Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 5
Reading: Ch. 14 of *Principles of Biochemistry*, "Glycolysis, Gluconeogenesis, & Pentose Phosphate Pathway."

PENTOSE PHOSPHATE PATHWAY

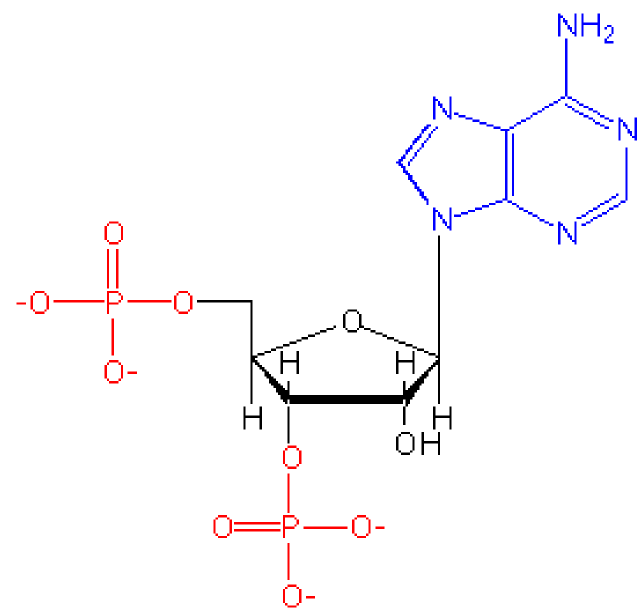
This pathway produces ribose from glucose, and it also generates 2 NADPH.

Two Phases: [1] Oxidative Phase & [2] Non-oxidative Phase

$$\textbf{Glucose 6-Phosphate} + 2\ \textbf{NADP}^+ + \textbf{H}_2\textbf{O} \leftrightarrow \textbf{Ribose 5-Phosphate} + 2\ \textbf{NADPH} + \textbf{CO}_2 + 2\textbf{H}^+$$

