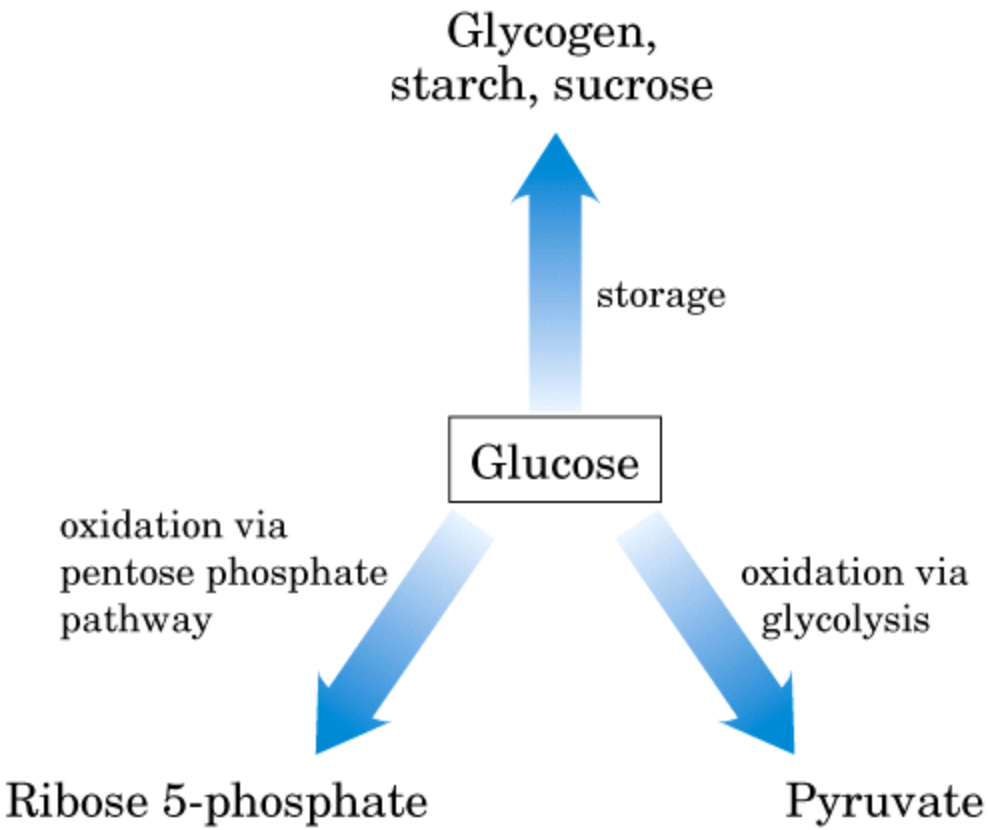
Pentose Phosphate Pathway


Glycogen,
starch, sucrose
storage
Glucose
oxidation via
pentose phosphate
pathway
oxidation via
glycolysis
Ribose 5-phosphate
Pyruvate

Pentose phosphate pathway

Glucose catabolism with major emphasis on generation of ATP: Oxidation of Glucose to carbon dioxide via glycolysis, PyrDC, TCA generates ATP and reducing equivalents such as NADH and FADH2 which are further oxidized in ETC generating ATP by oxidative phosphorylation.

Cells require ATP as well as reducing power for exergonic synthetic reactions. In most mammalian cells NADH produced by glycolysis and TCA is efficiently utilized by oxidative phosphorylation for ATP generation.

Thus the NAD^+/NADH ratio is always around 1000 (i.e. high concentrations of NAD^+). Therefore NADH is not the best reducing equivalent for synthetic reaction.

- **In order providing reducing power for synthetic reactions, cells produce NADPH in a special pathway of oxidation of glucose 6 phosphate called pentose phosphate shunt.**

- **$NADP^+$/NADPH ration is 0.01 in most cells as it is not used in oxidative phosphorylation and it is available exclusively for reduction reactions required for synthetic purposes.**

- **NADPH: an other currency for reducing power in synthetic reaction.**

- **This pathway generates NADPH and Ribose-5-P which is used for nucleic acid synthesis.**

Pentose phosphate pathway

This pathway also leads to the formation of pentose sugar intermediates such as Ribose 5 phosphate, Ribulose 5 phosphate that are essential for nucleic acid synthesis.

The overall reaction:

$$3G6P + 6NADP^{+} + 3H_2O = 6NADPH + 6H^{+} + 3CO_2 + 2F6P + GAP$$

There are seven enzymes involved in three different steps of this pathway;

1. Oxidative reactions:
   a. Glucose 6 phosphate dehydrogenase
   B 6-phosphogluconolactonase
   C. 6-phosphogluconate dehydrogenase

2. Isomerization and epimerization reactions
   a. Ribulose 5 phosphate isomerase
   b. Ribulose 5 phosphate epimerase

3. C-C bond cleavage and formation reactions
   a. Transketolase
   b. Transaldolase

Pentose Phosphate Pathway

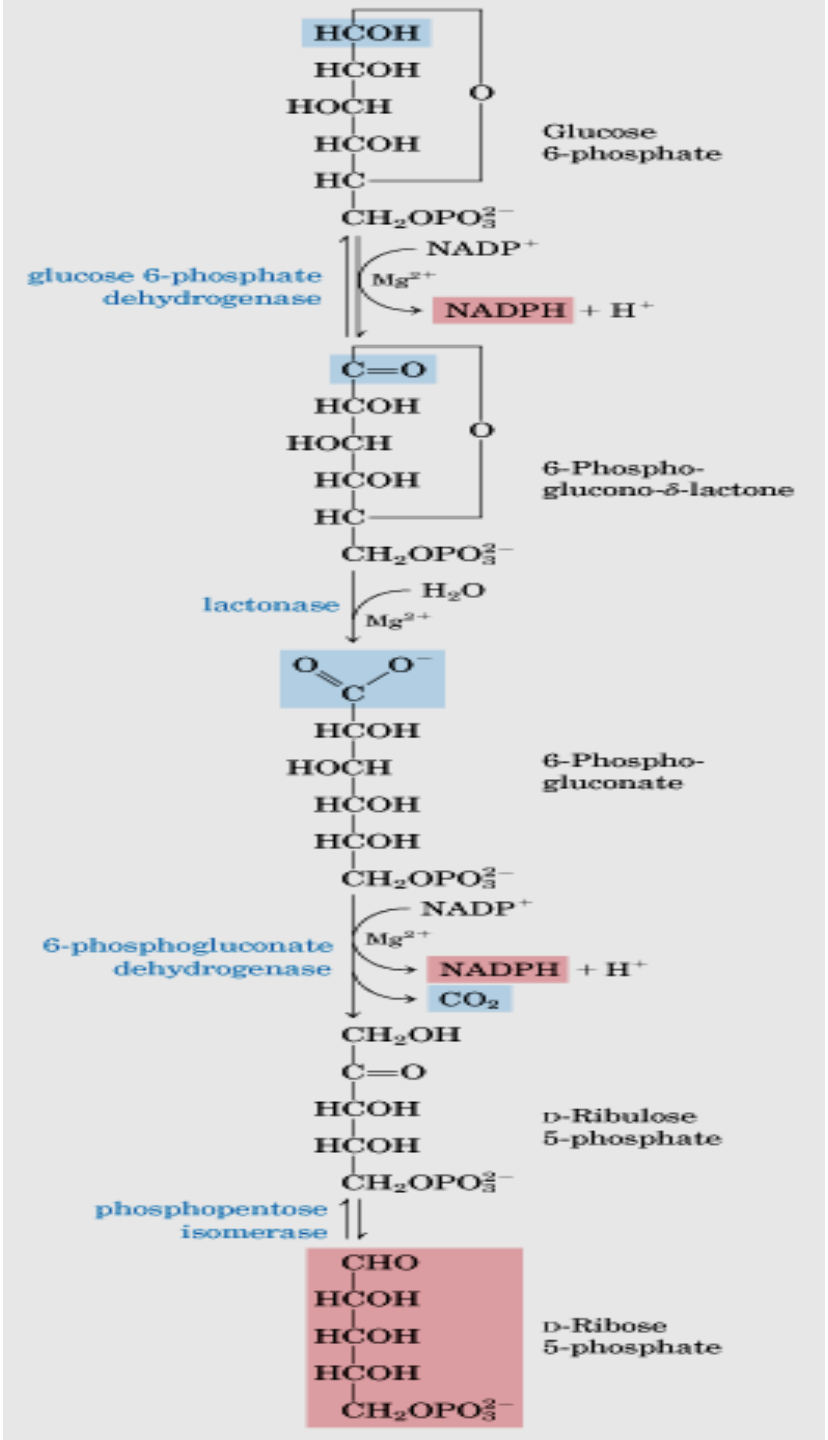
Pentose Phosphate Pathway

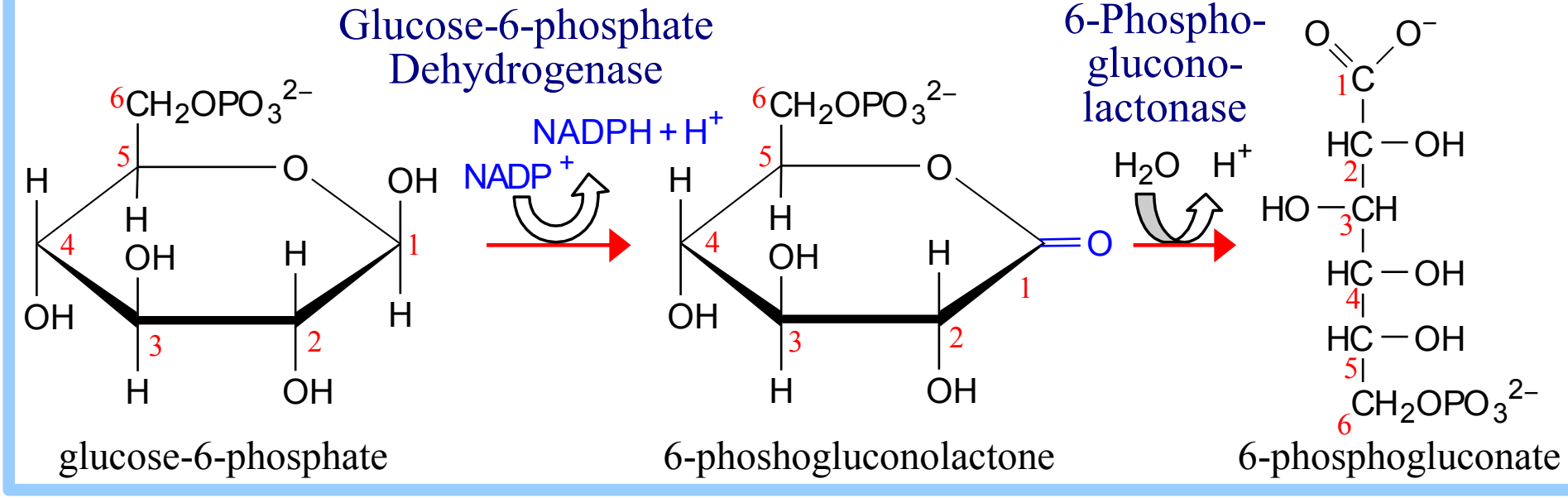
- Other names:

  Phosphogluconate Pathway

  Hexose Monophosphate Shunt

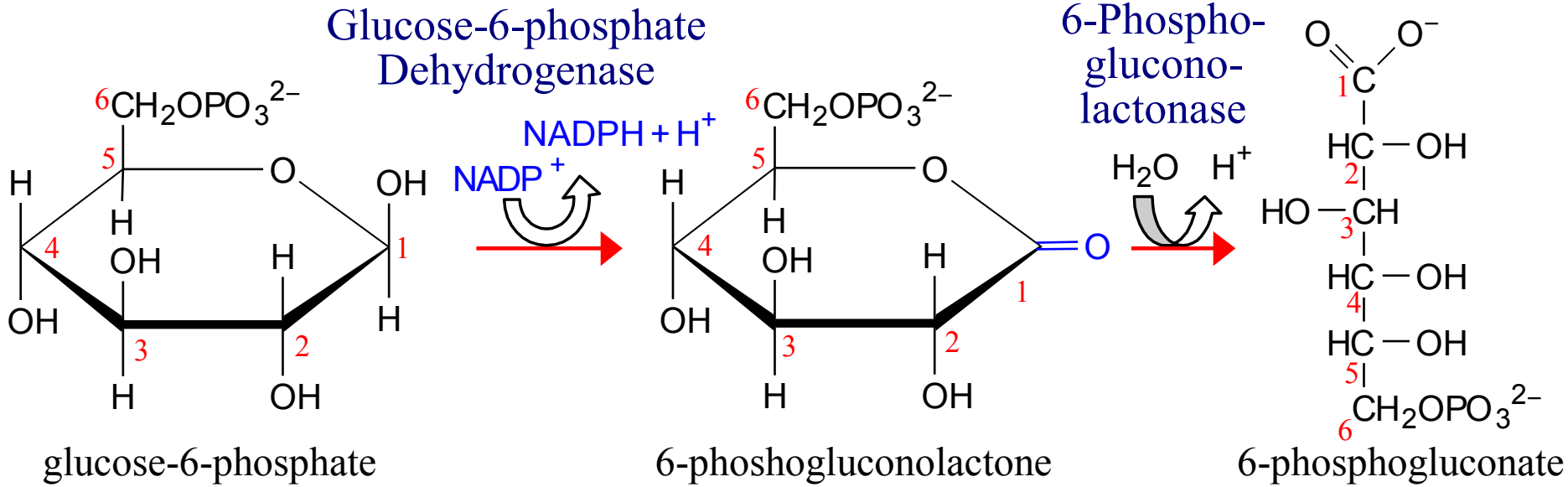
- The linear part of the pathway carries out oxidation and decarboxylation of the 6-C sugar glucose-6-P, producing the 5-C sugar ribulose-5-P.


