Assist. Prof. Dr. Shakir Faris Tuleab

Ph. D. Biochemistry

University of Anbar

College Of Education For Pure Sciences

Chemistry Department

Molecular Biochemistry II

Pentose Phosphate Pathway

Rensselaer

Pentose Phosphate Pathway

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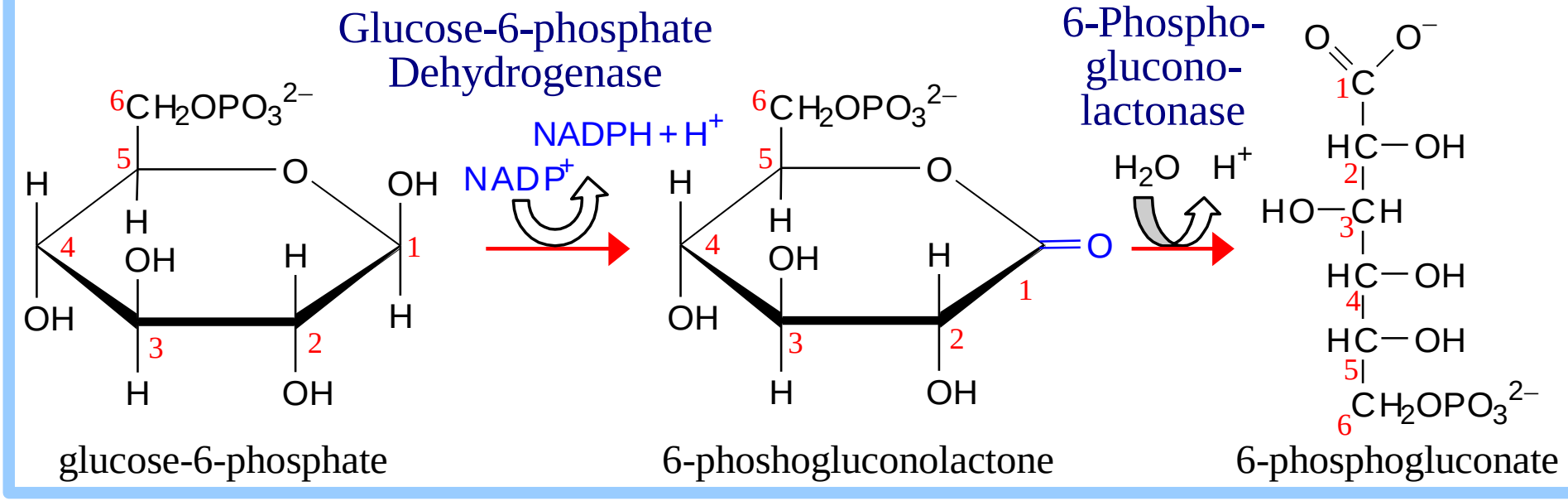
- Other names:

  Phosphogluconate Pathway

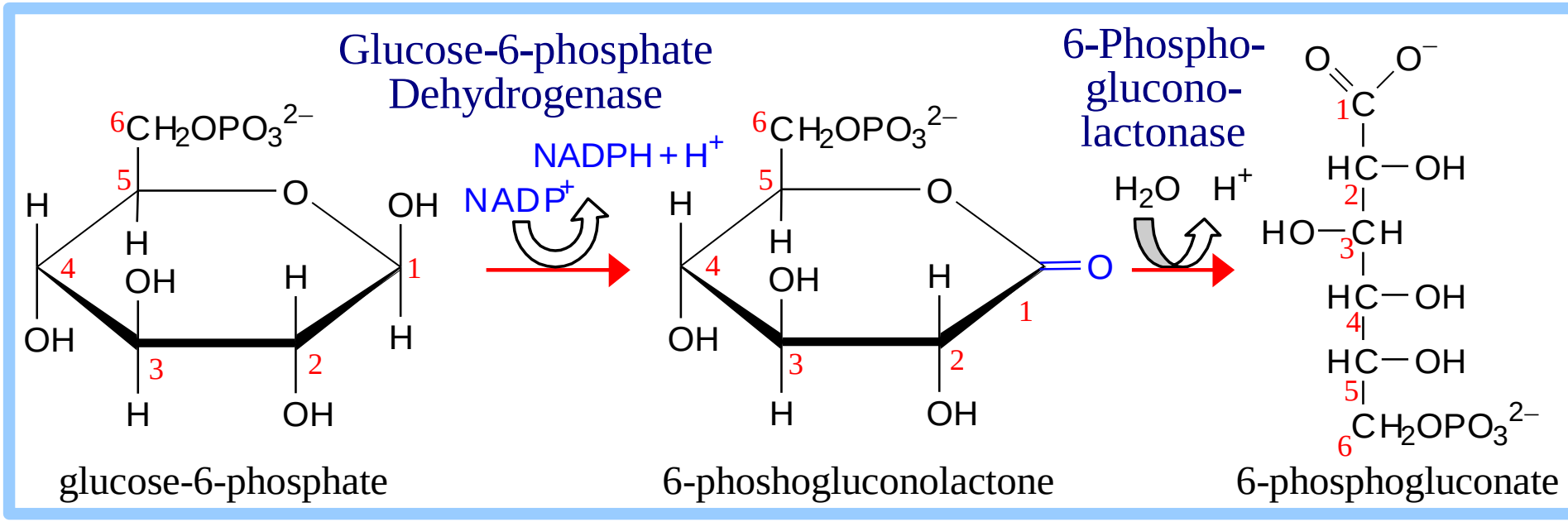
  Hexose Monophosphate Shunt

- The linear part of the pathway carries out oxidation and decarboxylation of the 6-C sugar glucose-6-P, producing the 5-C sugar ribulose-5-P.



Glucose-6-phosphate Dehydrogenase catalyzes **oxidation** of the aldehyde (hemiacetal), at **C1** of glucose-6-phosphate, to a **carboxylic acid**, in ester linkage (lactone).

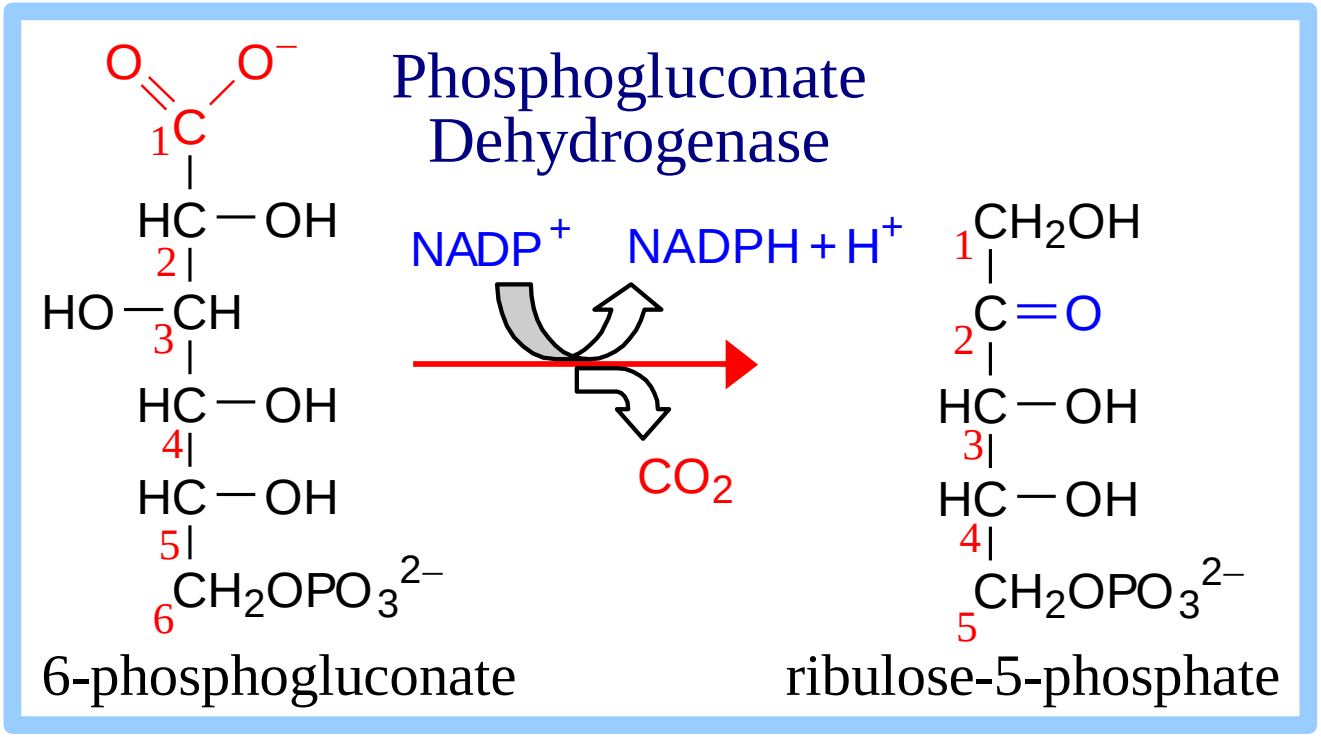
NADP$^+$ serves as electron acceptor.



6-Phosphogluconolactonase catalyzes **hydrolysis** of the ester linkage, resulting in **ring opening**.

The product is **6-phosphogluconate**.

Although ring opening occurs in the absence of a catalyst, 6-Phosphogluconolactonase speeds up the reaction, decreasing the lifetime of the highly reactive, and thus potentially toxic, 6-phosphogluconolactone.