● *What are pentoses? Why do we need them?*

- ◦ DNA & RNA
- ◦ Cofactors in enzymes

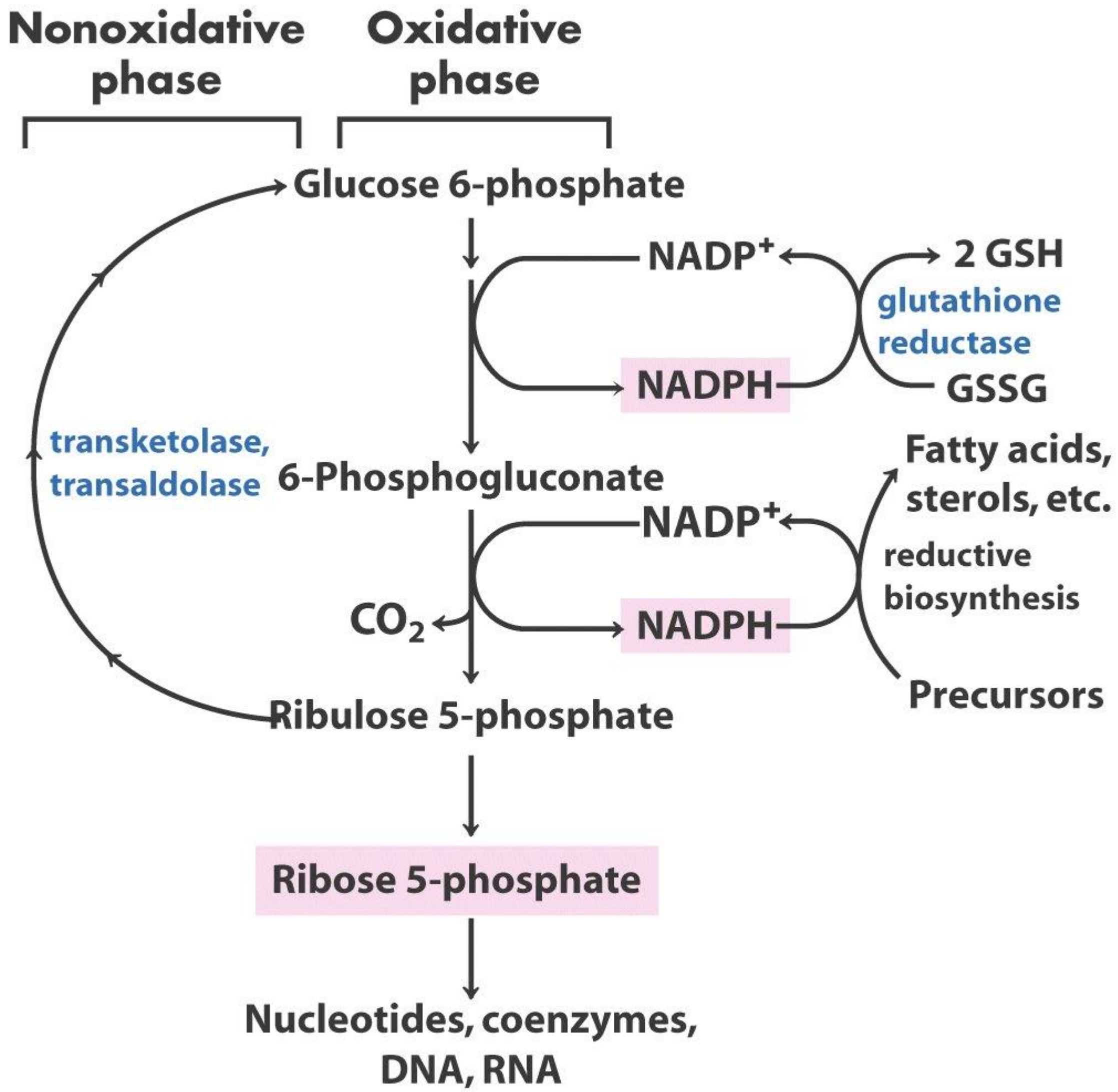


● *Where do we get them?* Diet and from glucose (and other sugars) via the Pentose Phosphate Pathway.

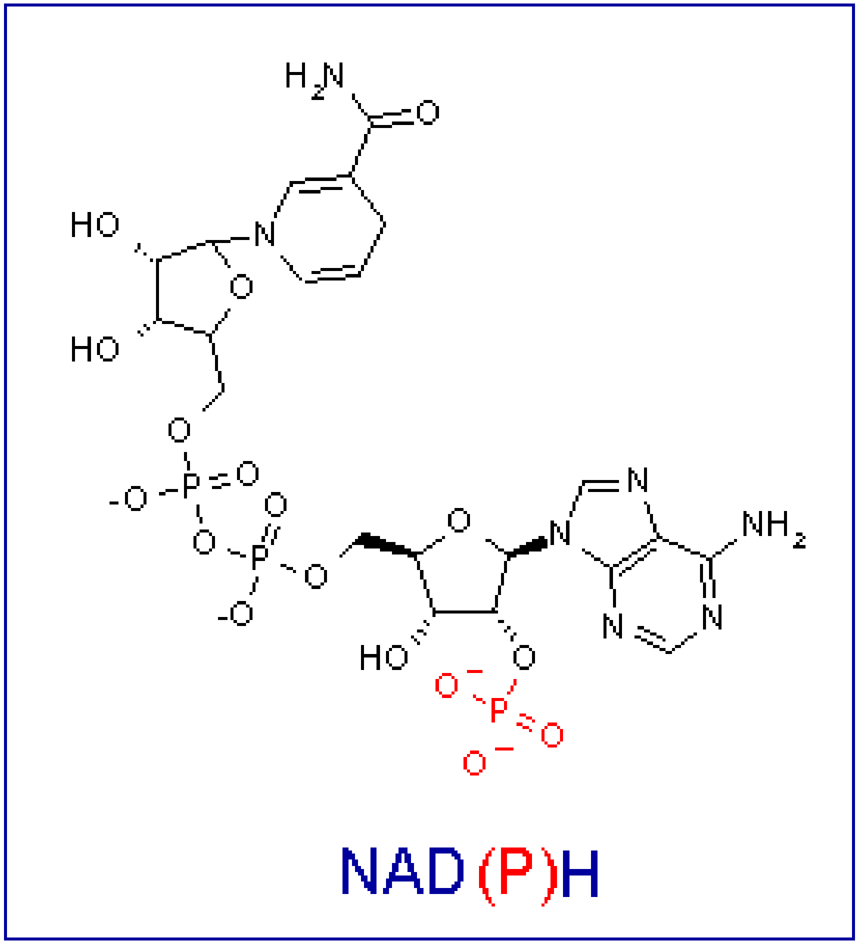
● *Is the Pentose Phosphate Pathway just about making ribose sugars from glucose?* (1) Important for biosynthetic pathways using NADPH, and (2) a high cytosolic reducing potential from NADPH is sometimes required to advert oxidative damage by radicals, *e.g.*,