Glucose 6-phosphate
glucose 6-phosphate dehydrogenase
NADP+
Mg2+
NADPH + H+
6-Phospho-glucono-δ-lactone
lactonase
H2O
Mg2+
6-Phospho-gluconate
6-phosphogluconate dehydrogenase
NADP+
Mg2+
NADPH + H+
CO2
D-Ribulose 5-phosphate
phosphopentose isomerase
D-Ribose 5-phosphate



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

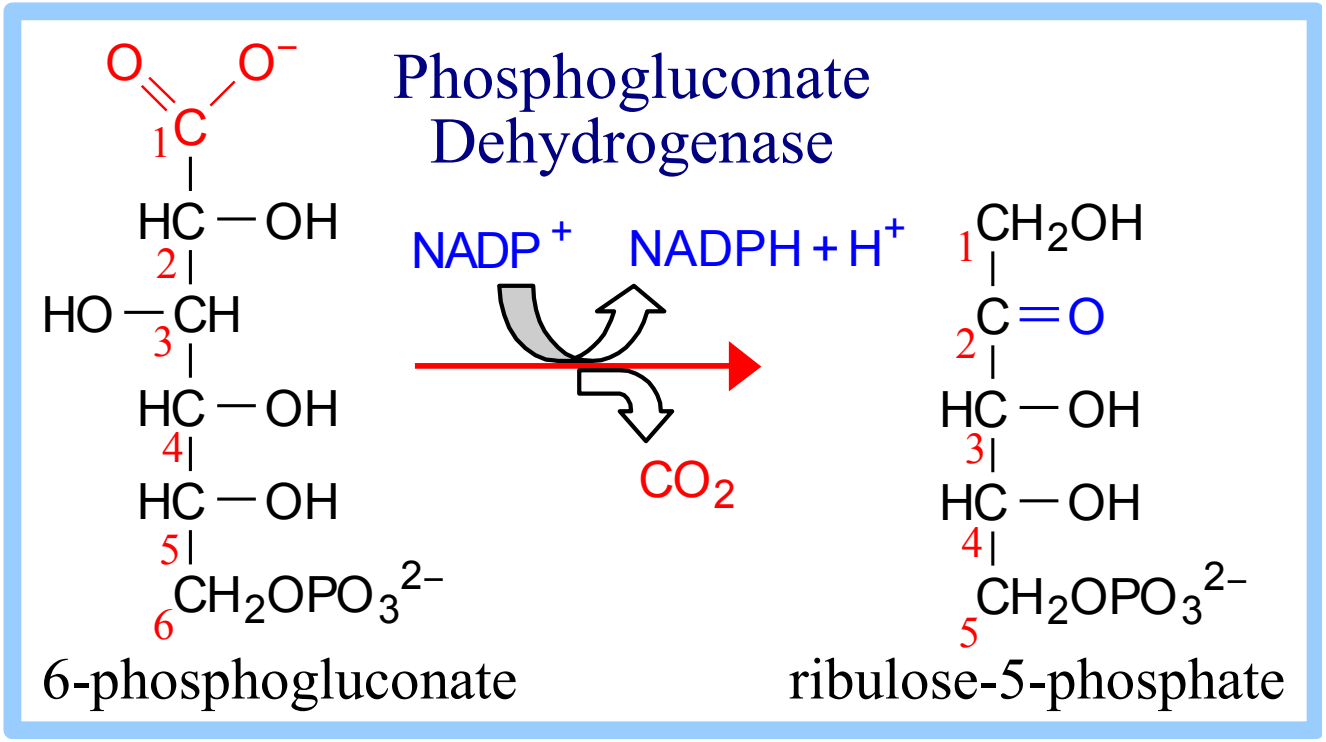
NADP$^+$ serves as electron acceptor.



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.



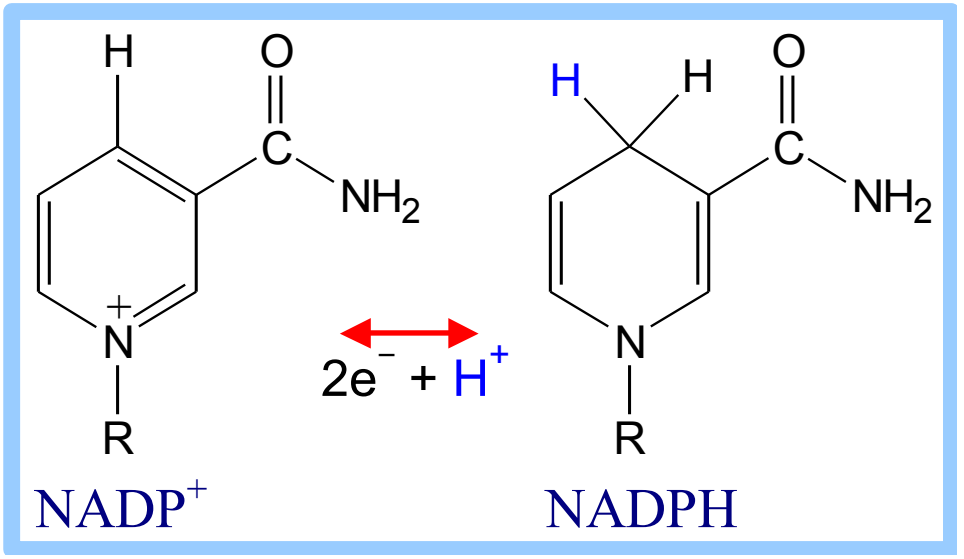
Phosphogluconate Dehydrogenase catalyzes **oxidative decarboxylation** of 6-phosphogluconate, to yield the **5-C** ketose **ribulose-5-phosphate**.

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 $2\,e^-$ and $1\,H^+$ 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.

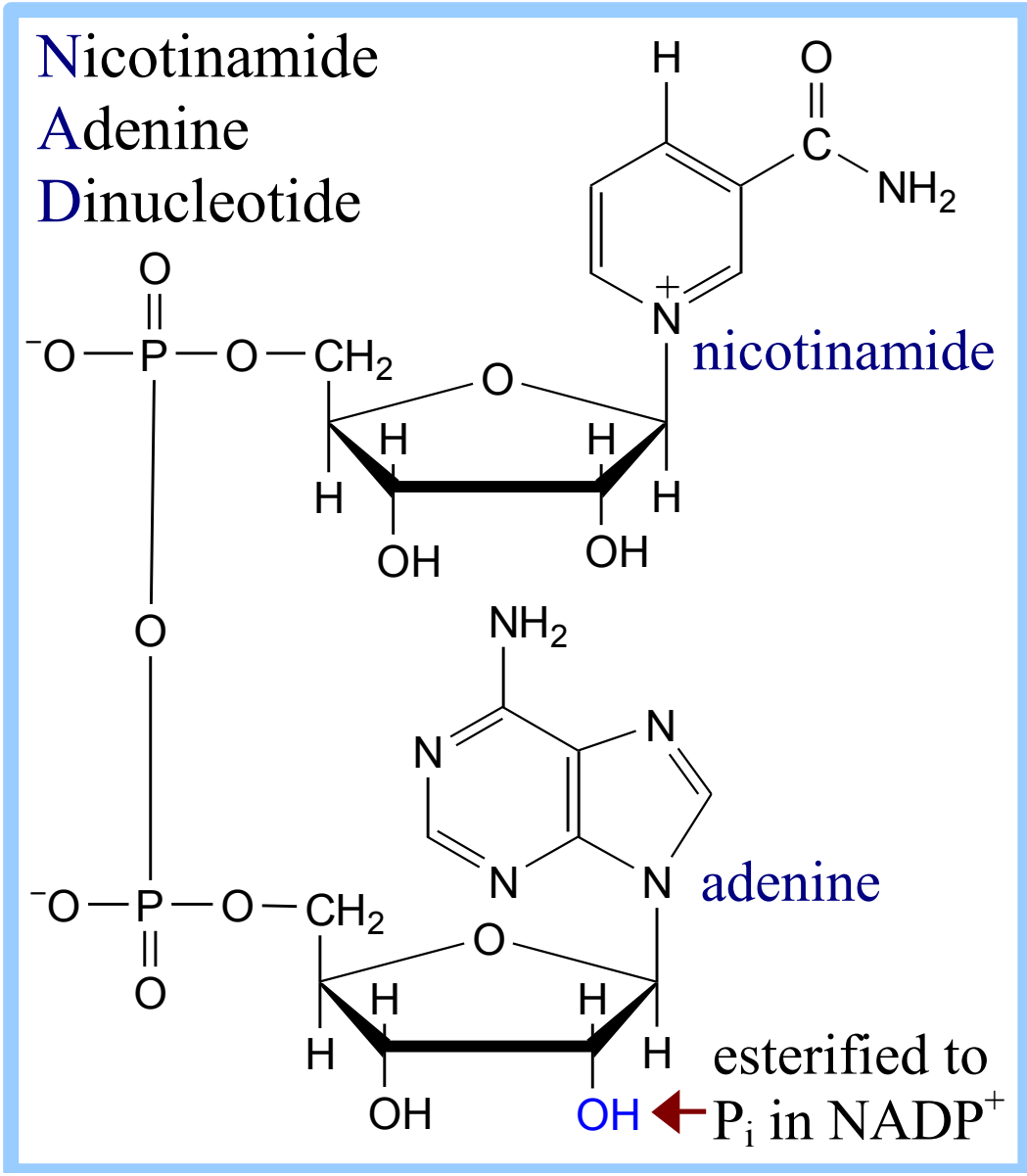
- **NAD^+** serves as electron acceptor in **catabolic** pathways, in which metabolites are oxidized.

  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.

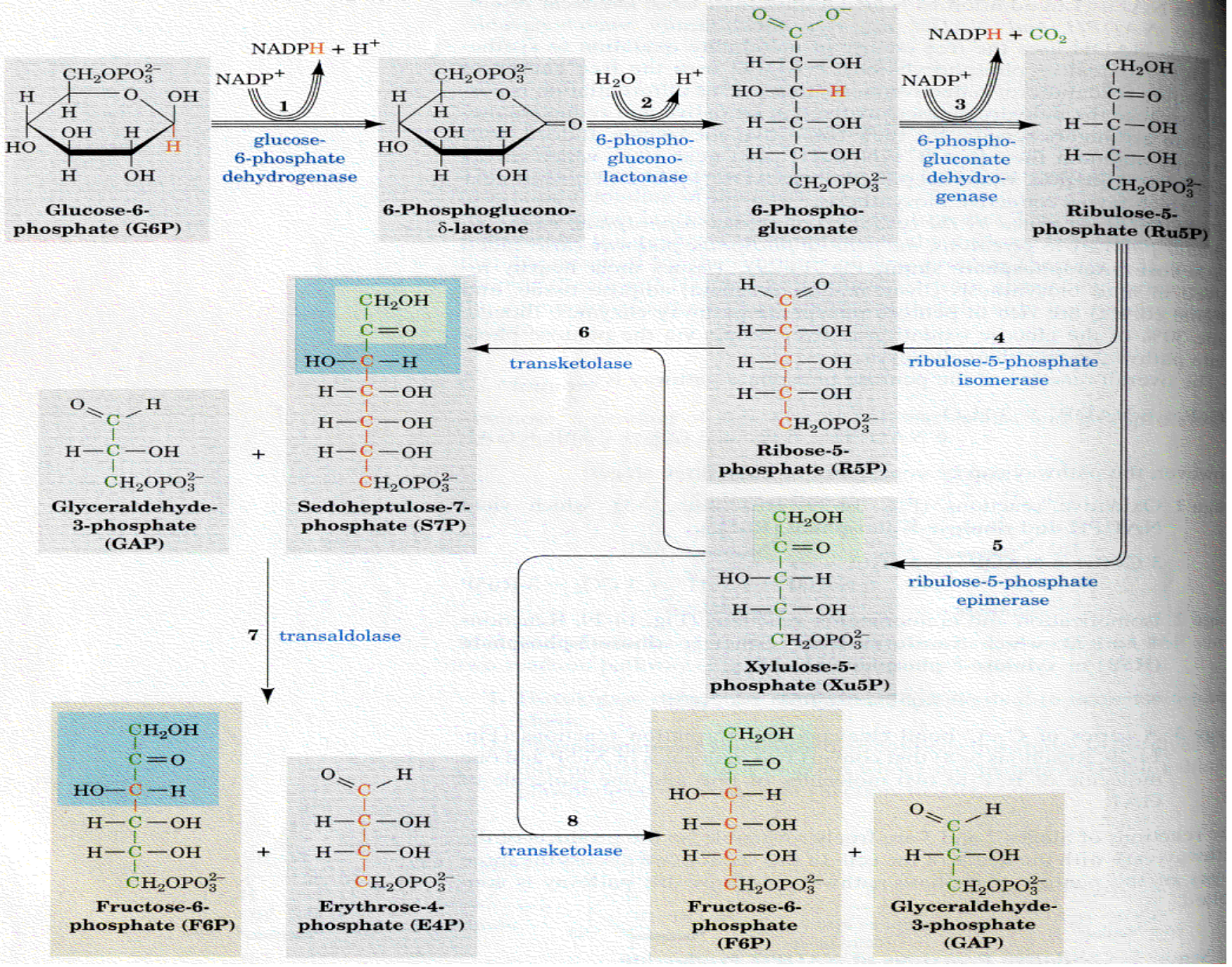


Regulation of Glucose-6-phosphate Dehydrogenase:

- 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.