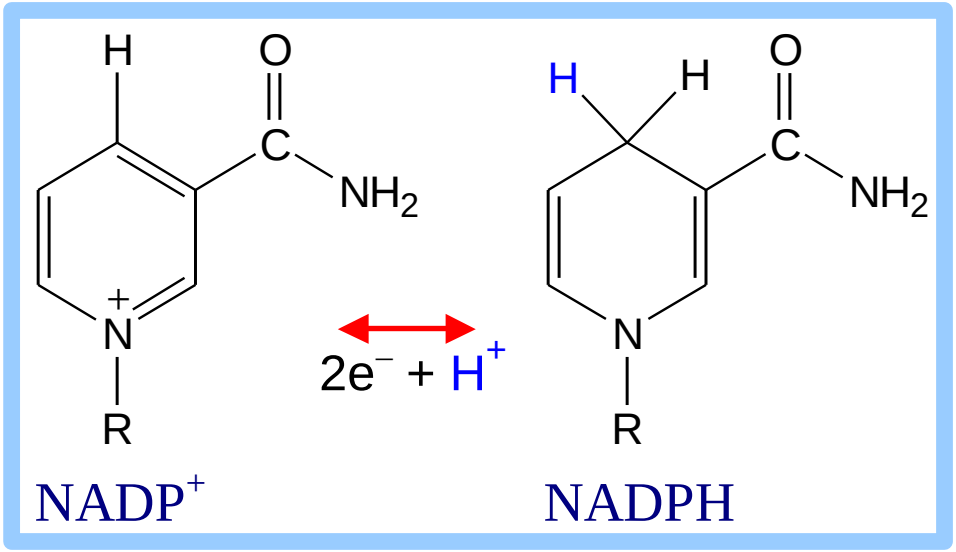
Phosphogluconate Dehydrogenase catalyzes **oxidative decarboxylation** of 6-phosphogluconate, to yield the **5-C** ketose **ribulose-5-phosphate**.

The **OH** at C**3** (C**2** of product) is oxidized to a **ketone**.

This promotes loss of the carboxyl at C**1** as **CO_2**.

$NADP^+$ serves as oxidant.

Reduction of $NADP^+$ (as with NAD^+) involves transfer of $2e^-$ and $1H^+$ to the nicotinamide moiety.



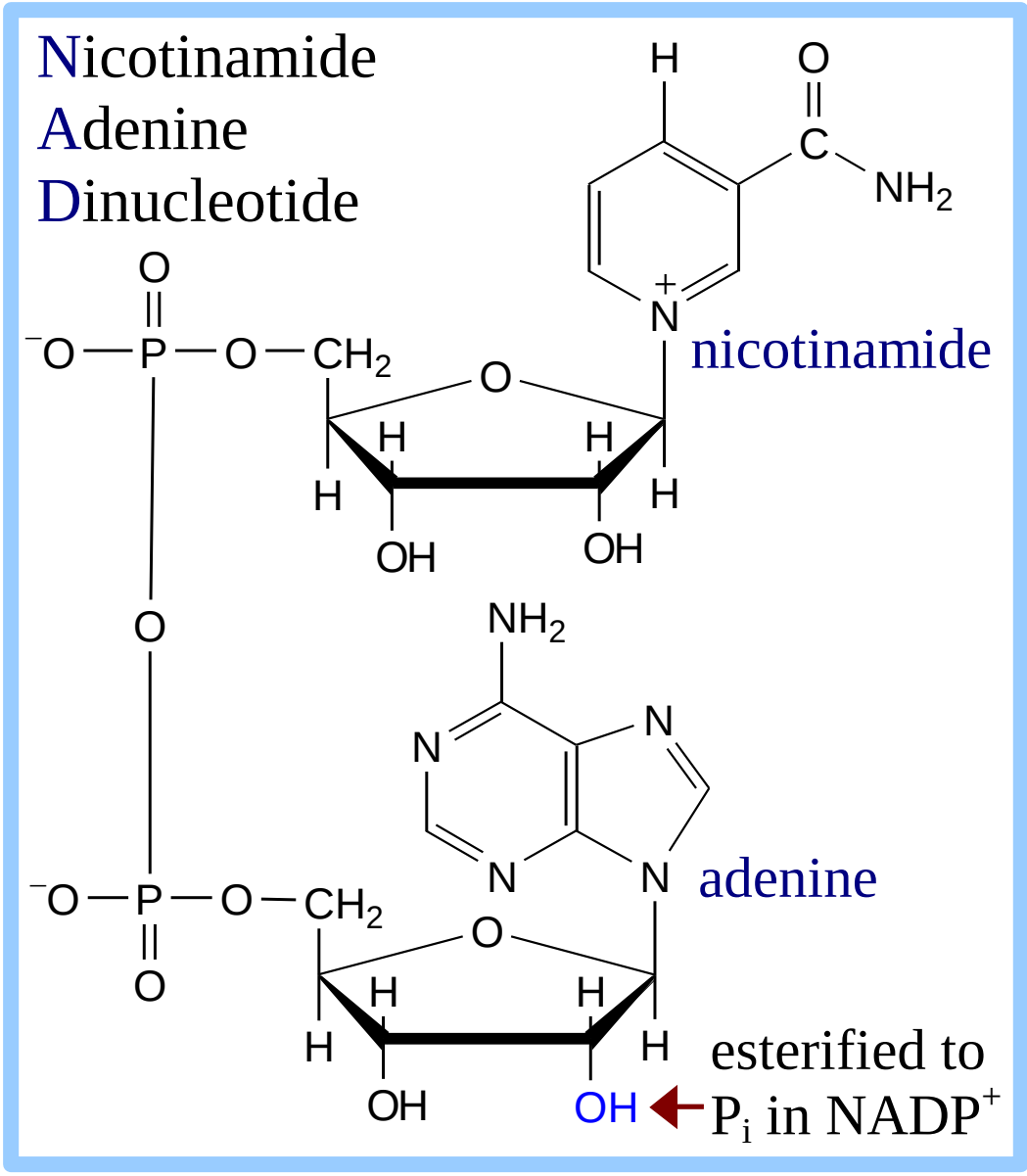
- **NADPH**, a product of the Pentose Phosphate Pathway, functions as a reductant in **anabolic** (synthetic) pathways, e.g., fatty acid synthesis.
- **NAD^+** serves as electron acceptor in **catabolic** pathways, in which metabolites are oxidized.

  The resultant NADH is reoxidized by the respiratory chain, producing ATP.

NAD$^+$ & **NADP$^+$** differ only in the presence of an extra **phosphate** on the adenosine ribose of NADP$^+$.

This difference has little to do with redox activity, but is recognized by substrate-binding sites of enzymes.

It is a mechanism for separation of **catabolic** and **synthetic** pathways.



Regulation of Glucose-6-phosphate Dehydrogenase:

- Glucose-6-phosphate Dehydrogenase is the **committed step** of the Pentose Phosphate Pathway. This enzyme is regulated by availability of the substrate **$NADP^+$**.
- As NADPH is utilized in reductive synthetic pathways, the increasing concentration of $NADP^+$ stimulates the Pentose Phosphate Pathway, to replenish NADPH.

The rest of the pathway converts ribulose-5-P to the **5-C** product ribose-5-P, or to **3-C** glyceraldehyde-3-P & **6-C** fructose-6-P.

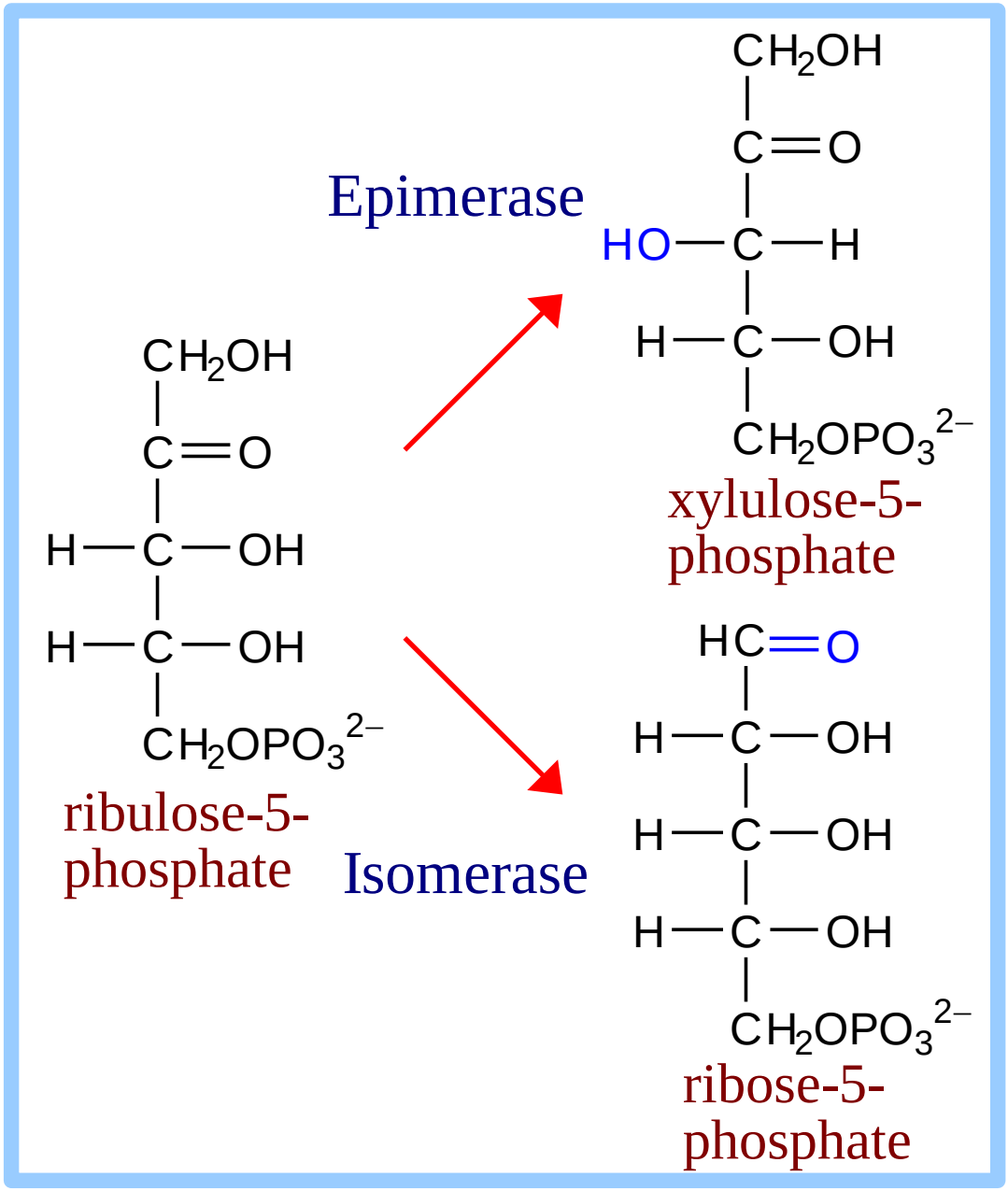
Additional enzymes include an Isomerase, Epimerase, Transketolase, and Transaldolase.