$$^{\bullet}\mathbf{O_2^-} \quad \text{and} \quad \mathbf{H{-}O^{\bullet}}$$

Two Phases of the Pentose Pathway

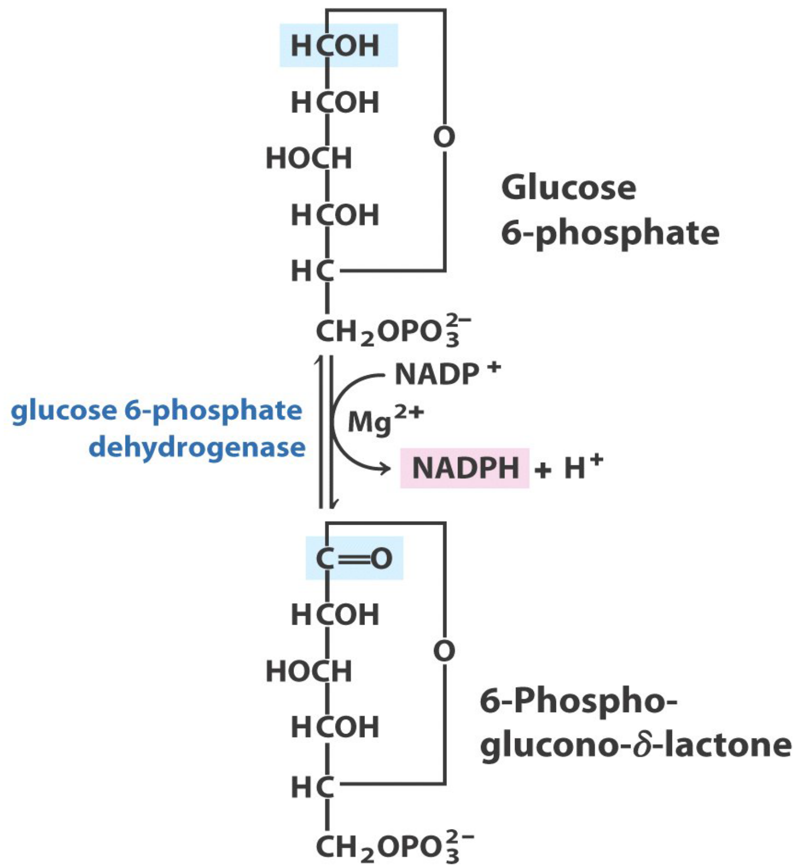


NADPH vs. NADH



Oxidative Phase: Glucose-6-P → Ribose-5-P

Glucose 6-phosphate dehydrogenase. First enzymatic step in oxidative phase, converting $NADP^+$ to NADPH.



Glucose 6-phosphate + $NADP^+$ ←→

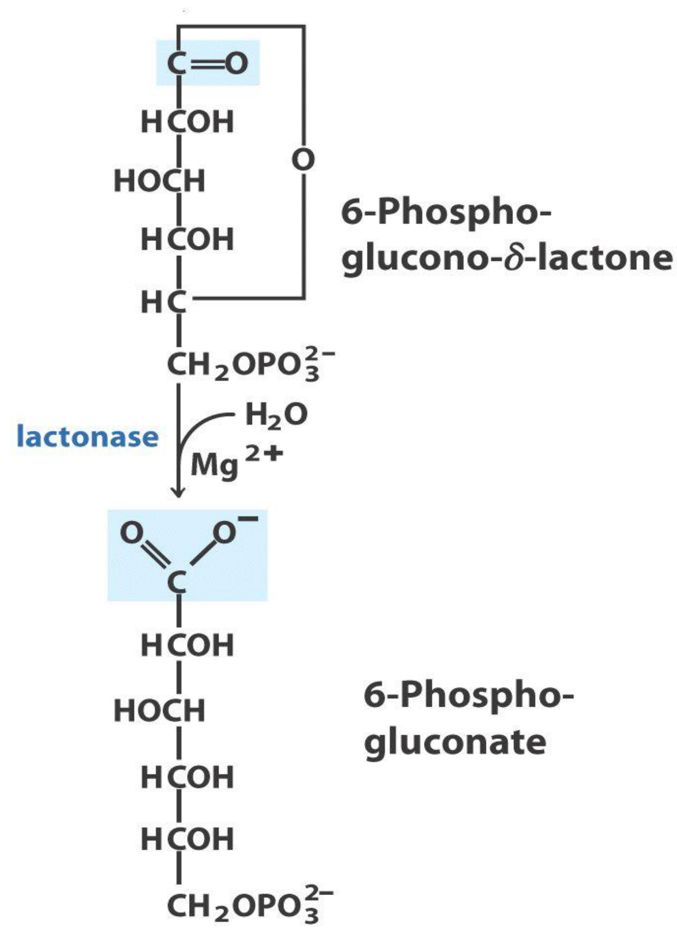
6-Phosphoglucono-δ-lactone + NADPH + H^+

Mechanism. Oxidation reaction of C1 position. Hydride transfer to the $NADP^+$, forming a **lactone**, which is an intra-molecular ester.

Energetics. Very favorable reaction in the forward direction for producing NADPH.

Lactonase. A specific enzyme that targets 6-Phosphoglucono-δ-lactone for hydrolysis.