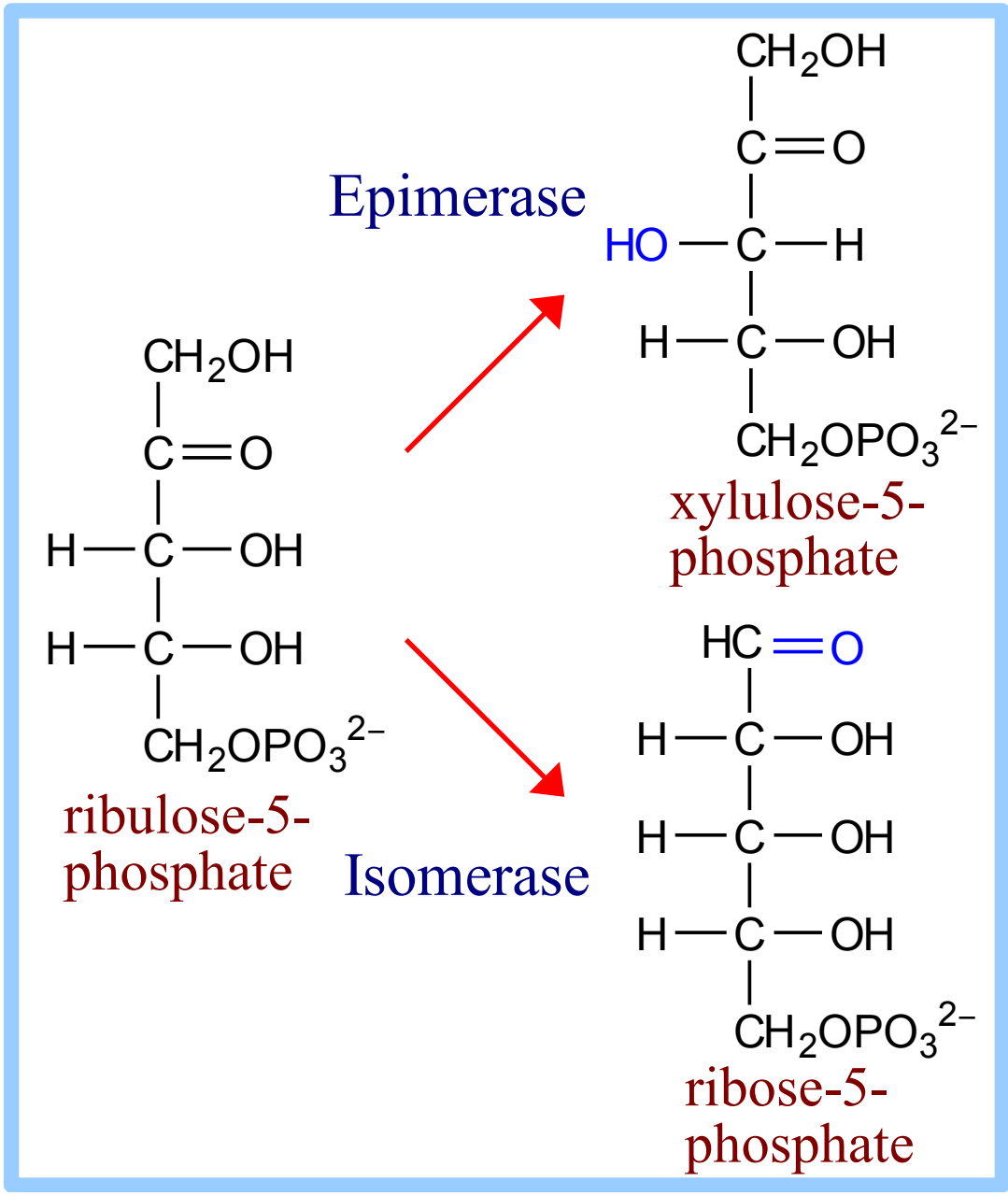

Glucose-6-phosphate (G6P)
NADPH + H+
NADP+
1
glucose-6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone
H2O
H+
2
6-phospho-gluconolactonase
6-Phospho-gluconate
NADPH + CO2
NADP+
3
6-phospho-gluconate dehydro-genase
Ribulose-5-phosphate (Ru5P)
4
ribulose-5-phosphate isomerase
Ribose-5-phosphate (R5P)
5
ribulose-5-phosphate epimerase
Xylulose-5-phosphate (Xu5P)
6
transketolase
Sedoheptulose-7-phosphate (S7P)
Glyceraldehyde-3-phosphate (GAP)
7
transaldolase
Fructose-6-phosphate (F6P)
Erythrose-4-phosphate (E4P)
8
transketolase
Fructose-6-phosphate (F6P)
Glyceraldehyde-3-phosphate (GAP)

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.



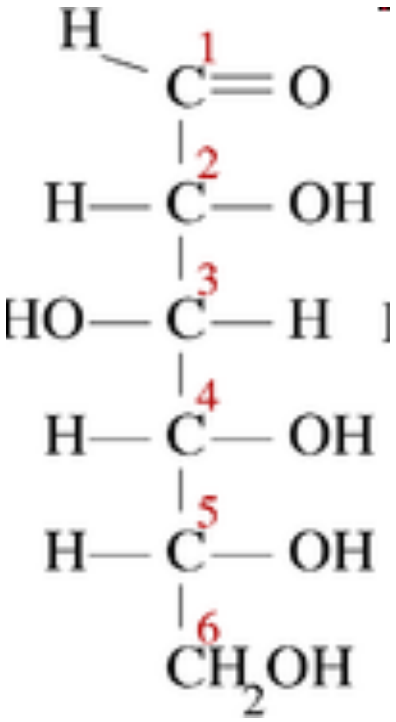
ISOMERI

- Due composti con uguale formula bruta si dicono *isomeri*. **Isomeri costituzionali** (o **strutturali**), se hanno formula bruta identica ma diversa connettività. In altre parole, sono composti aventi la stessa formula molecolare ma diversa formula di struttura
- **Isomeri di gruppo funzionale**, pur avendo formula bruta uguale, presentano gruppi funzionalmente diversi, ed hanno quindi proprietà chimiche e fisiche molto differenti

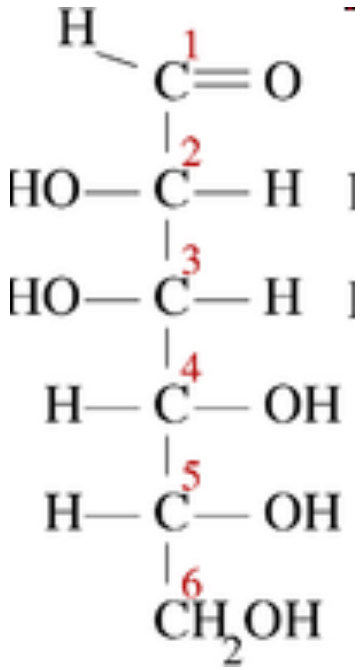
EPIMERI

- In chimica si definiscono come **epimeri** distereoisomeri che presentano una differente configurazione presso un solo stereocentro. Se un epimero viene incorporato all'interno di una struttura ad anello, esso viene chiamato anomero

Esempio di Epimeri (in C2)