Epimerase inter-converts stereoisomers ribulose-5-P and xylulose-5-P.

Isomerase converts the ketose ribulose-5-P to the aldose ribose-5-P.

Both reactions involve deprotonation to an **endiolate** intermediate followed by specific reprotonation to yield the product.

Both reactions are reversible.

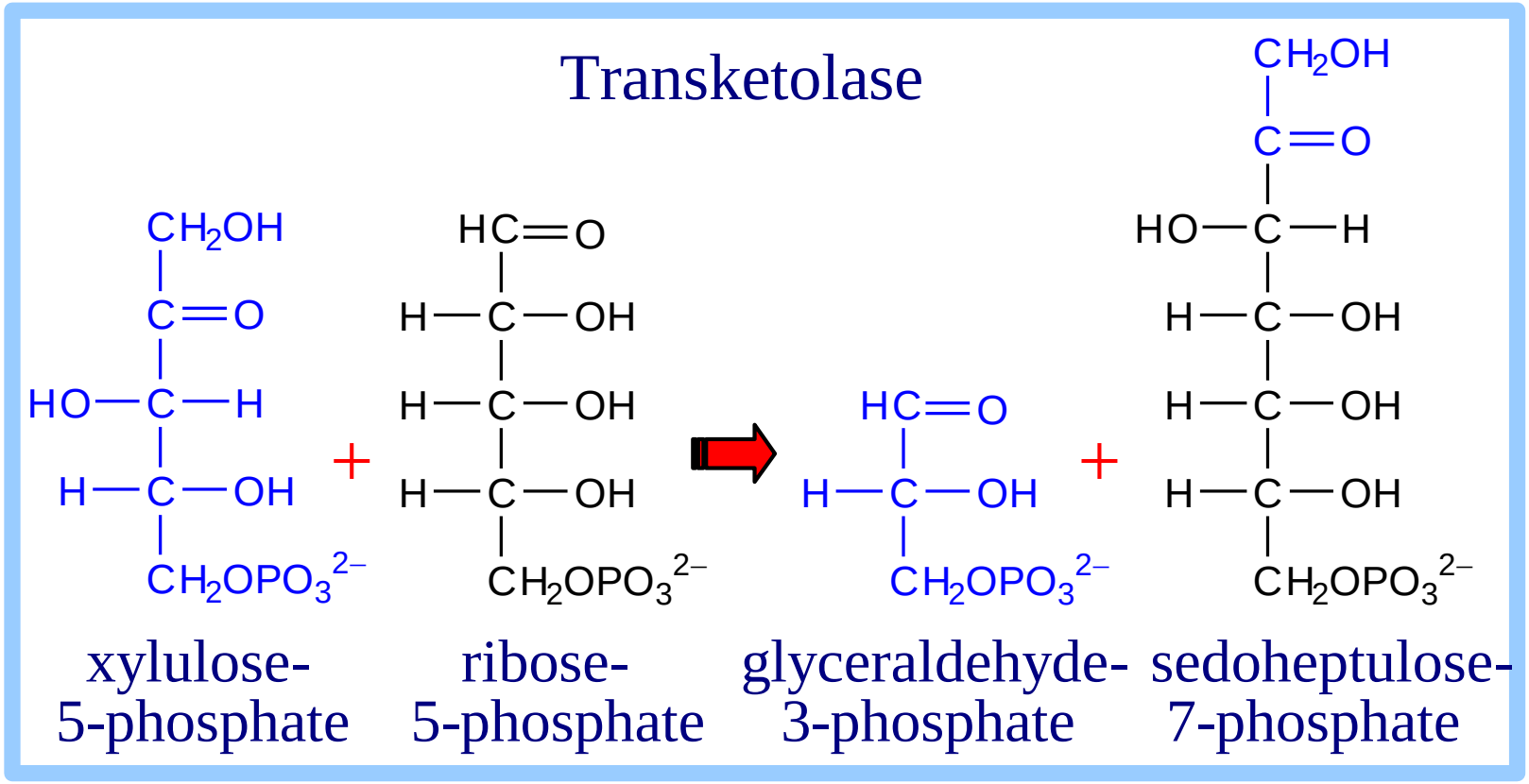


Transketolase & **Transaldolase** catalyze transfer of 2-C or 3-C molecular fragments respectively, in each case from a ketose donor to an aldose acceptor.

D. E. Nicholson has suggested that the **names** of these enzymes should be changed, since

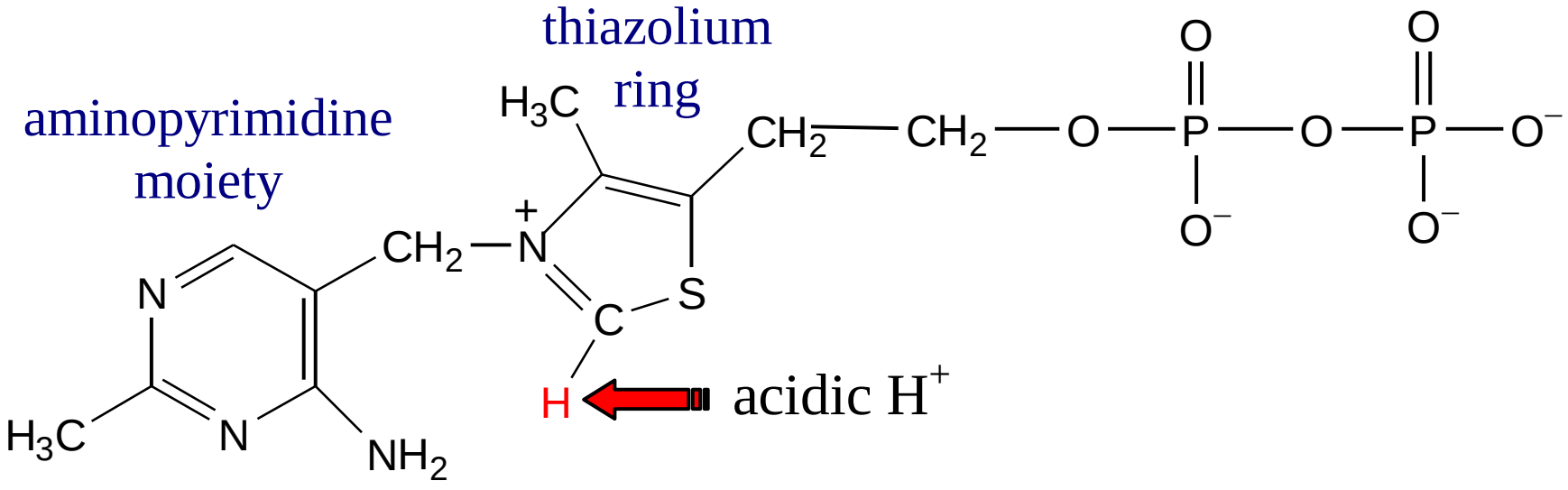
- Transketolase actually transfers an aldol moiety (glycoaldehyde), and
- Transaldolase actually transfers a ketol moiety (dihydroxyacetone).

However the traditional enzyme names are used here.



- **Transketolase** transfers a **2-C fragment** from xylulose-5-P to either ribose-5-P or erythrose-4-P.
- Transketolase utilizes as prosthetic group **thiamine pyrophosphate (TPP)**, a derivative of **vitamin B_1**.

  Pyruvate Dehydrogenase of Krebs Cycle also utilizes TPP as prosthetic group.