6-Phosphoglucono-δ-lactone ←→ 6-Phosphogluconate



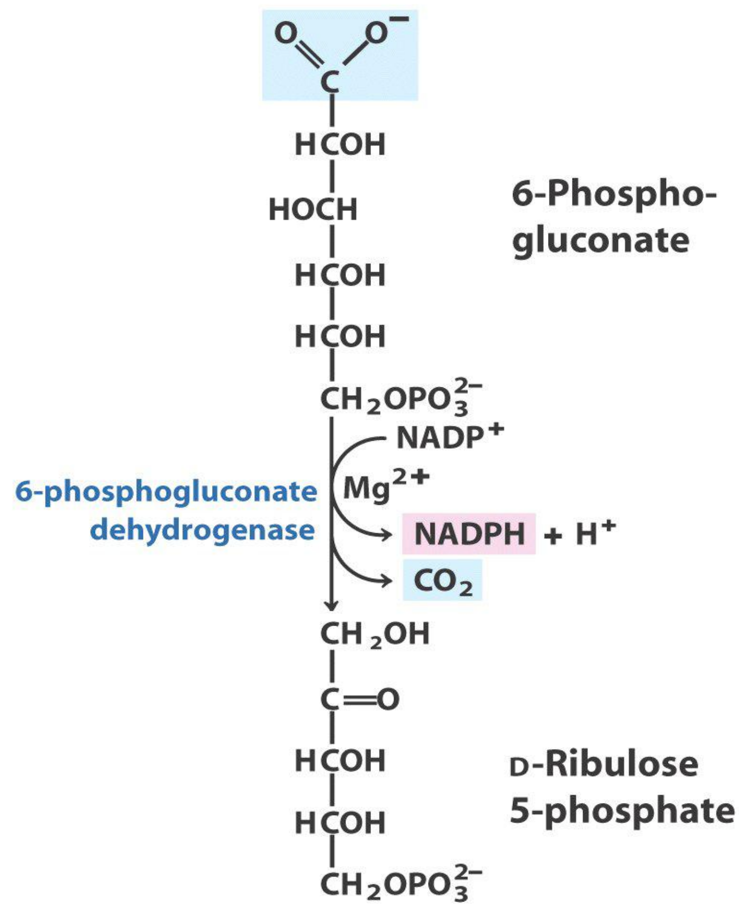
Mechanism. The lactone is opened by **hydrolysis**, the addition of water to cleave a bond, usually a type of amide or ester. In this case, since the lactone (by definition) is intra-molecular, then 6-phosphoglucono-δ-lactone is opened up to the acid form, gluconate.

Energetics. Often these types of hydrolysis reactions are very favorable.

<u>6-Phosphogluconate dehydrogenase.</u> Performs oxidative decarboxylation converting the 6-carbon skeleton to a pentose.

$$\textbf{6-Phosphogluconate + NADP}^{+} \leftrightarrow \textbf{D-Ribulose 5-phosphate + NADPH + H}^{+} \textbf{ + CO}_2$$

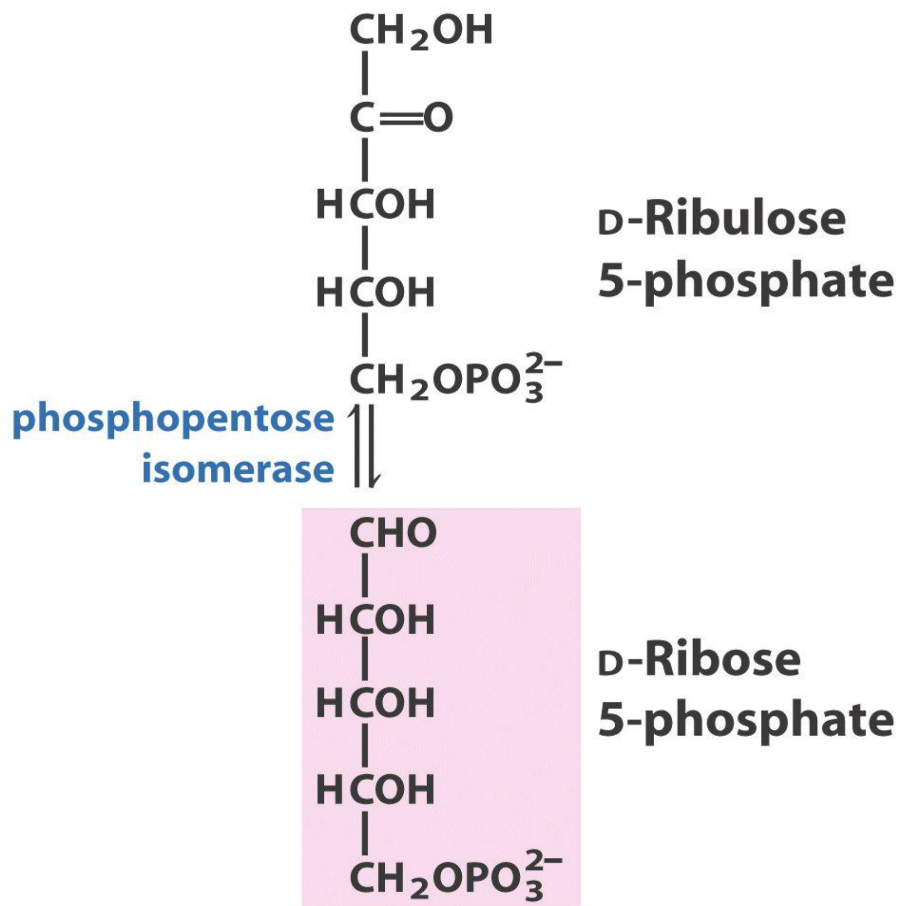
<u>Mechanism.</u> The C1 carboxylate is removed as the C3 position is oxidized to a ketone, making 5-carbon ketose, ribulose.



