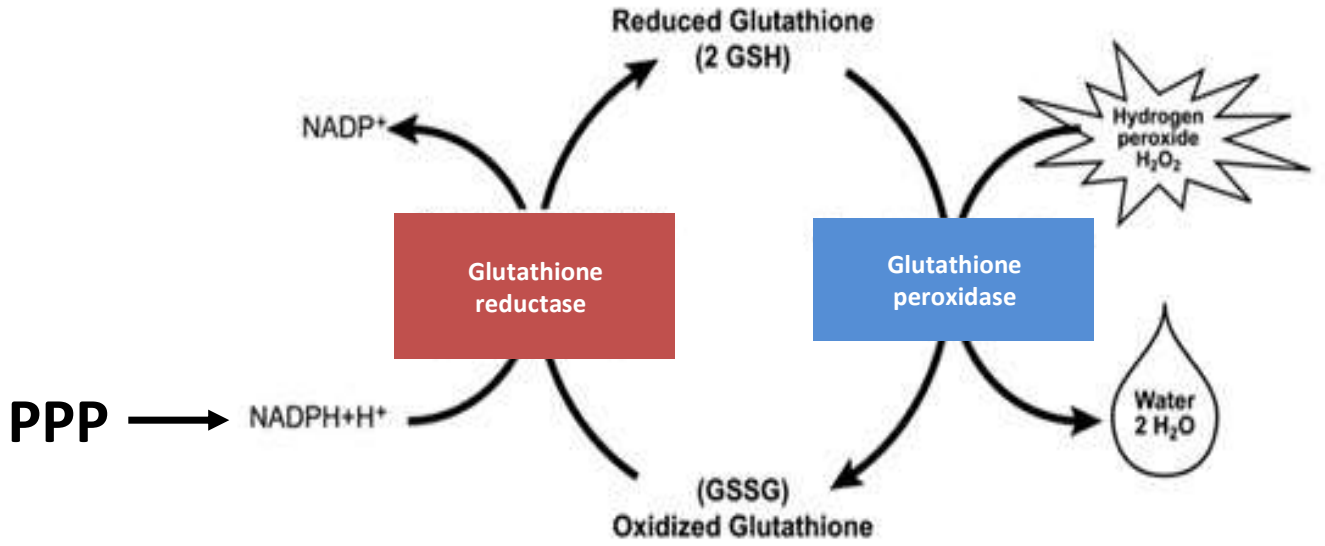


Favism

G6P Dehydrogenase Deficiency

G6P Dehydrogenase Deficiency

- Defects in PPP results in reduced intracellular NADPH level which participates in the glutathione cycle to protect cells against hydrogen peroxide
- G6PD enzyme prevents oxidative damage
- G6PD deficiency is characterized by hemolytic anemia

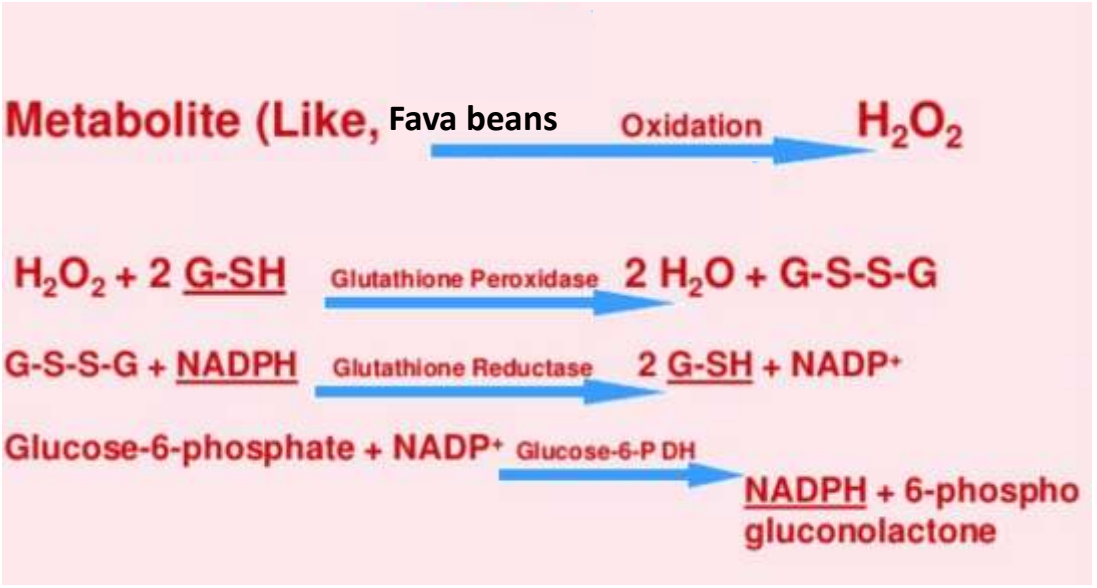


G6P Dehydrogenase Deficiency

- PPP is active pathway in RBCs for generation of reducing power. Actually, NADPH in RBCs is important to keep a high ratio of the reduced glutathione which is vital to protect cells from damaging effect of ROS (detoxification process)
- People with this deficiency are asymptomatic until stressed
- People with G6PD deficiency are at risk of hemolytic anemia (destruction of RBCs) in state of oxidative stress such as exposure to infection, some medications and certain foods (e.g. broad or fava beans)
- Oxidative stress is due to imbalance between the generation of ROS or free radicals (e.g. H_2O_2, ·OH,…) and the removal by specific cellular enzymes (antioxidants) like glutathione peroxidase (enzyme abundant in cells)

G6P Dehydrogenase Deficiency

- Oxidative stress depletes the reduced form of glutathione (GSH) and G6P dehydrogenase deficiency disorder can not supply enough NADPH to regenerate GSH from the oxidized one (GSSG)



- Damaged RBCs are recycled to the spleen. The hemoglobin is metabolized to bilirubin causing jaundice in high concentration