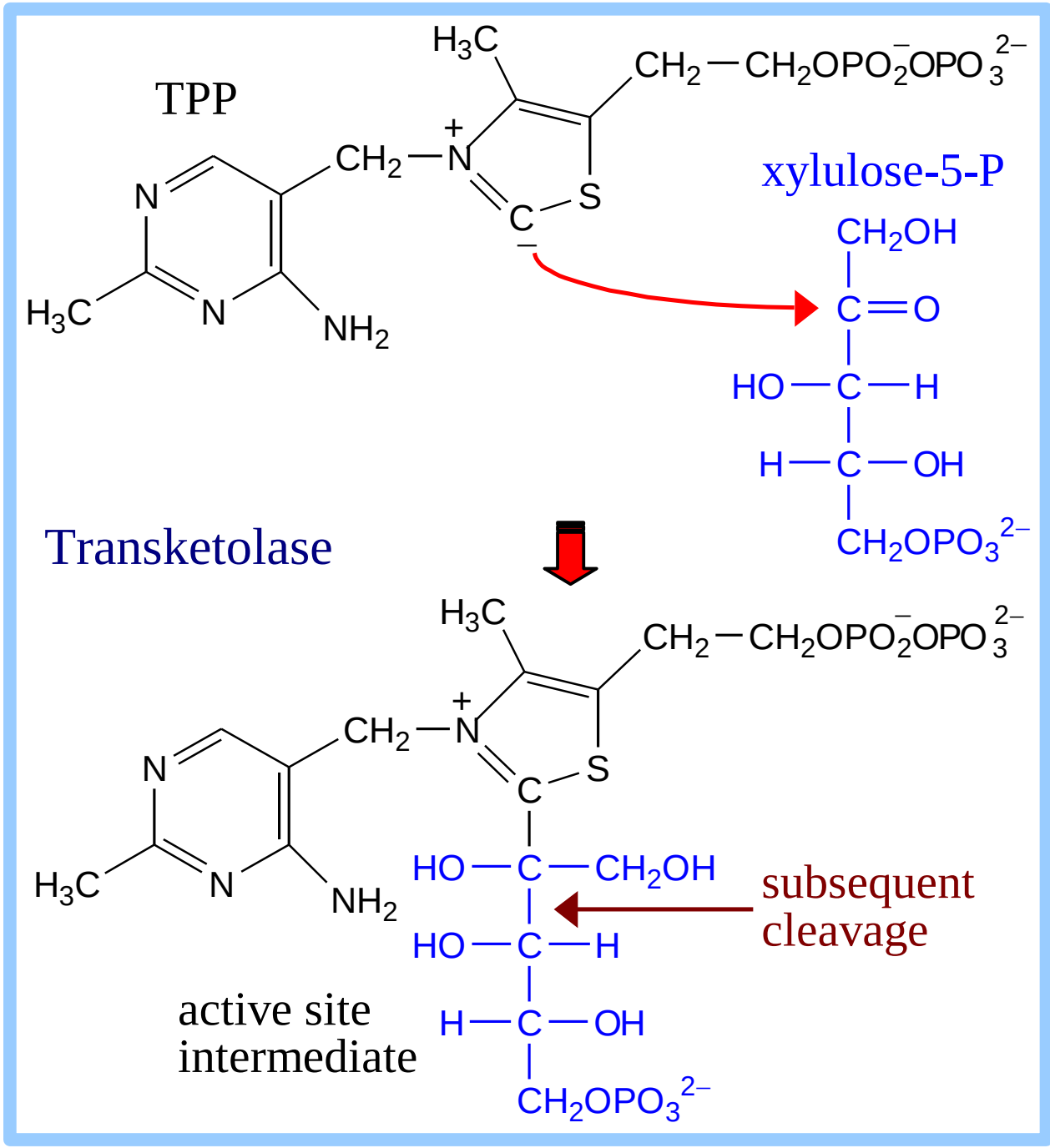


- TPP binds at the active site in a "**V**" conformation.
- **H^+ dissociates** from the **C** between **N** & **S** in the thiazolium ring.
- The aminopyrimidine **amino** group is near the dissociable H^+, & serves as **H^+ acceptor**.

  This H^+ transfer is promoted by a Glu residue adjacent to the pyrimidine ring.

The thiazolium **carbanion** reacts with the carbonyl **C** of xylulose-5-P to form an addition compound.

N^+ in the thiazole ring acts as an **e^- sink**, promoting **C-C** bond cleavage.

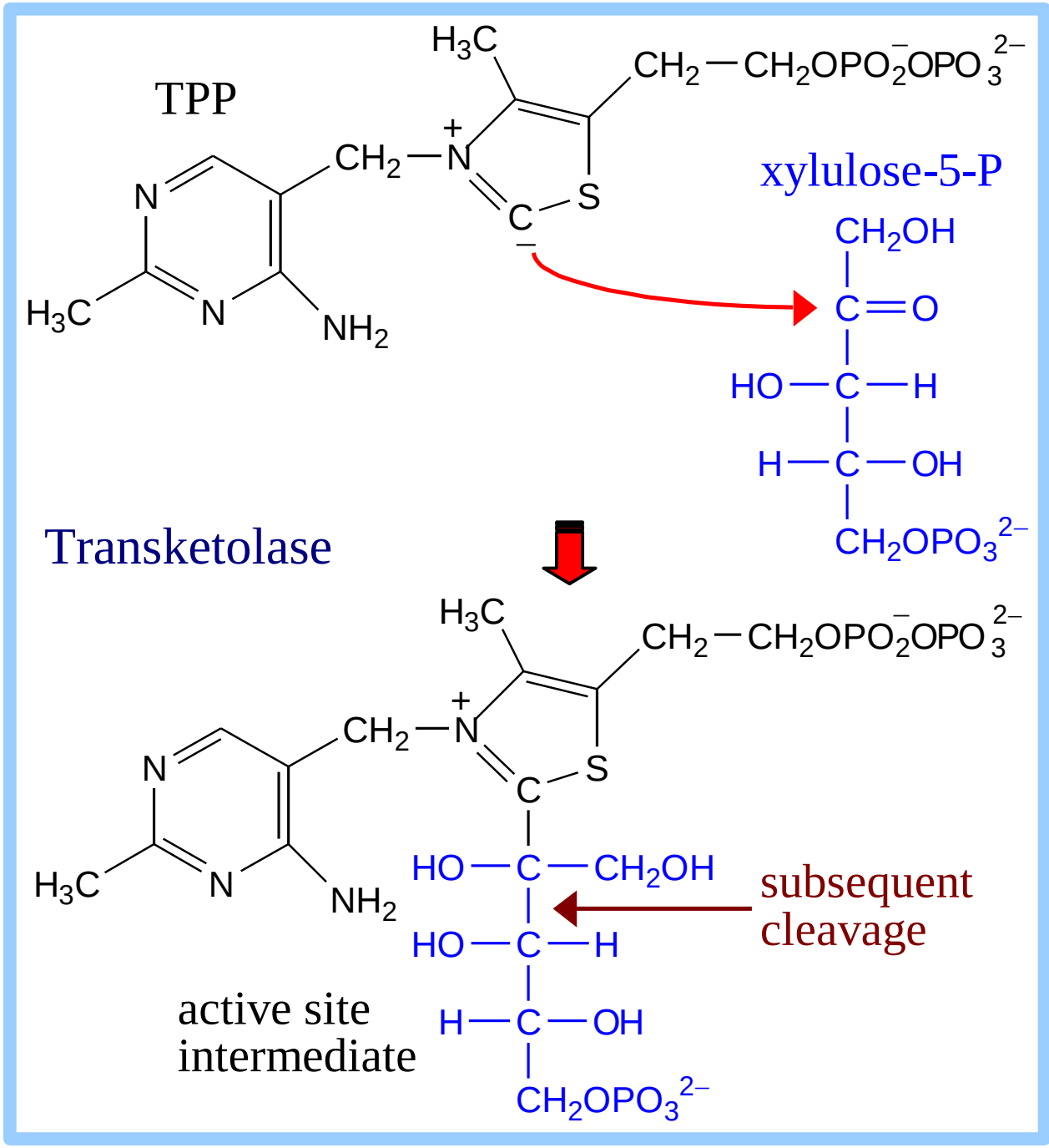


The 3-C aldose glyceraldehyde-3-P is released.