Phosphopentose Isomerase.

D-Ribulose 5-phosphate ←→ D-Ribose 5-phosphate

Mechanism. Typical ketose to aldose conversion. Another example of a general acid base catalyzed reaction.

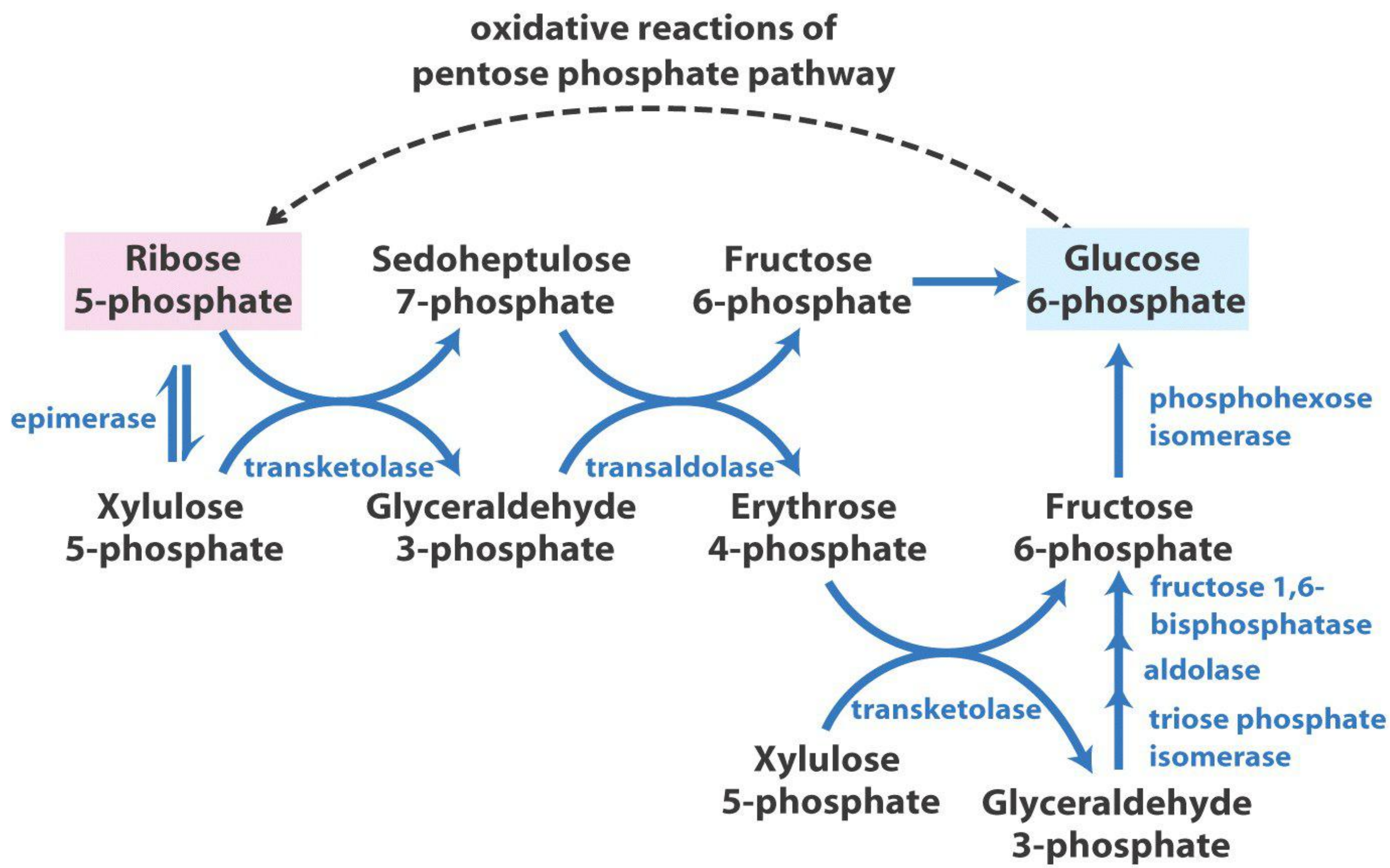


Non-oxidative Phase: Recycling Pentose Phosphates to Glucose 6-Phosphate

What if the cell needs much more NADPH than it needs pentose? D-ribose 5-phosphate has to be converted back to glucose 6-phosphate in multiple enzyme catalyzed steps.