D-GLUCOSIO



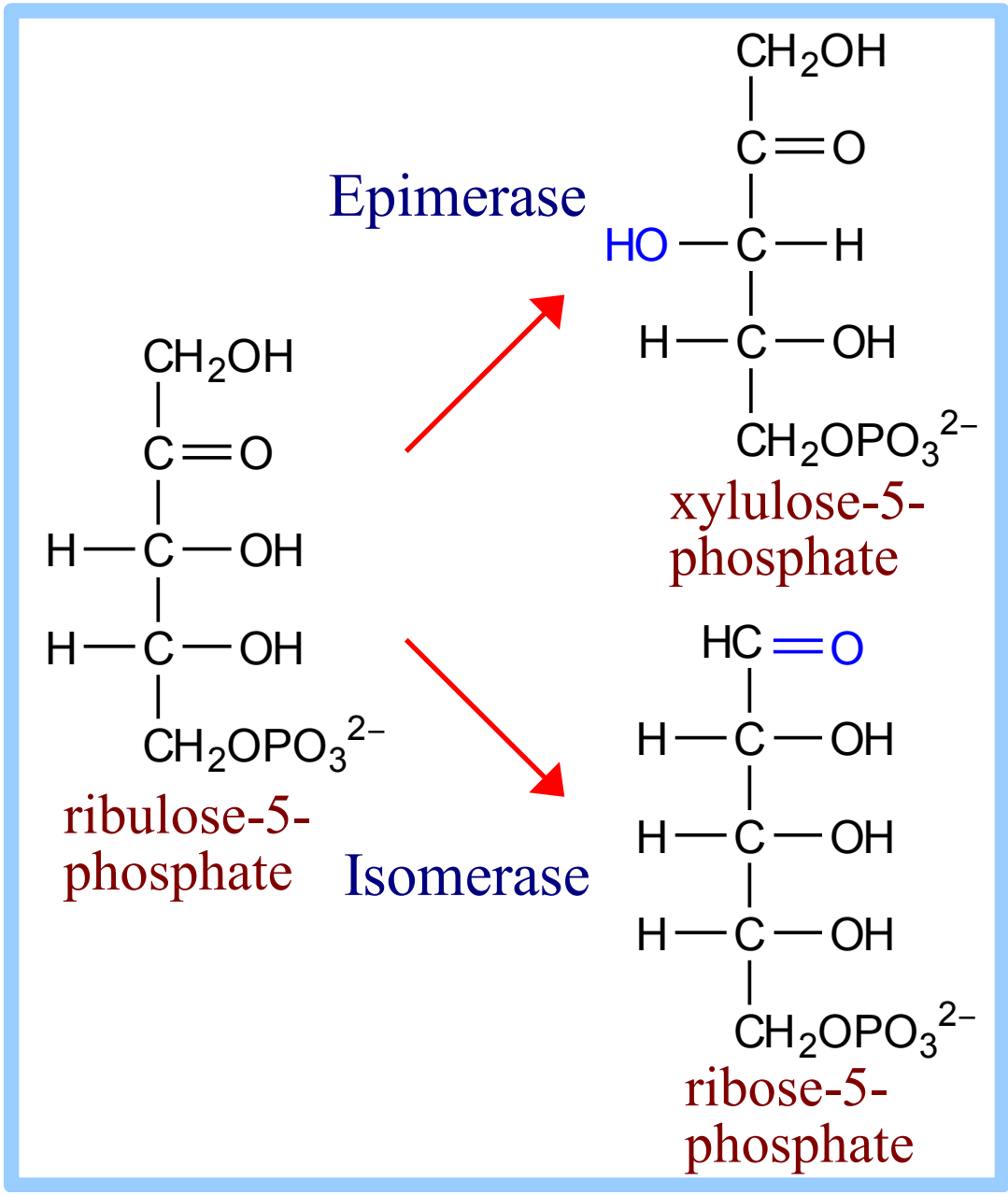
D-MANNOSIO

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

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

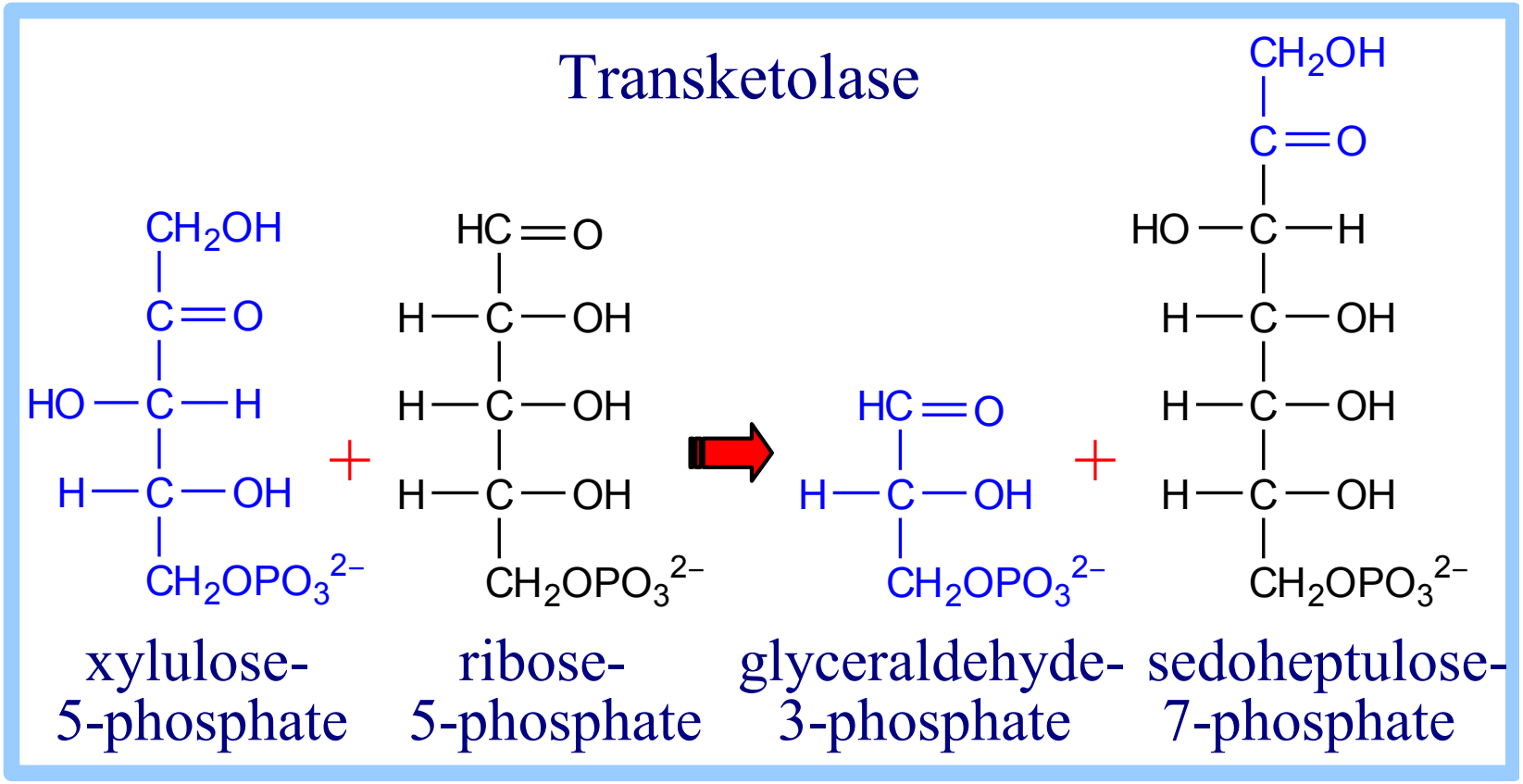
Both reactions involve deprotonation to an **endiolate** intermediate followed by specific reprotonation to yield the product.

Both reactions are reversible.

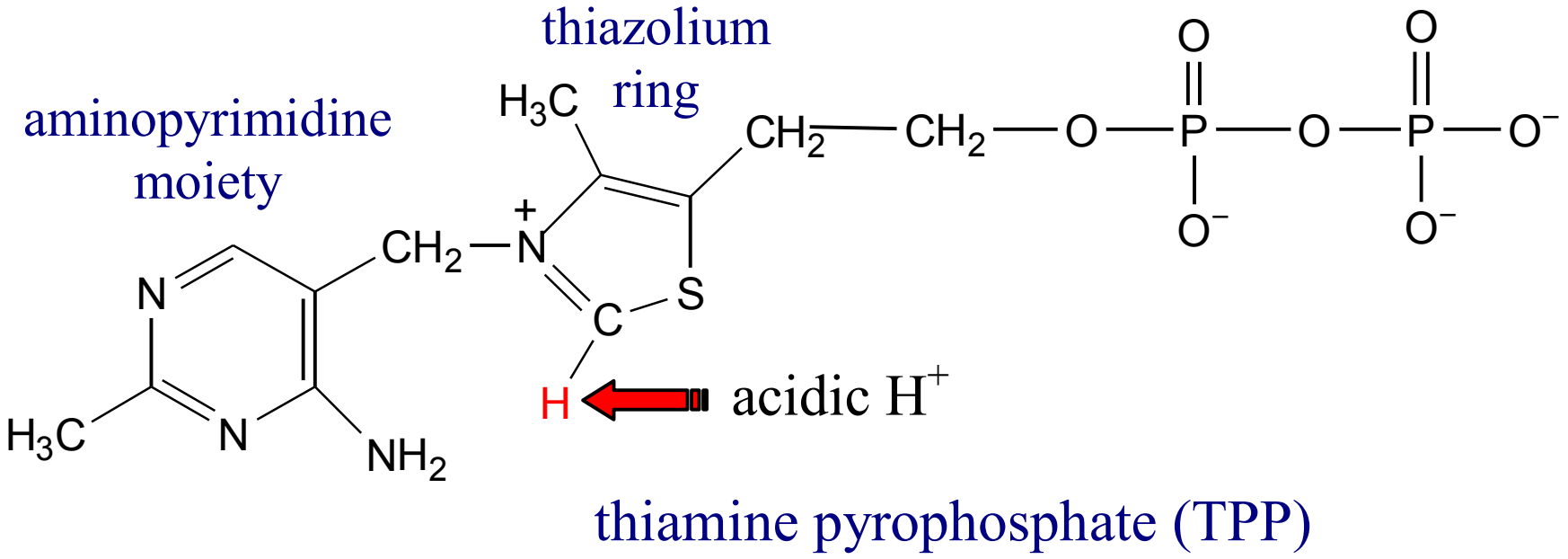


Transketolase & **Transaldolase** catalyze transfer of 2-C or 3-C molecular fragments respectively, in each case from a ketose donor to an aldose acceptor.

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



Transketolase transfers a **2-C fragment** from xylulose-5-phosphate to either ribose-5-phosphate or erythrose-4-phosphate.

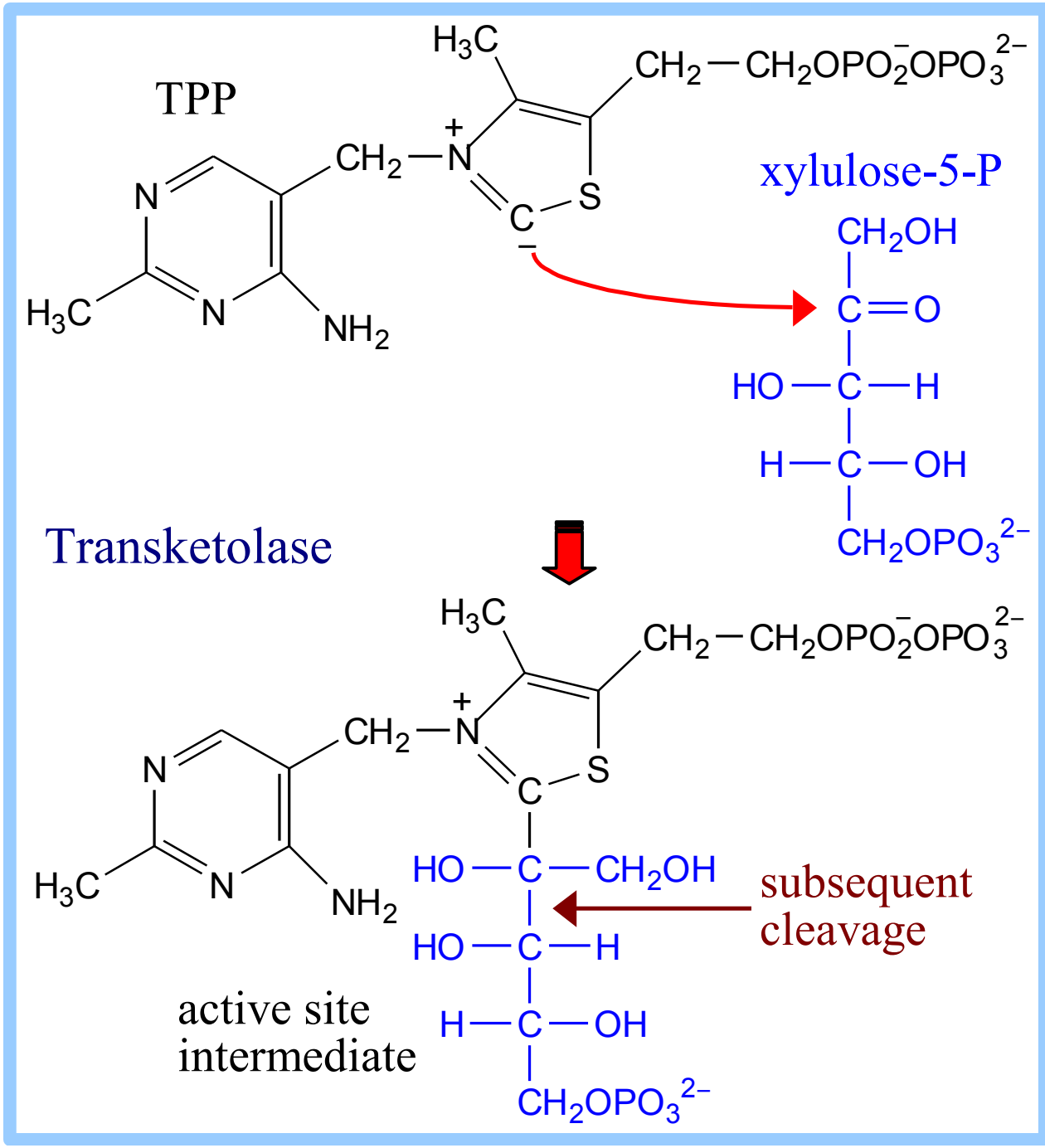


Transketolase utilizes as prosthetic group **TPP** (thiamine pyrophosphate), a derivative of vitamin B_1.

- **H^+** dissociates from the **C** between **N** & **S** in the thiazolium ring.
- The aminopyrimidine amino 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.