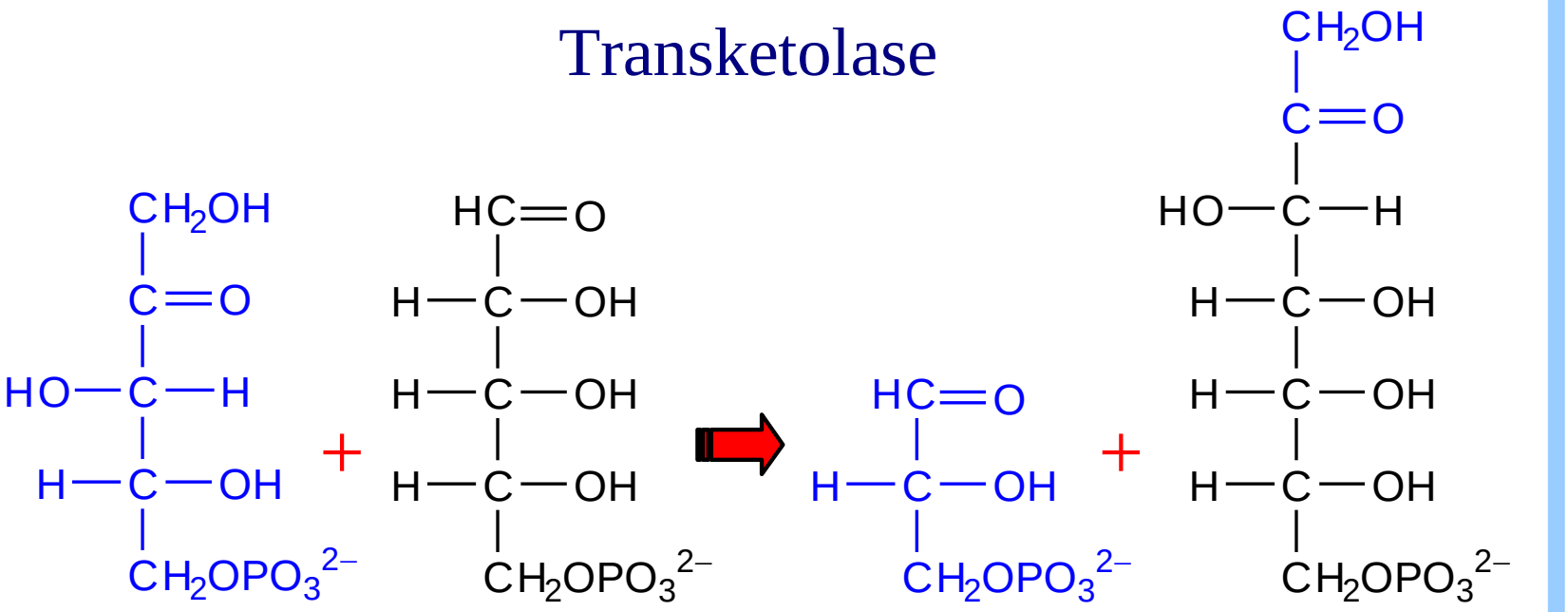
A **2-C fragment** remains on TPP.

Completion is by **reversal** of these steps.

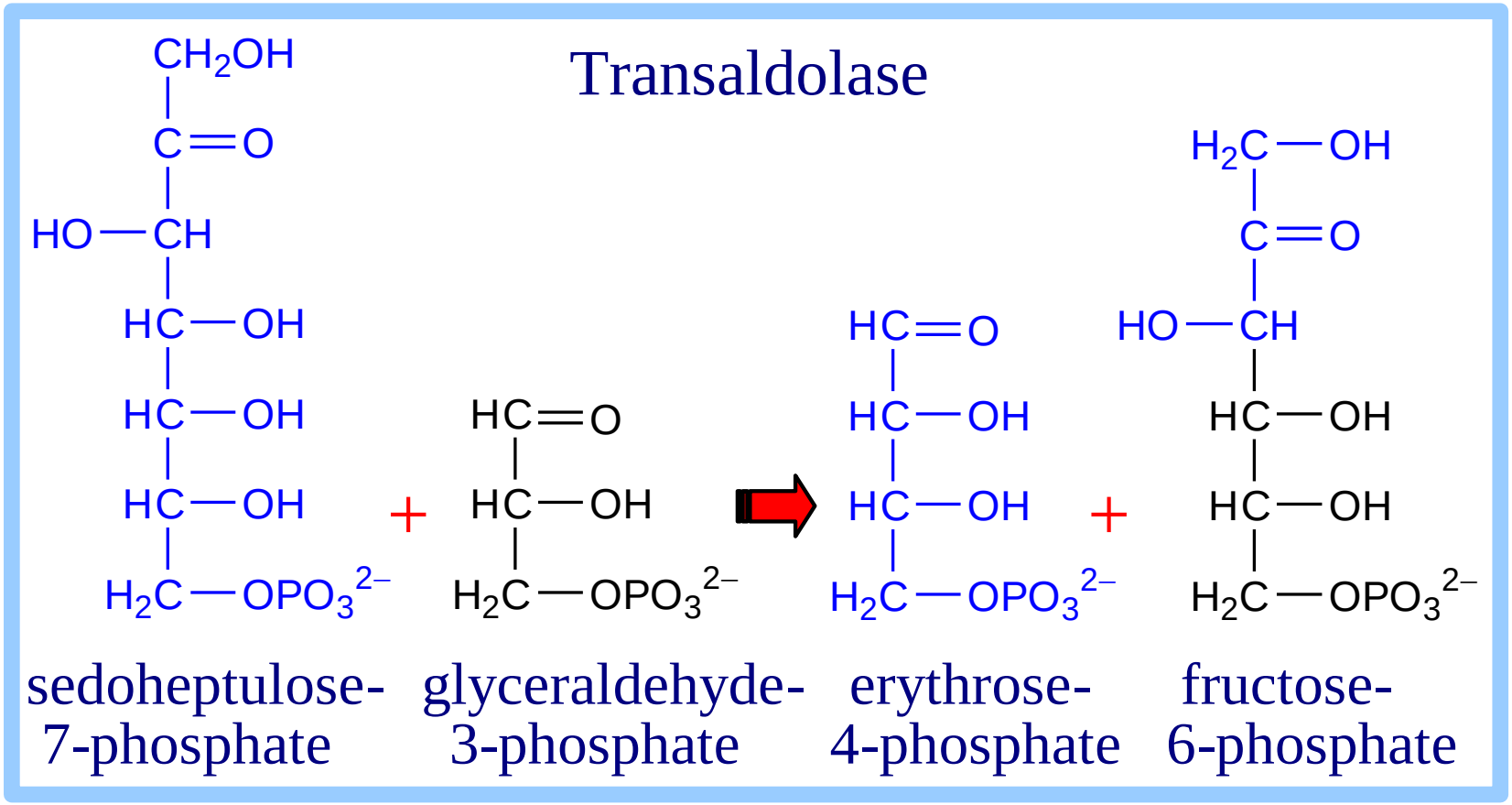
The **2-C** fragment condenses with one of the aldoses erythrose-4-P (4-C) or ribose-5-P (5-C) to form a ketose-P product.



Transketolase

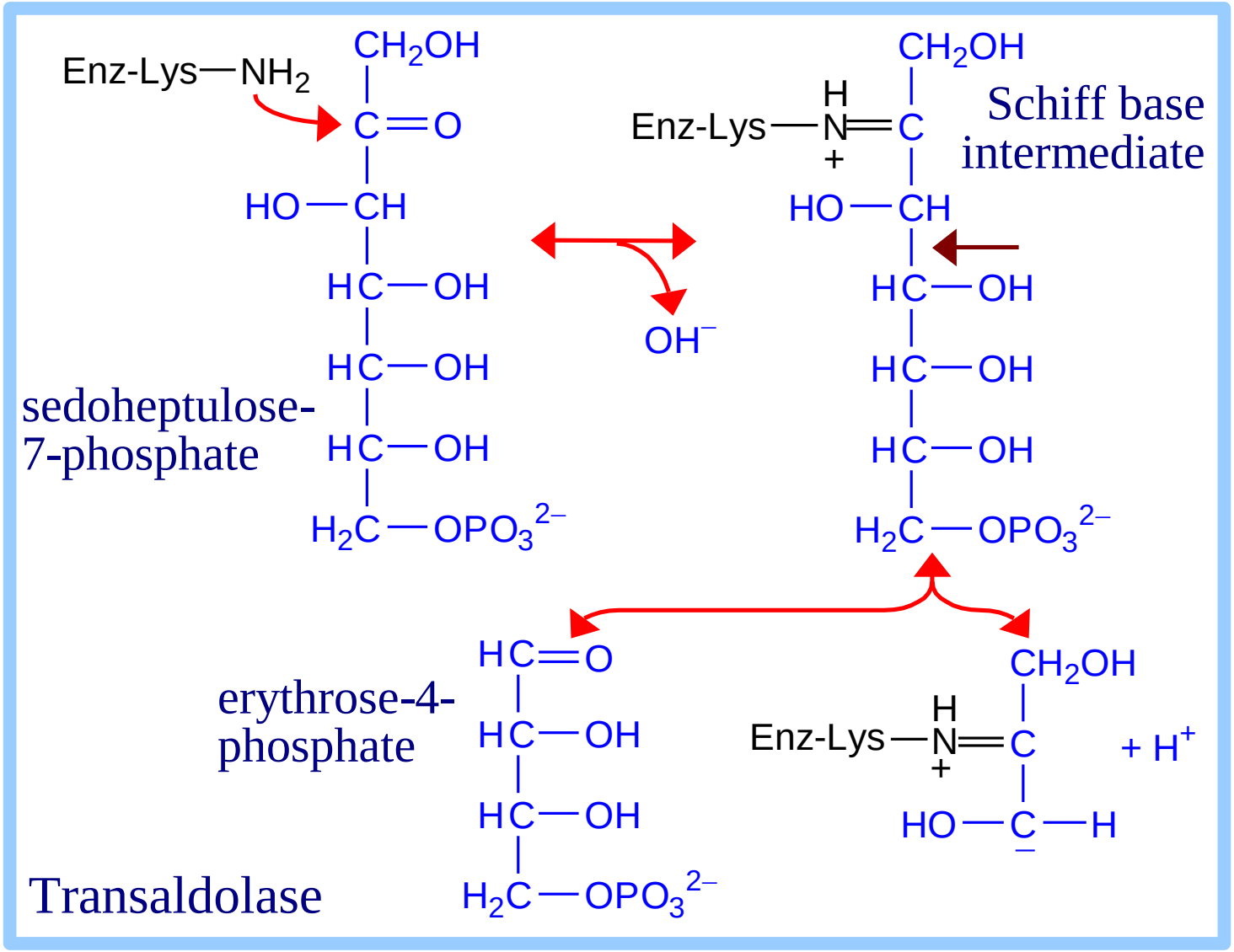


- Transfer of the 2-C fragment to the 5-C aldose ribose-5-phosphate yields sedoheptulose-7-phosphate.
- Transfer of the 2-C fragment instead to the 4-C aldose erythrose-4-phosphate yields fructose-6-phosphate.