The recycling of 5-carbon skeletons as expected for the stoichiometry below is complex:

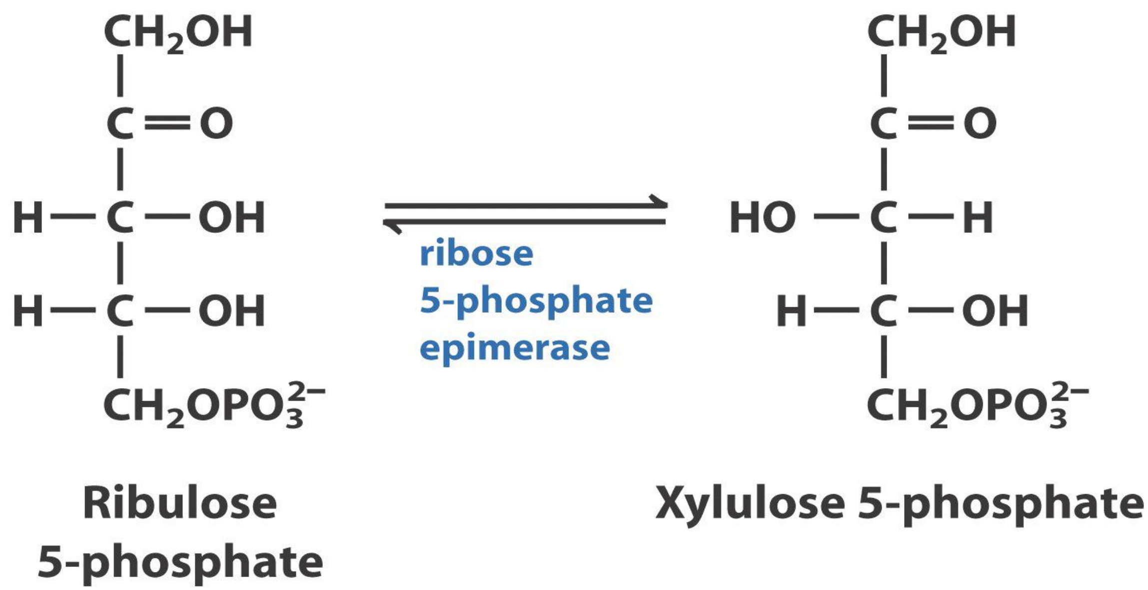
6 Pentose ←→ 5 Hexose



Ribulose 5-Phosphate Epimerase.

Ribulose 5-phosphate ←→ Xylulose 5-phosphate

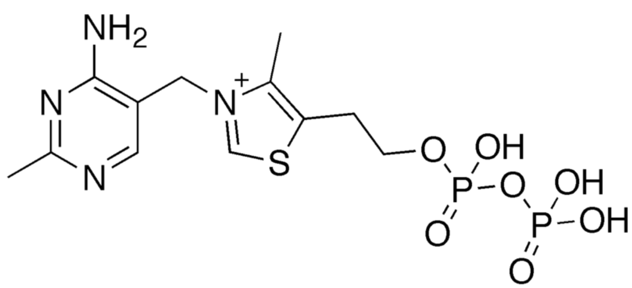
Mechanism. **Epimerization** reaction is the flipping of a stereo center in the substrate. An **epimer** of a compound occurs when only *one* stereo center is flipped.