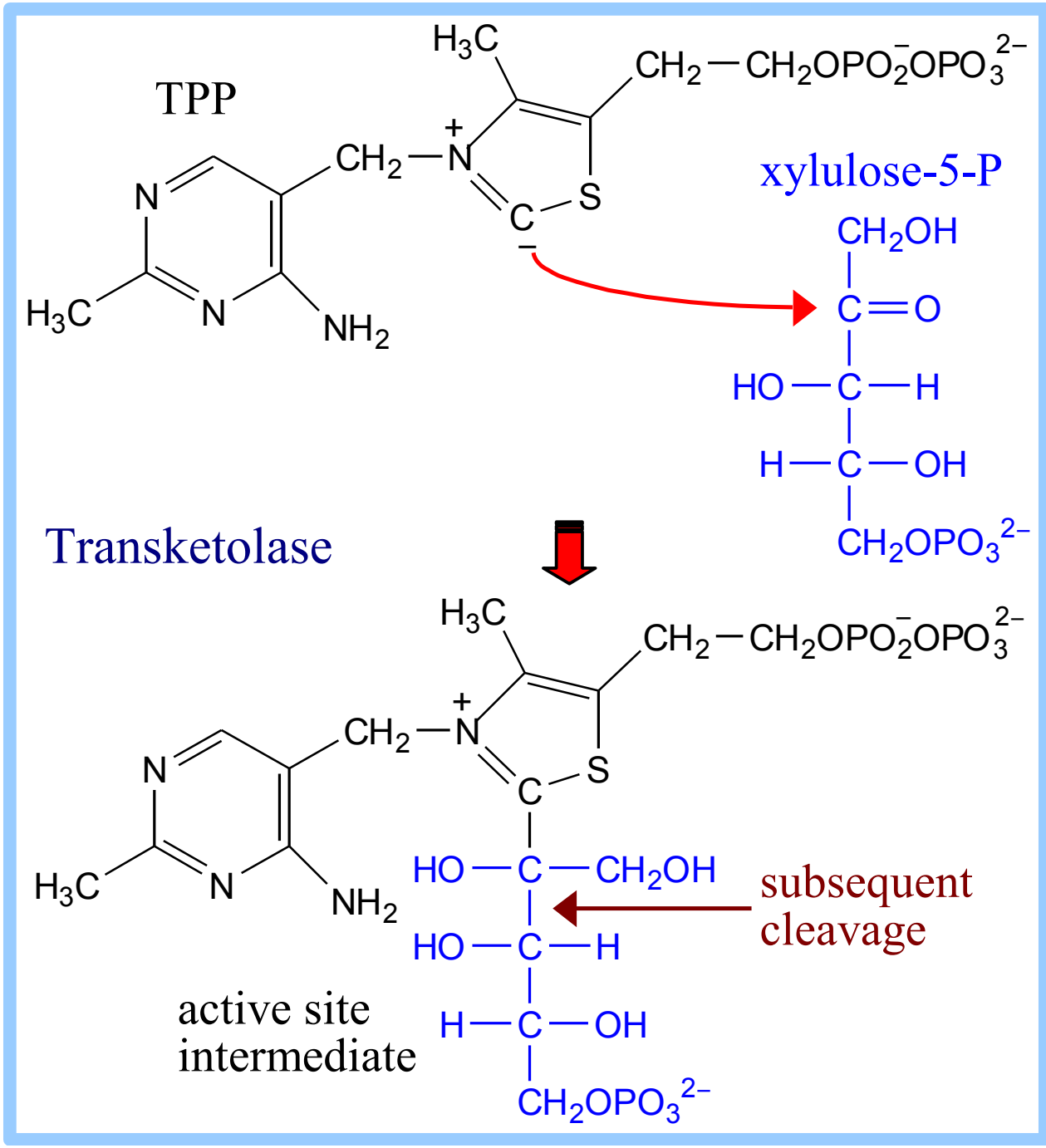


The 3-C aldose glyceraldehyde-3-P is released.

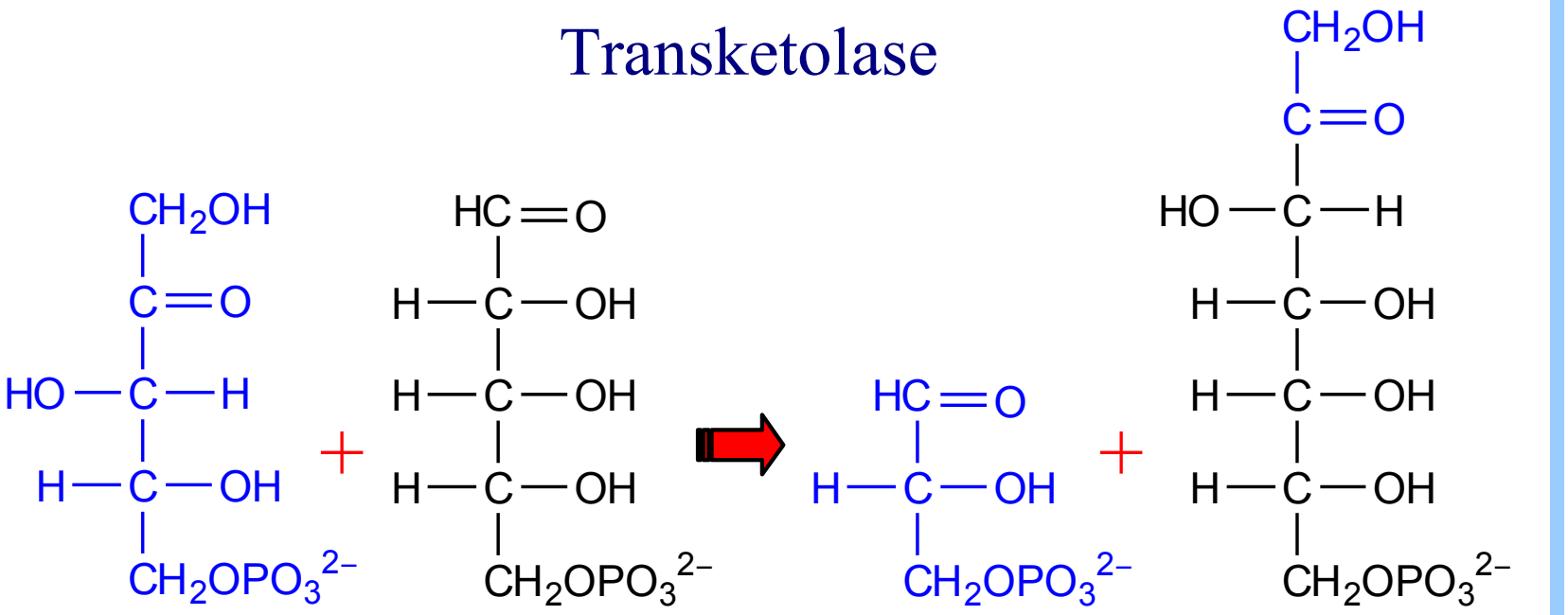
A **2-C fragment** remains on TPP.

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

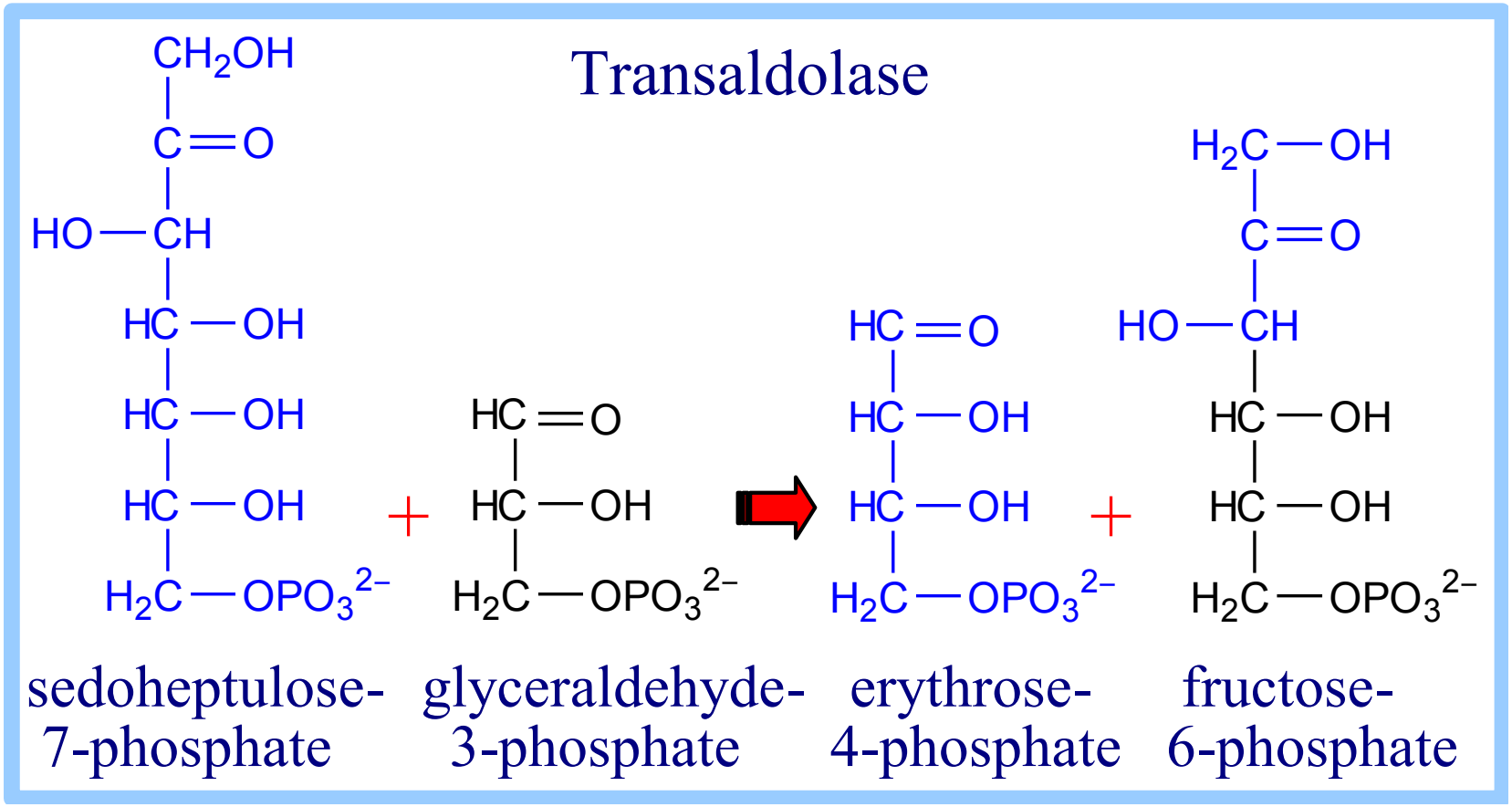
The **2-C** fragment condenses with one of the aldoses erythrose-4-P (4-C) or ribose-5-P (5-C) to form a ketose-P product.



Transketolase

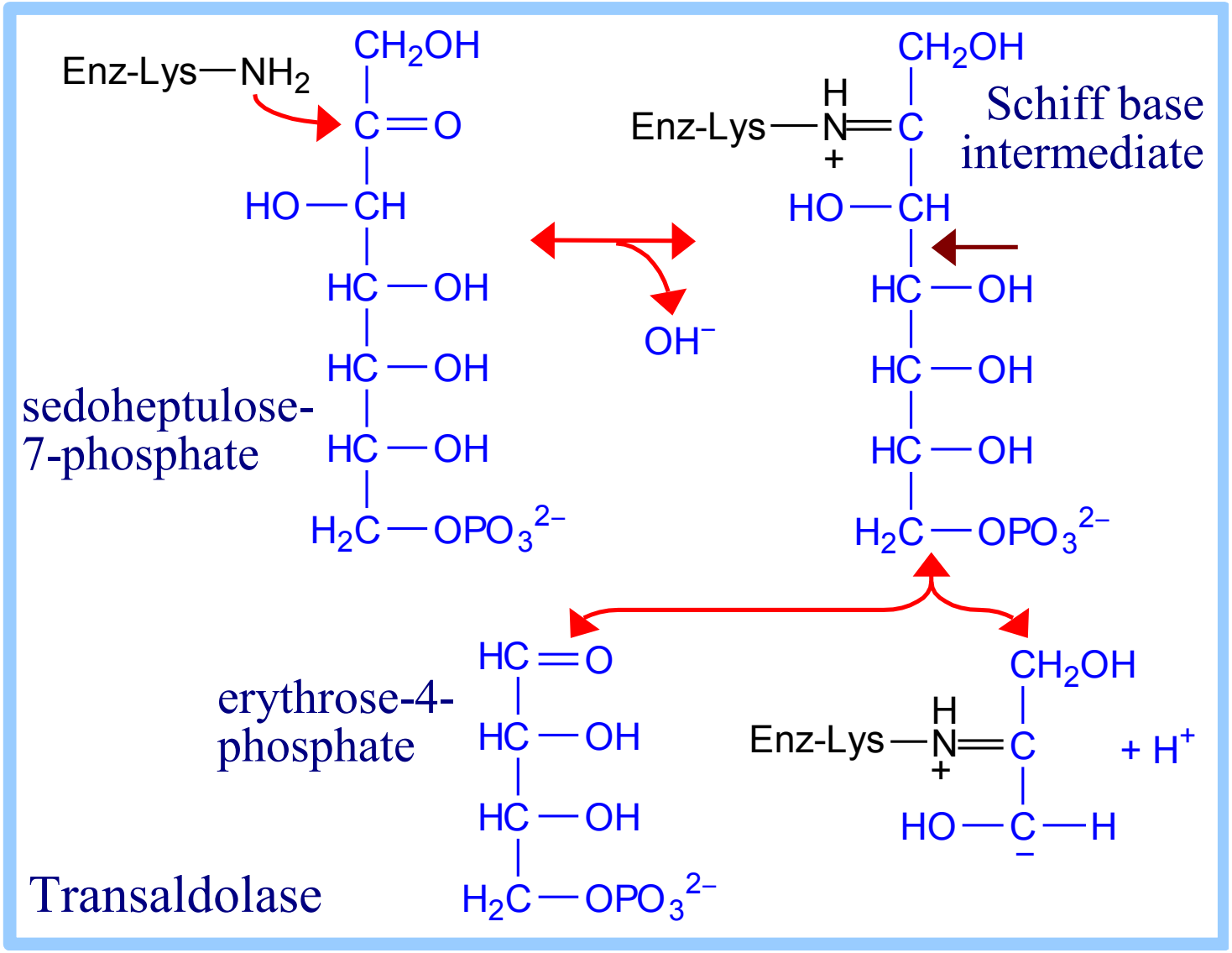


- Transfer of the 2-C fragment to the 5-C aldose ribose-5-phosphate yields sedoheptulose-7-phosphate.
- Transfer of the 2-C fragment instead to the 4-C aldose erythrose-4-phosphate yields fructose-6-phosphate.