Transaldolase catalyzes transfer of a **3-C** dihydroxyacetone moiety, from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate.

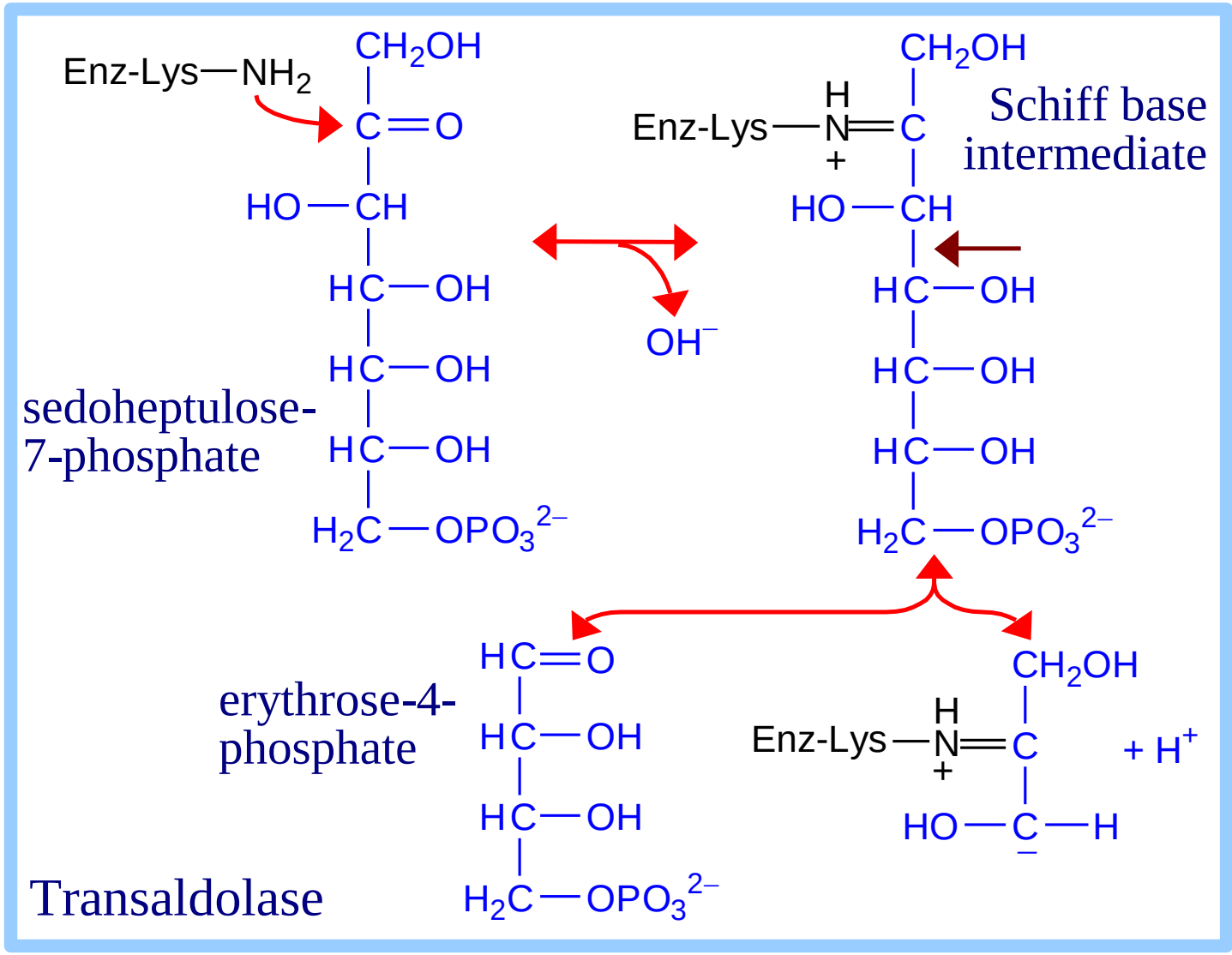
Transaldolase has an **α,β barrel** structure.



In **Transaldolase**, the ε-amino group of a **lysine** residue reacts with the carbonyl **C** of sedoheptulose-7-P to form a protonated **Schiff base** intermediate.

Aldol cleavage releases erythrose-4-phosphate.

The Schiff base stabilizes the carbanion on C3.



Completion of the reaction is by **reversal**, as the carbanion attacks instead the aldehyde carbon of the 3-C aldose glyceraldehyde-3-P to yield the 6-C fructose-6-P.

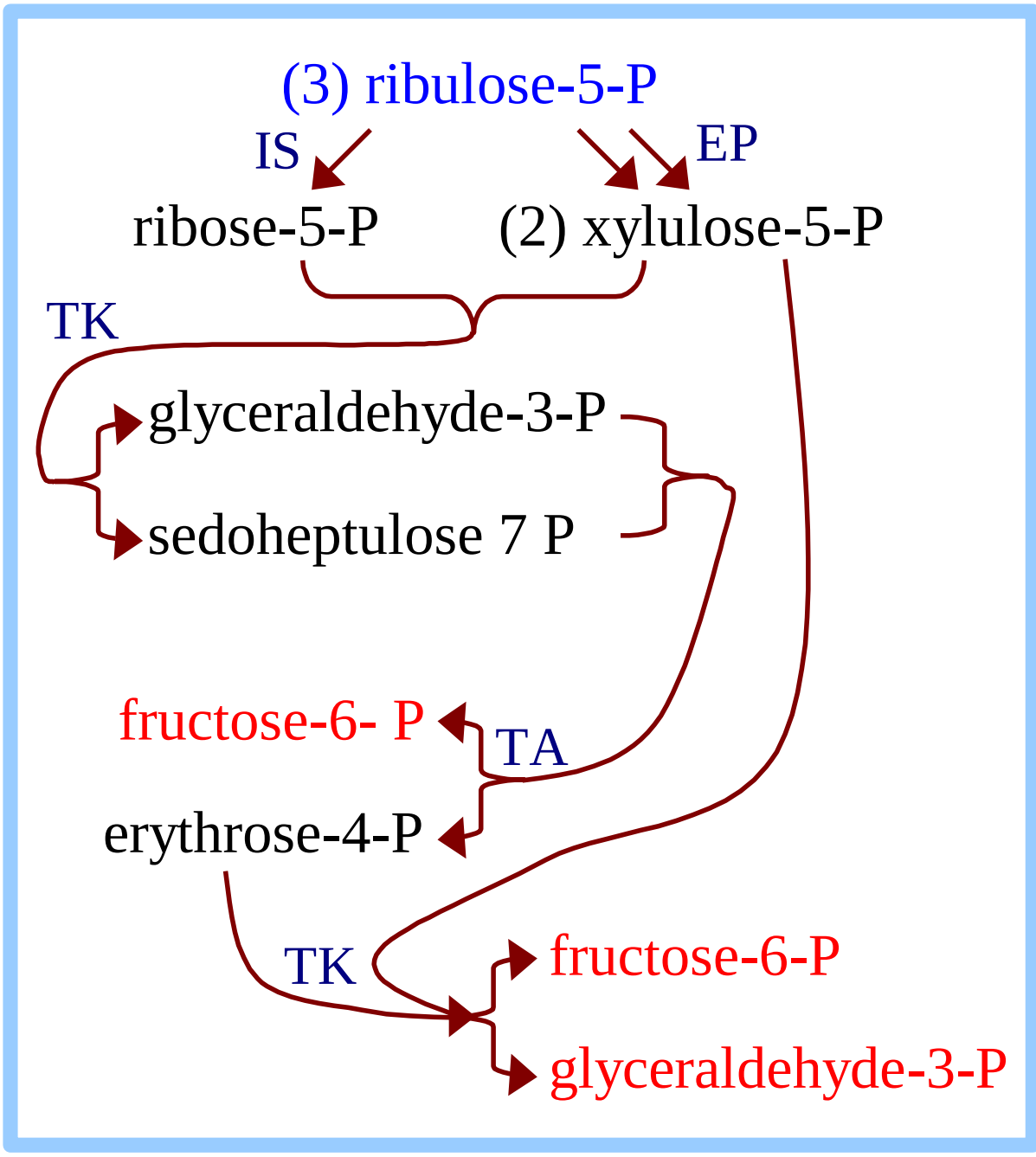
The diagram at right summarizes flow of 15 **C** atoms through Pentose Phosphate Pathway reactions by which **5-C** sugars are converted to **3-C** and **6-C** sugars.