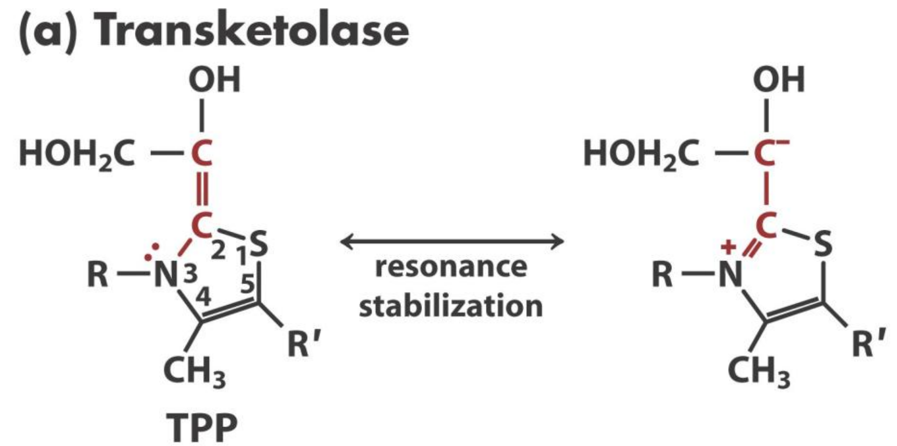


Transketolase & Transaldolase

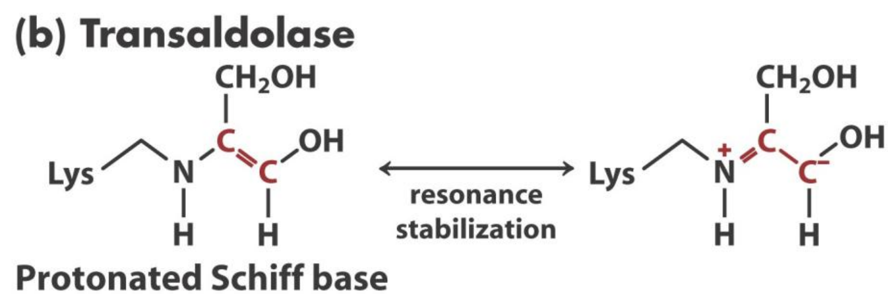
A myriad of steps are required to transform pentoses back to glucose, but besides the epimerase, only two other *new* enzymes are needed: **[1] transketolase** and **[2] transaldolase.**

Transketolase. This enzyme uses a cofactor, called **thiamine pyrophosphate (TPP)**. TPP is used to stabilize a 2-carbon carbanion intermediate.





Transaldolase. This enzyme forms a protonated Schiff base intermediate with a ketose, stabilizing a 3-carbon carbanion intermediate, allowing an aldehyde based sugar to react with the enzyme-linked ketose. The mechanism is similar to aldolase.



[1] <u>Transketolase + TPP</u>

Ribulose 5-phosphate + Xylulose 5-phosphate ←→

Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate (G3P)

[2] <u>Transaldolase</u>

Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate (G3P) ←→

Fructose 6-phosphate + Erythrose 4-phosphate

[3] <u>Transketolase + TPP</u>

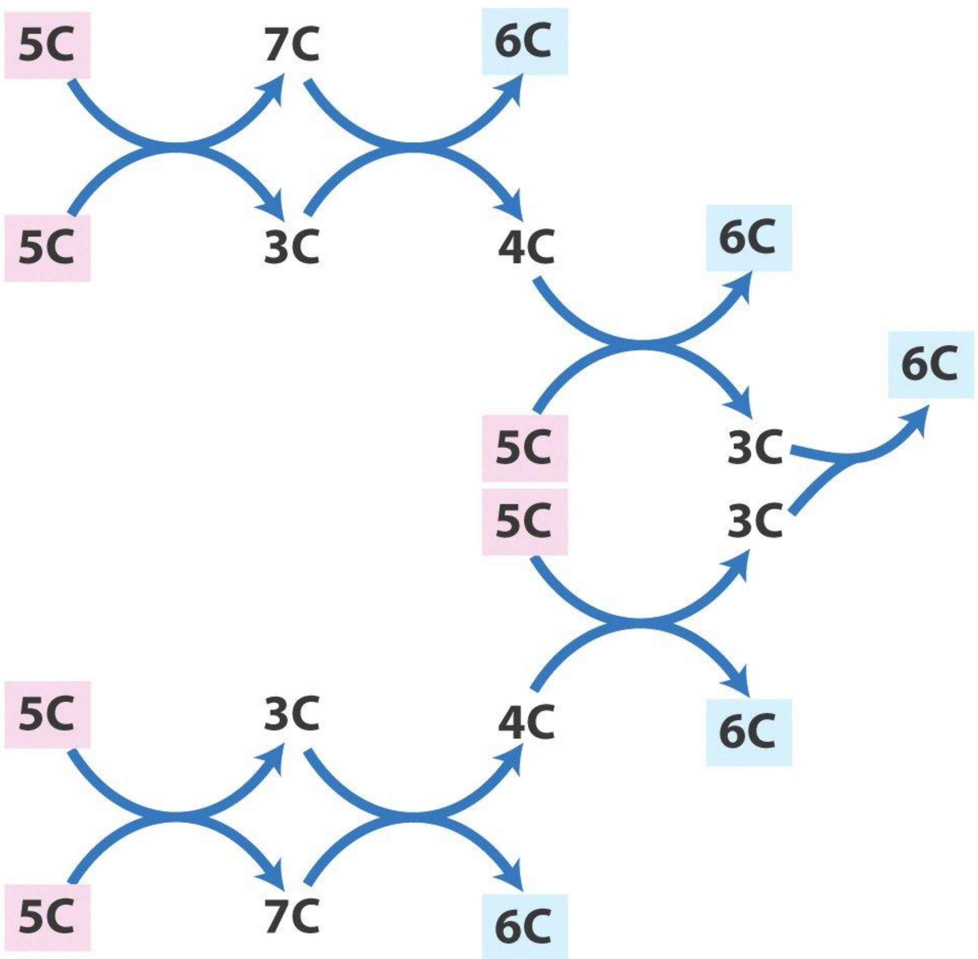
Erythrose 4-phosphate + Xylulose 5-phosphate←→