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

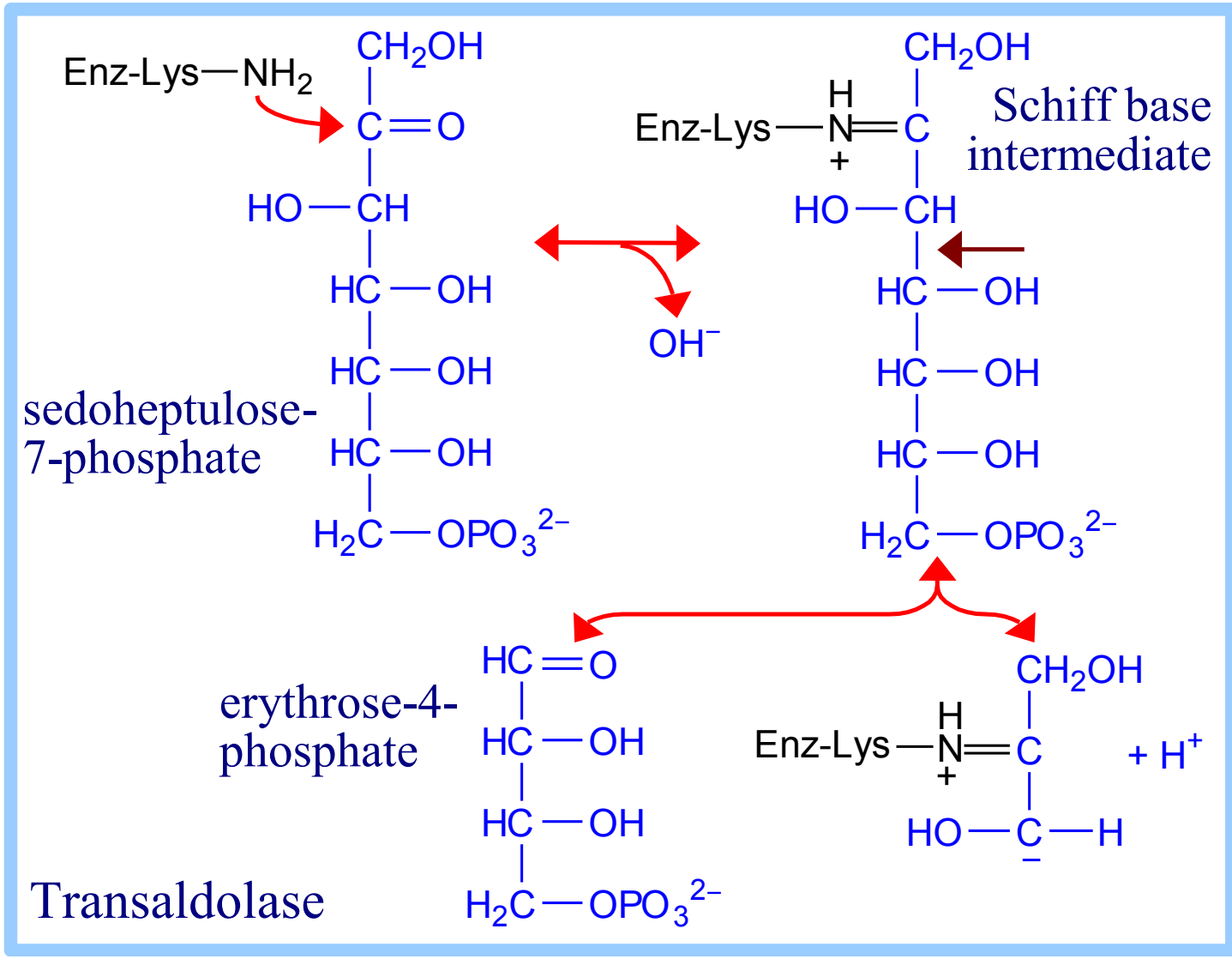
Transaldolase has an **α,β barrel** structure.



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.

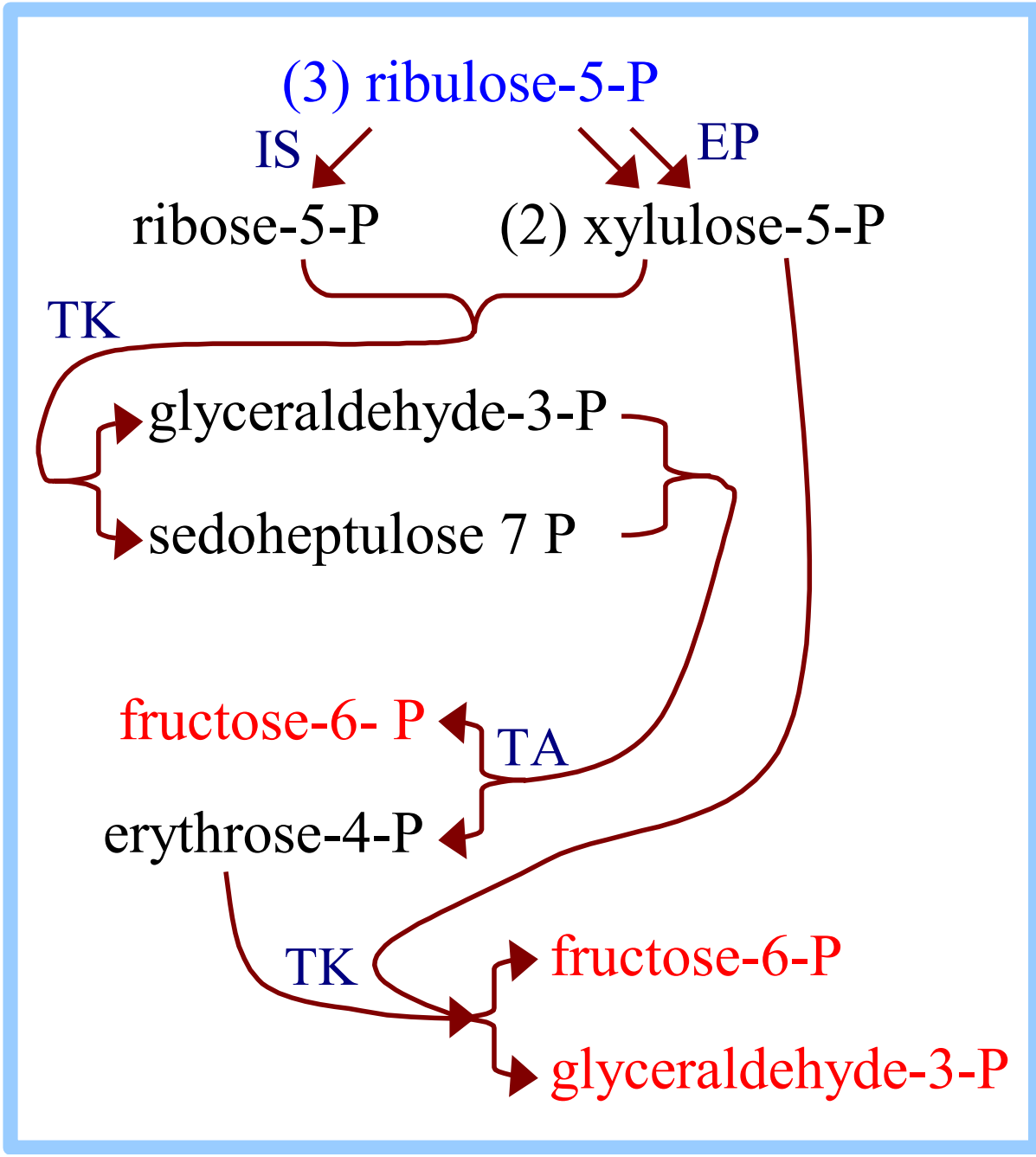
The diagram at right summarizes flow of 15 **C** atoms through Pentose Phosphate Pathway reactions by which **5-C** sugars are converted to **3-C** and **6-C** sugars.