IS = Isomerase

EP = Epimerase

TK = Transketolase

TA = Transaldolase



The **balance sheet** below summarizes flow of 15 **C** atoms through Pentose Phosphate Pathway reactions by which **5-C** sugars are converted to **3-C** and **6-C** sugars.

$$C_5 + C_5 \rightarrow C_3 + C_7 \quad \text{(Transketolase)}$$

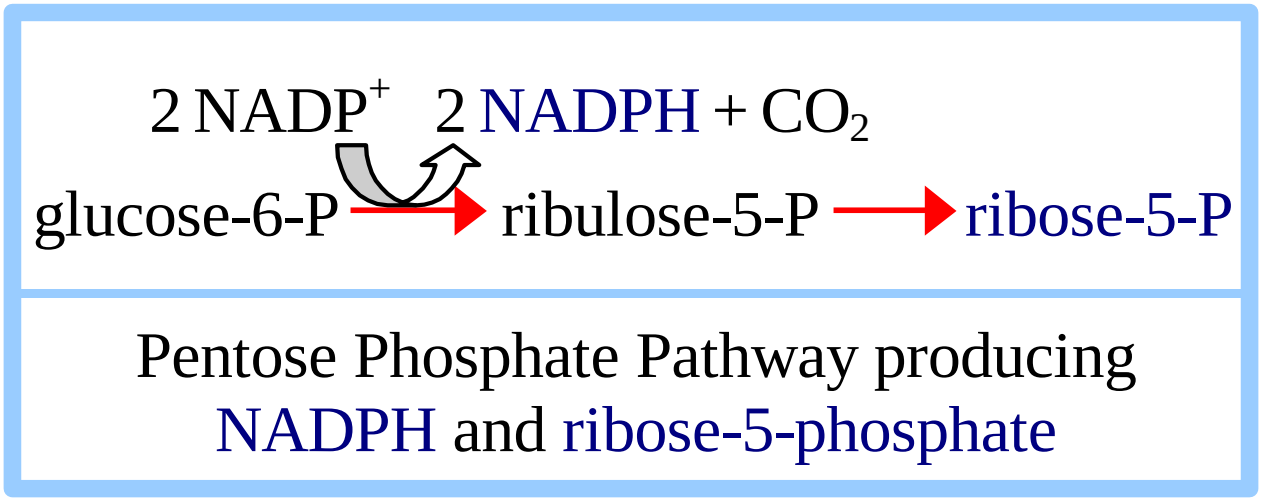
$$C_3 + C_7 \rightarrow C_6 + C_4 \quad \text{(Transaldolase)}$$

$$C_5 + C_4 \rightarrow C_6 + C_3 \quad \text{(Transketolase)}$$

$$3\,C_5 \rightarrow 2\,C_6 + C_3 \quad \text{(Overall)}$$

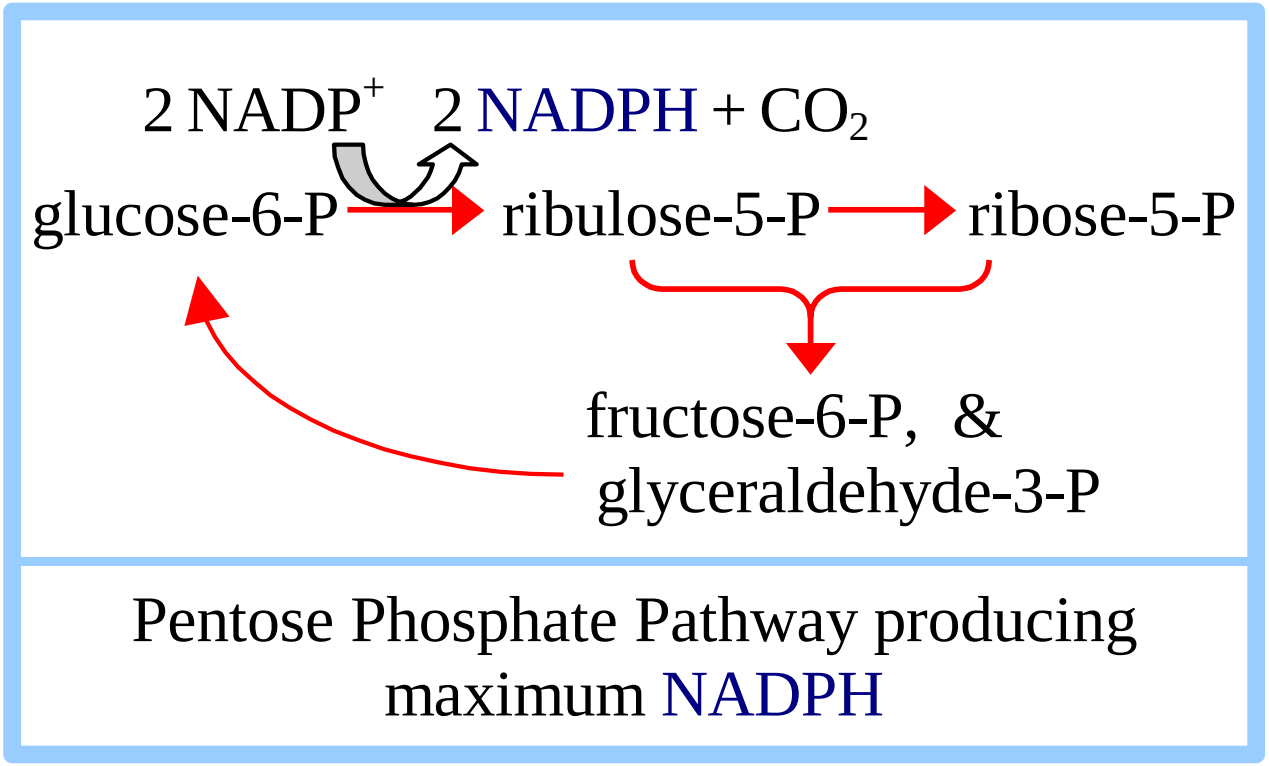
Glucose-6-phosphate may be regenerated from either the **3-C** glyceraldehyde-3-phosphate or the **6-C** fructose-6-phosphate, via enzymes of Gluconeogenesis.

Depending on needs of a cell for **ribose-5-phosphate**, **NADPH**, and **ATP**, the Pentose Phosphate Pathway can operate in various modes, to maximize different products. There are three major scenarios:

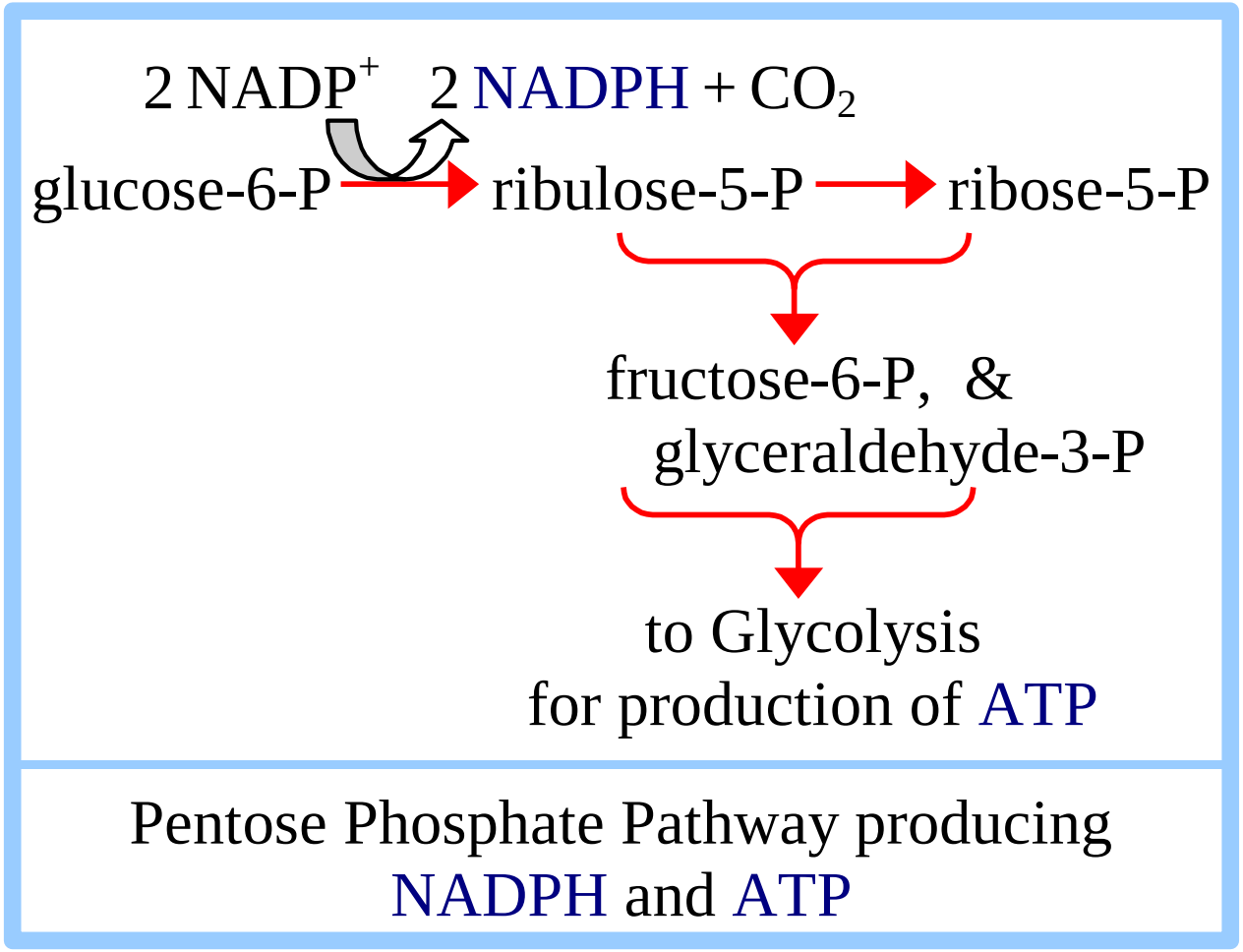


Ribulose-5-P may be converted to **ribose-5-phosphate**, a substrate for synthesis of **nucleotides** and nucleic acids.