Glyceraldehyde 3-phosphate (G3P) + Fructose 6-phosphate

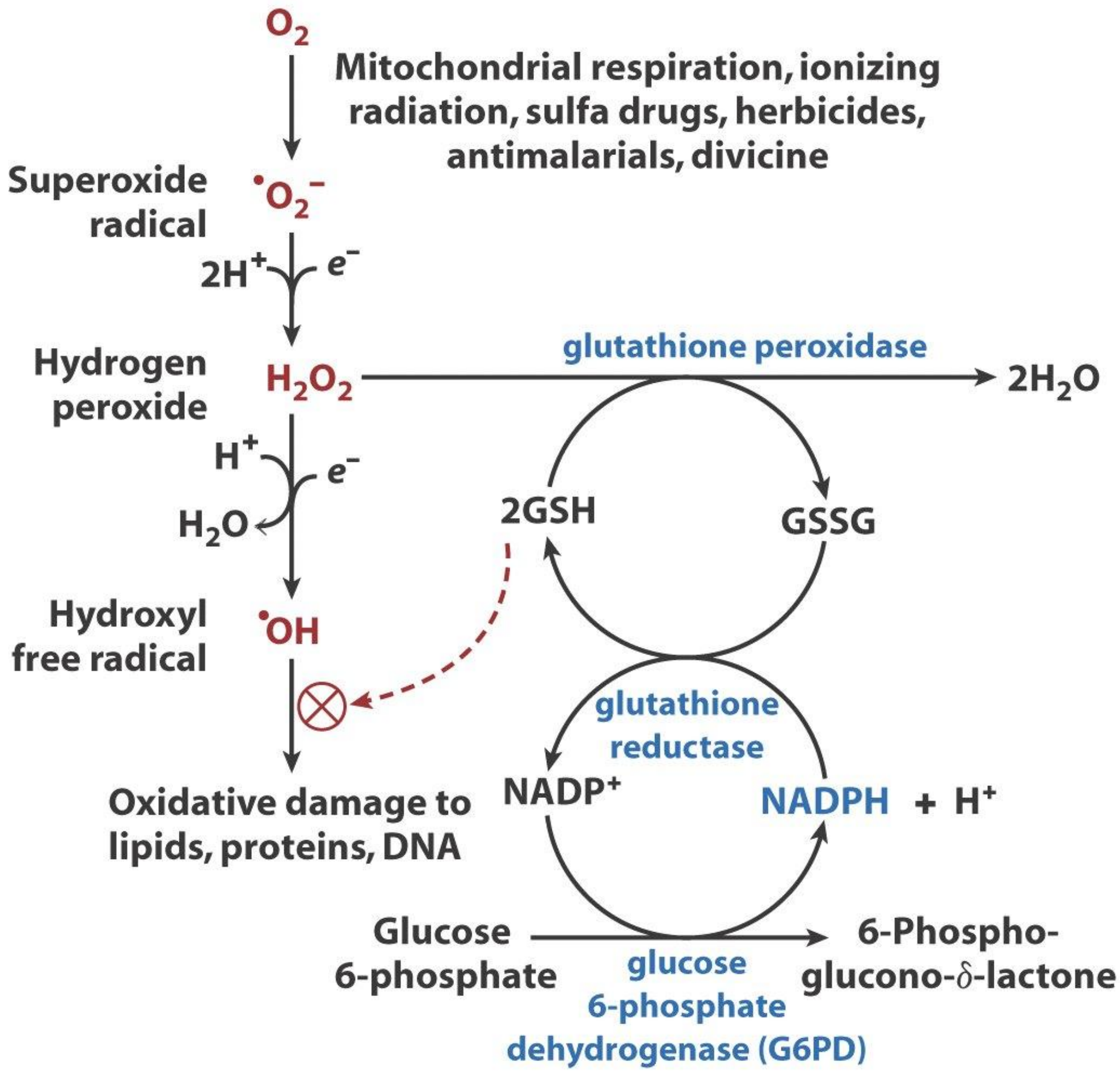
[4] <u>Reversal of Glycolysis</u>

G3P ←→DHAP + G3P ←→ Fructose 1,6-bisphosphate → Fructose 6-phosphate

Reactions Required to Convert 6 Pentoses to 5 Hexoses



Protection from Radical Damage



<u>Regulation of the Pentose Phosphate Pathway.</u>

How is this pentose phosphate pathway regulated? NADPH which is generated in the oxidative branch of the pathway can feed back and inhibit the pathway. Glucose 6-phosphate can still be utilized by glycolysis.