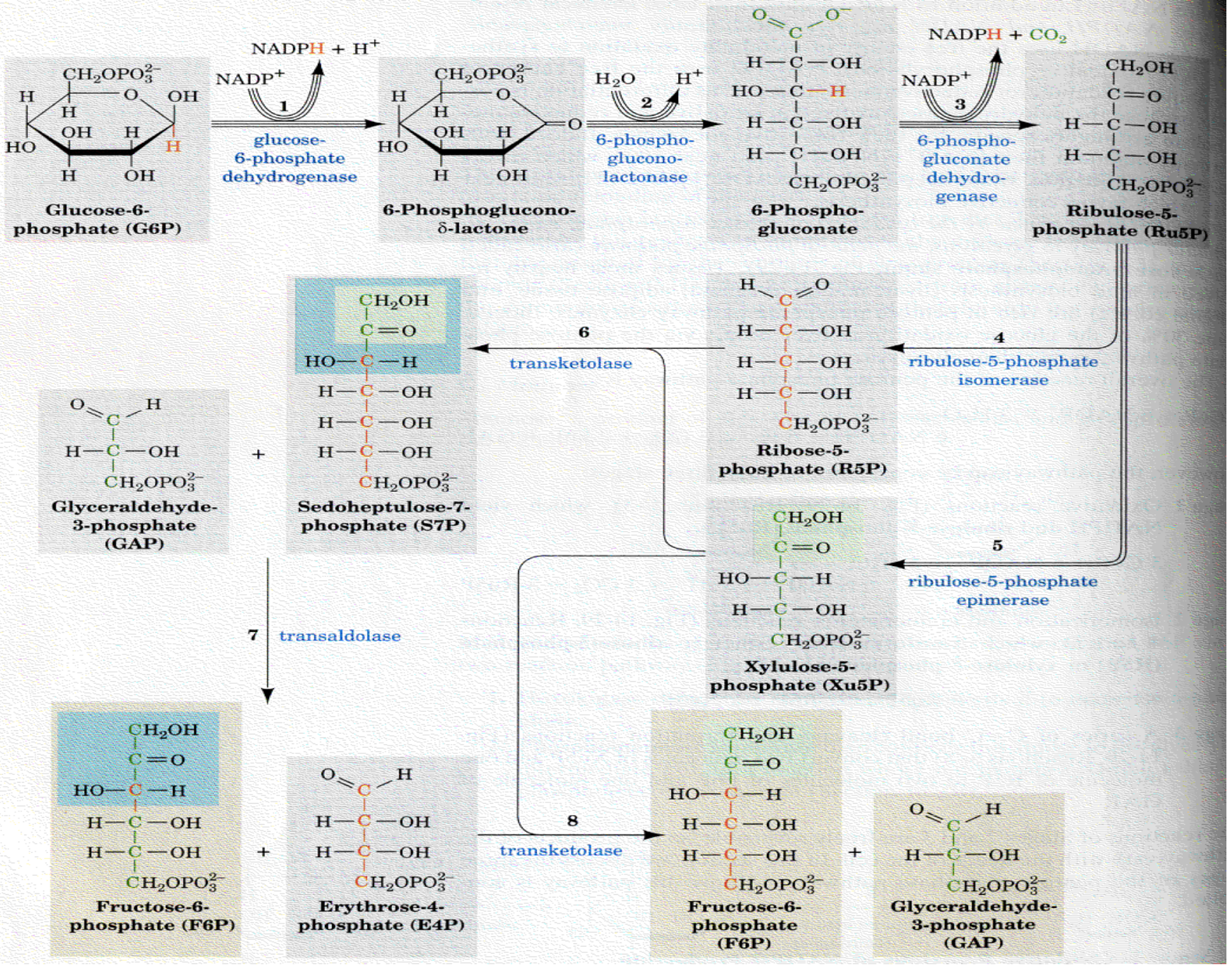
IS = Isomerase

EP = Epimerase

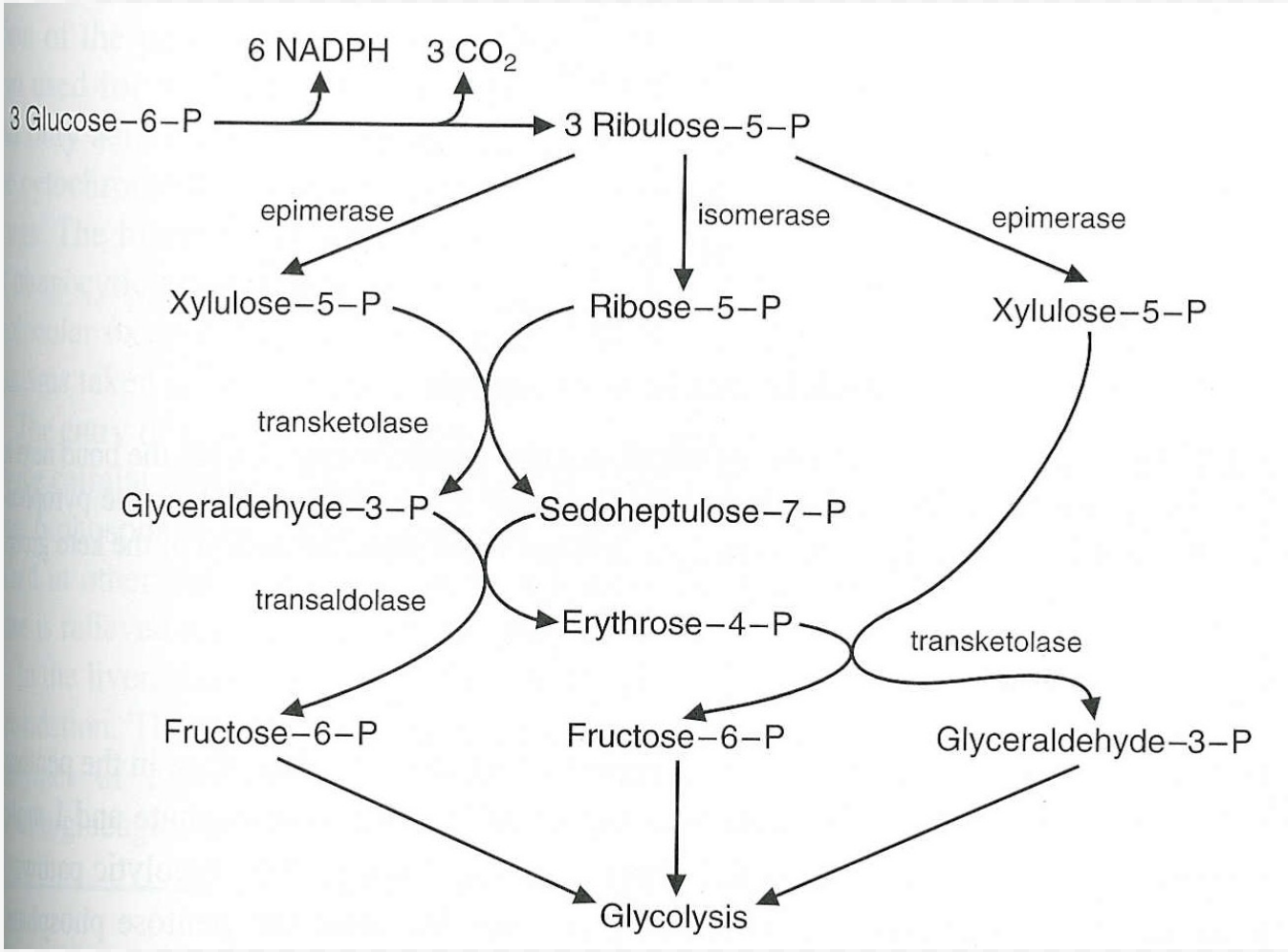
TK = Transketolase

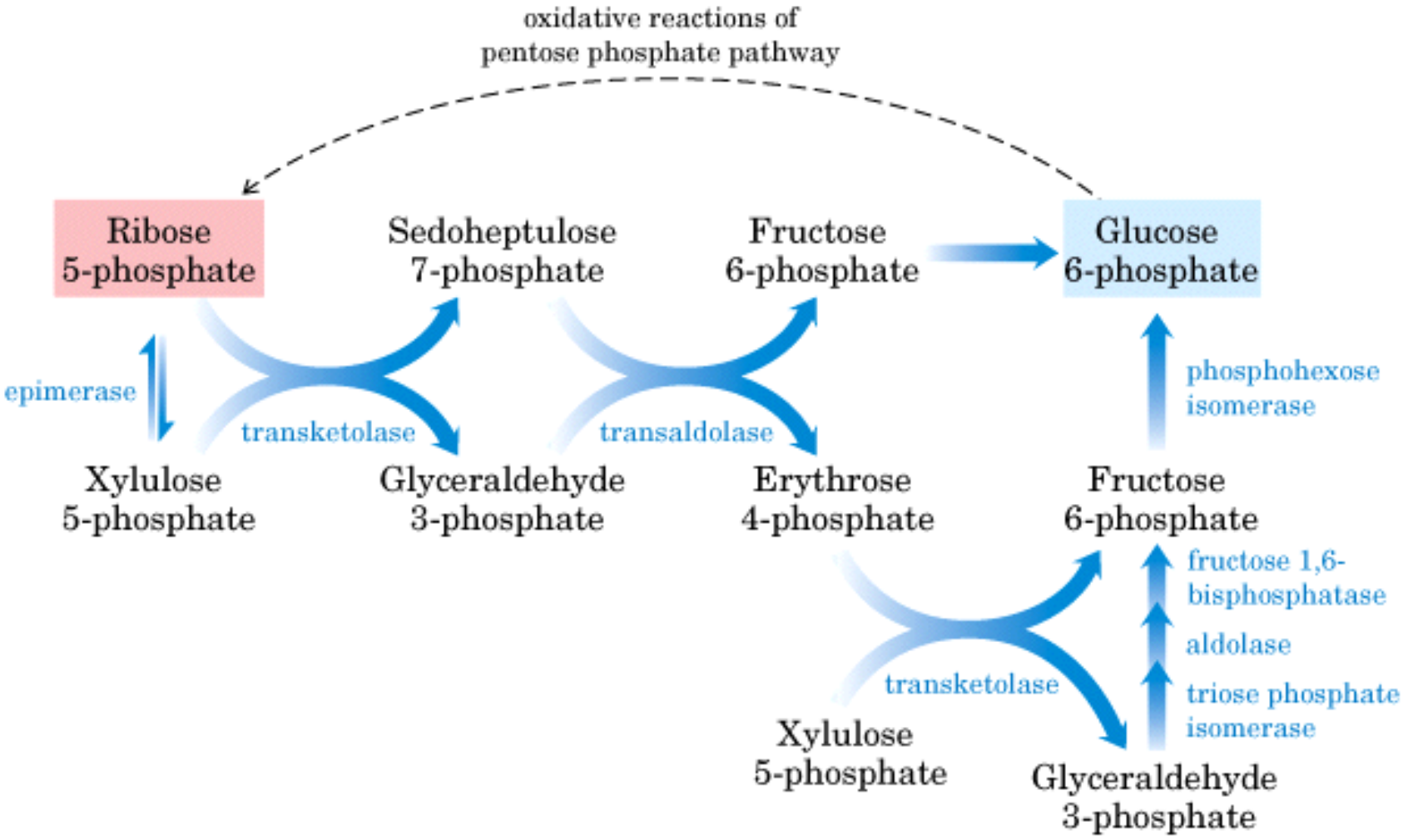
TA = Transaldolase



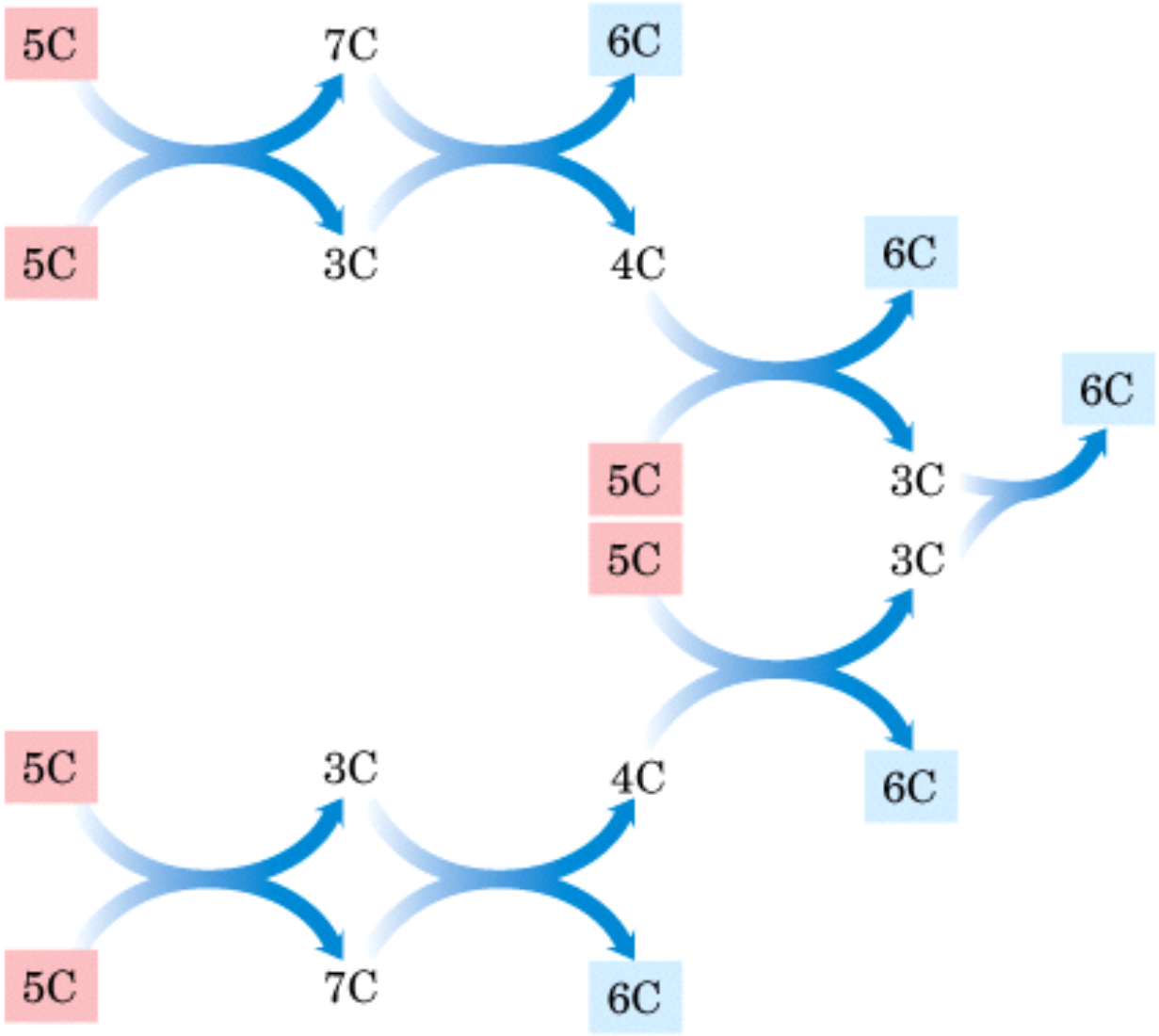

Glucose-6-phosphate (G6P)
NADPH + H+
NADP+
1
glucose-6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone
H2O
H+
2
6-phospho-gluconolactonase
6-Phospho-gluconate
NADPH + CO2
NADP+
3
6-phospho-gluconate dehydro-genase
Ribulose-5-phosphate (Ru5P)
4
ribulose-5-phosphate isomerase
Ribose-5-phosphate (R5P)
5
ribulose-5-phosphate epimerase
Xylulose-5-phosphate (Xu5P)
6
transketolase
Sedoheptulose-7-phosphate (S7P)
Glyceraldehyde-3-phosphate (GAP)
7
transaldolase
Fructose-6-phosphate (F6P)
Erythrose-4-phosphate (E4P)
8
transketolase
Fructose-6-phosphate (F6P)
Glyceraldehyde-3-phosphate (GAP)

The Pentose Phosphate Pathway: Non-oxidative phases




oxidative reactions of
pentose phosphate pathway
Ribose
5-phosphate
Sedoheptulose
7-phosphate
Fructose
6-phosphate
Glucose
6-phosphate
epimerase
transketolase
transaldolase
phosphohexose
isomerase
Xylulose
5-phosphate
Glyceraldehyde
3-phosphate
Erythrose
4-phosphate
Fructose
6-phosphate
fructose 1,6-
bisphosphatase
aldolase
triose phosphate
isomerase
transketolase
Xylulose
5-phosphate
Glyceraldehyde
3-phosphate

(a)



(b)

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.