The pathway also produces some **NADPH**.

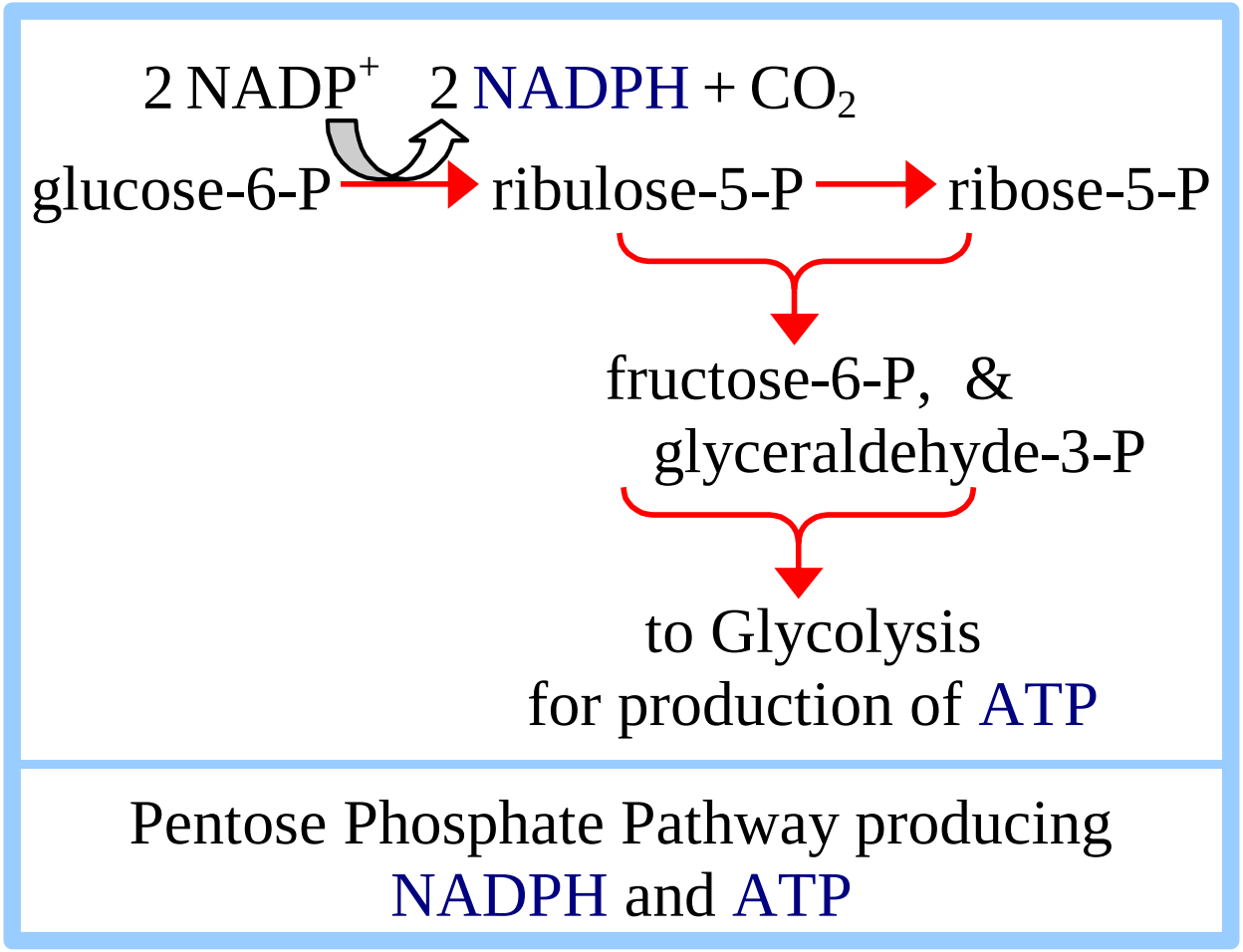


Glyceraldehyde-3-P and fructose-6-P may be converted to **glucose-6-P** for reentry to the linear portion of the Pentose Phosphate Pathway, maximizing formation of **NADPH**.



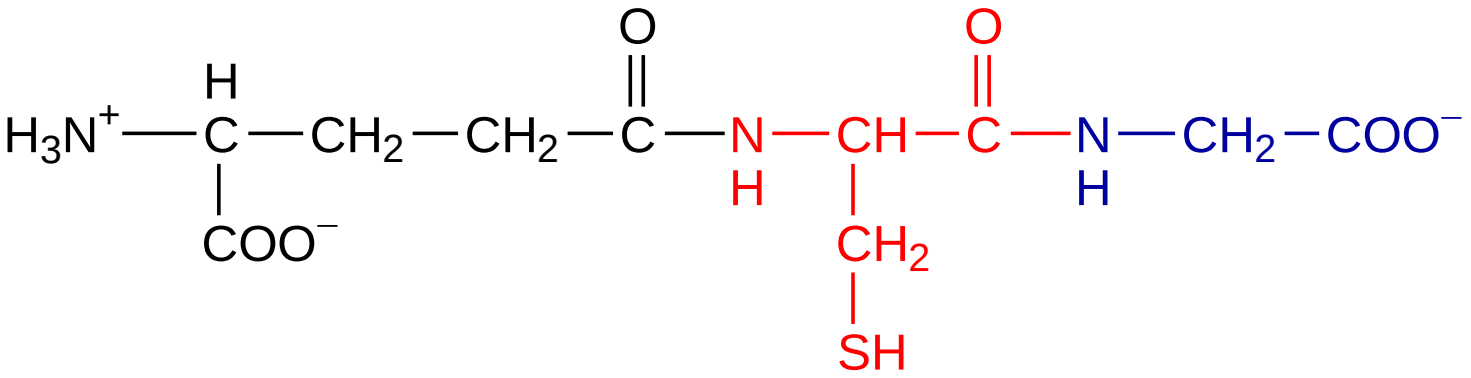
Glyceraldehyde-3-P and fructose-6-P, formed from 5-C sugar phosphates, may enter **Glycolysis** for **ATP** synthesis.

The pathway also produces some **NADPH**.



Ribose-1-phosphate generated during **catabolism of nucleosides** also enters Glycolysis in this way, after first being converted to ribose-5-phosphate.

Thus the Pentose Phosphate Pathway serves as an **entry into Glycolysis** for both 5-carbon & 6-carbon sugars.



γ-glutamyl-cysteinyl-glycine

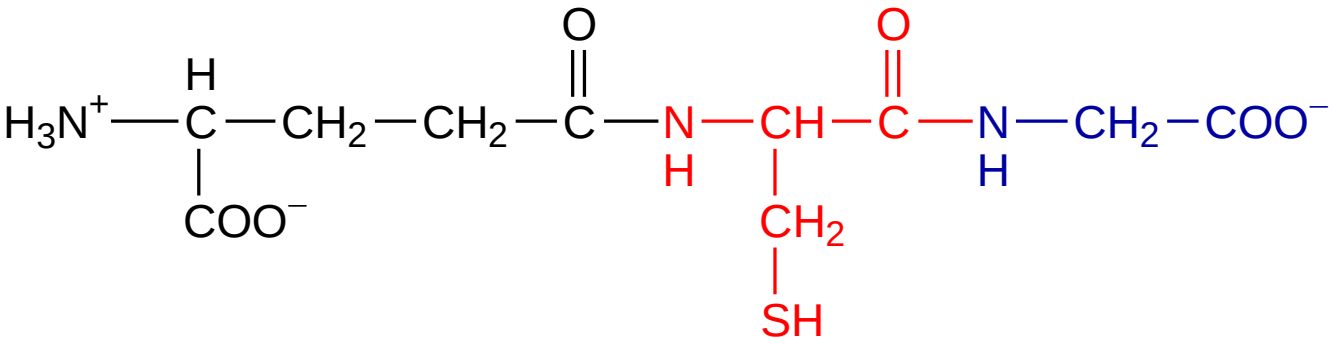
Glutathione

Glutathione is a tripeptide that includes a Glu linked by an isopeptide bond involving the side-chain carbonyl group.

Its functional group is a **cysteine thiol**.

One role of glutathione is **degradation of hydroperoxides**, that arise spontaneously in the oxygen-rich environment in red blood cells.

Hydroperoxides can react with double bonds in fatty acids of membrane lipids, making membranes leaky.



γ-glutamyl-cysteinyl-glycine

Glutathione

Glutathione Peroxidase catalyzes degradation of organic hydroperoxides by reduction, as two glutathione molecules (represented as GSH) are oxidized to a disulfide.

2 GSH + ROOH → GSSG + ROH + H_2O

Glutathione Peroxidase uses the trace element **selenium** as functional group.

The enzyme's primary structure includes an analog of cysteine, selenocysteine, with Se replacing S.

Regeneration of reduced glutathione requires NADPH, produced within erythrocytes in the Pentose Phosphate Pathway.

Glutathione Reductase catalyzes:

$$\mathbf{GSSG + NADPH + H^{+} \rightarrow 2\,GSH + NADP^{+}}$$

Genetic deficiency of Glucose-6-P Dehydrogenase can lead to hemolytic anemia, due to inadequate [NADPH] within red blood cells.

The effect of partial deficiency of Glucose-6-phosphate Dehydrogenase is exacerbated by substances that lead to increased production of peroxides (e.g., the antimalarial **primaquine**).