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

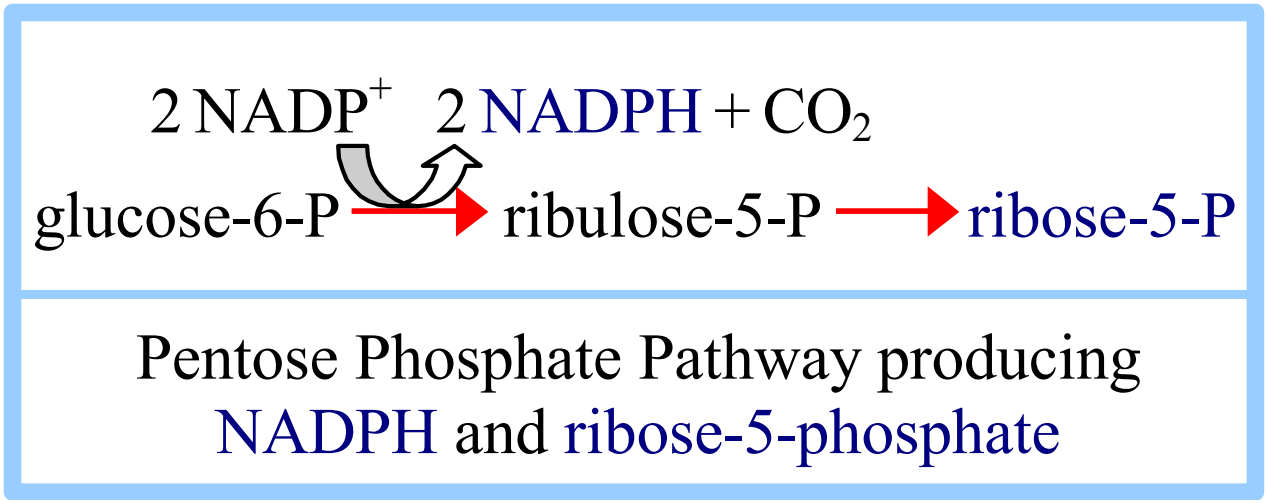
$$C_3 + C_7 \rightarrow C_6 + C_4 \quad \text{(Transaldolase)}$$

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

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

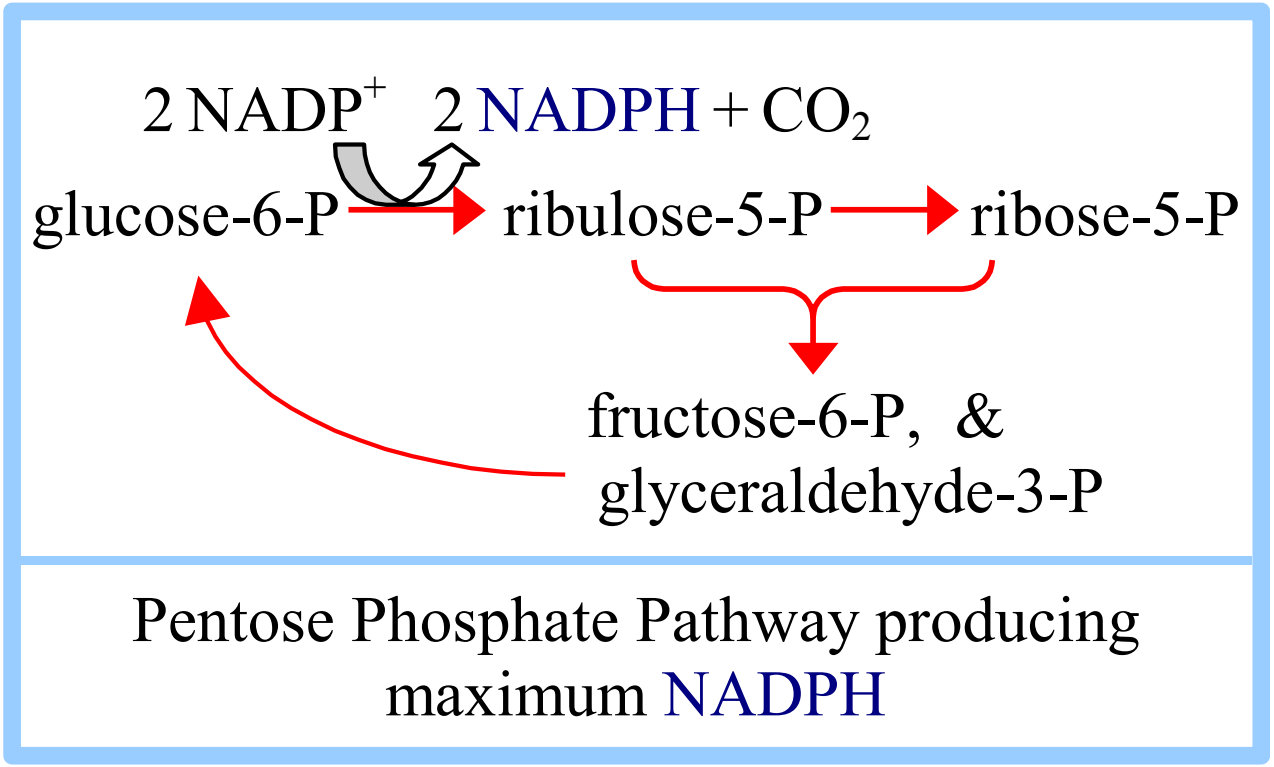
Glucose-6-phosphate may be regenerated from either the **3-C** glyceraldehyde-3-phosphate or the **6-C** fructose-6-phosphate, via enzymes of Gluconeogenesis.

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**:

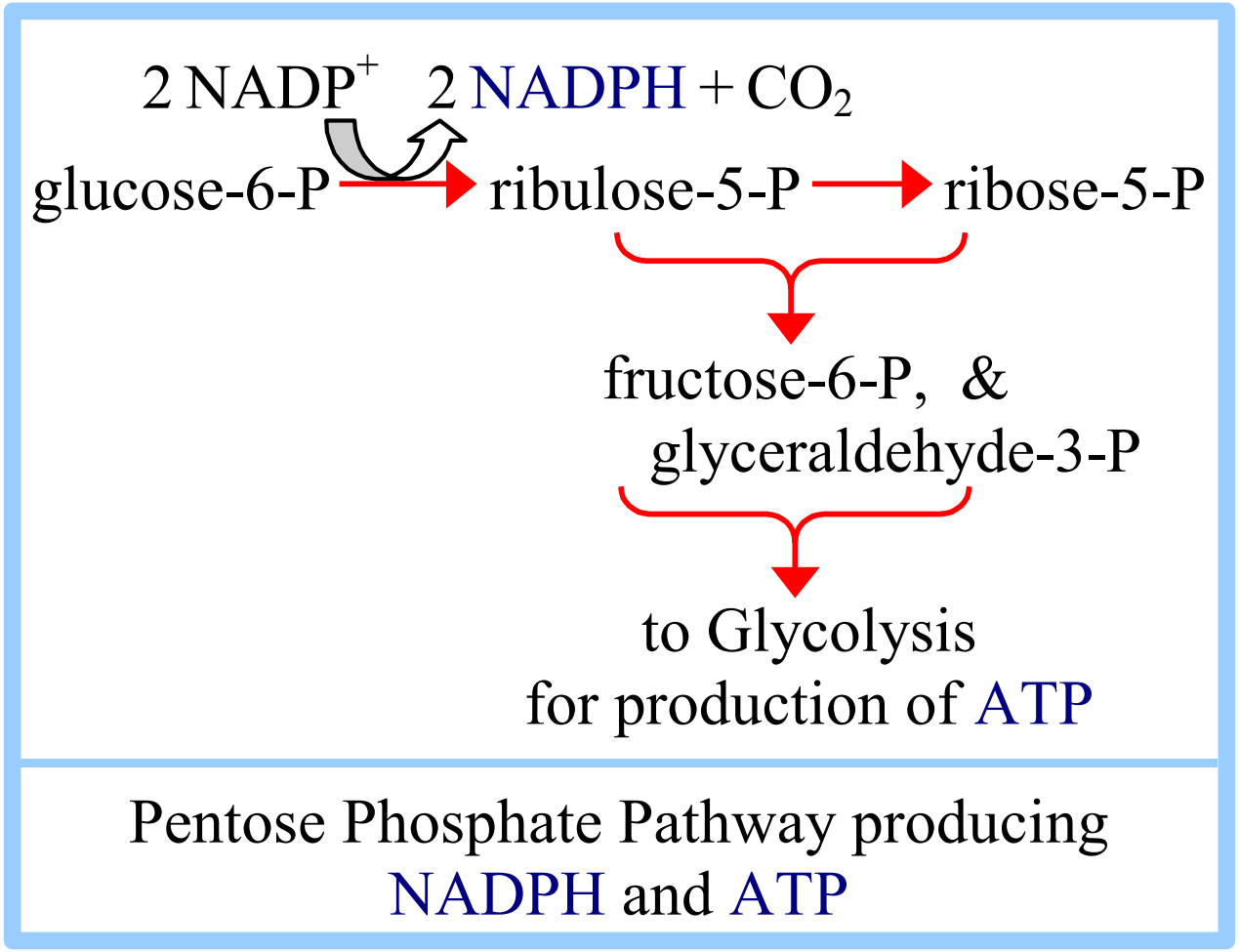


1. Ribulose-5-P may be converted to **ribose-5-phosphate**, a substrate for synthesis of **nucleotides** and nucleic acids.

The pathway also produces some **NADPH**.

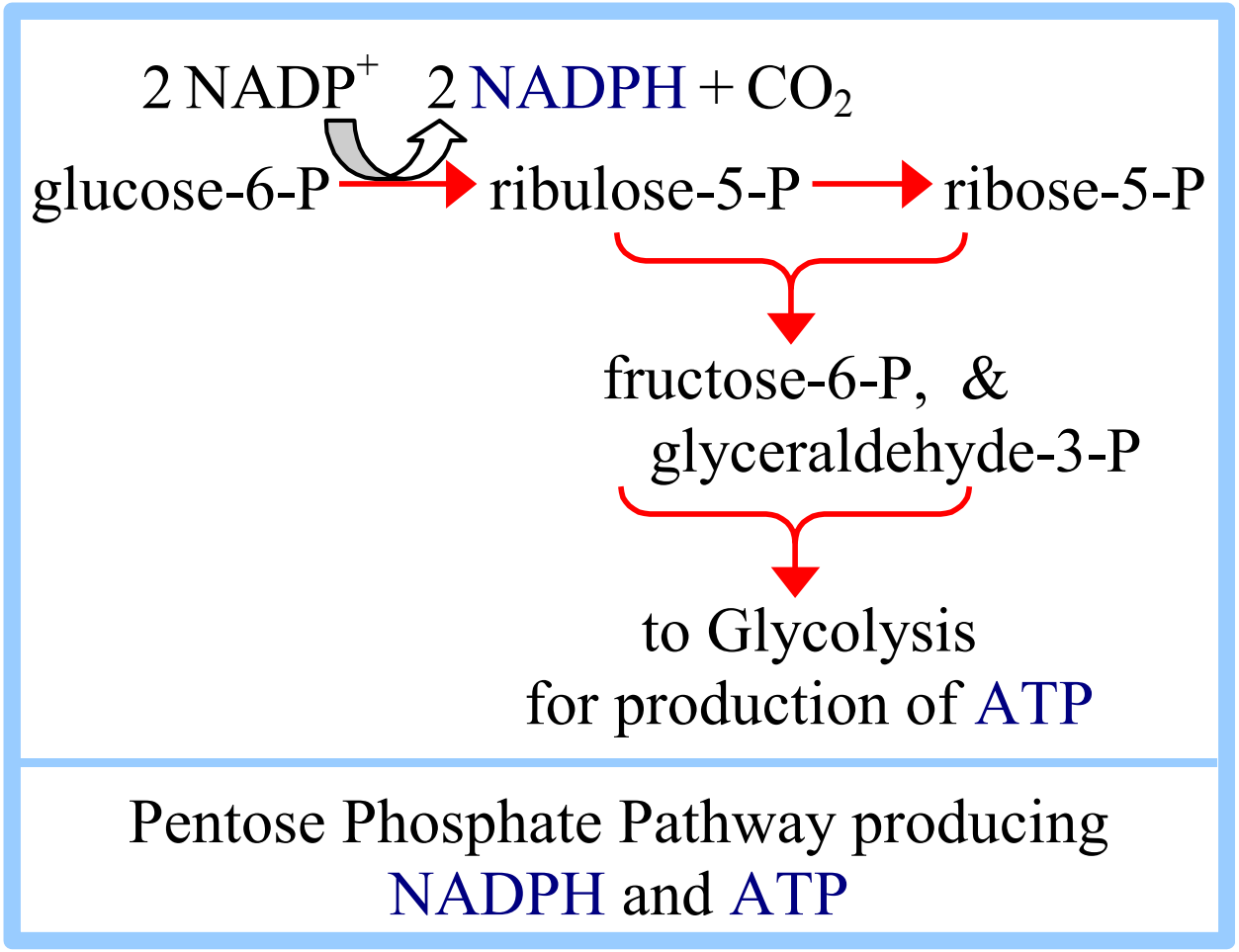


2. Glyceraldehyde-3-P and fructose-6-P may be converted to **glucose-6-P** for reentry to the linear portion of the Pentose Phosphate Pathway, maximizing formation of **NADPH**.



3a. 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**.



3b. Ribose-1-phosphate generated during **catabolism of nucleosides** also enters Glycolysis in this way, after first being converted to ribose-5-phosphate.

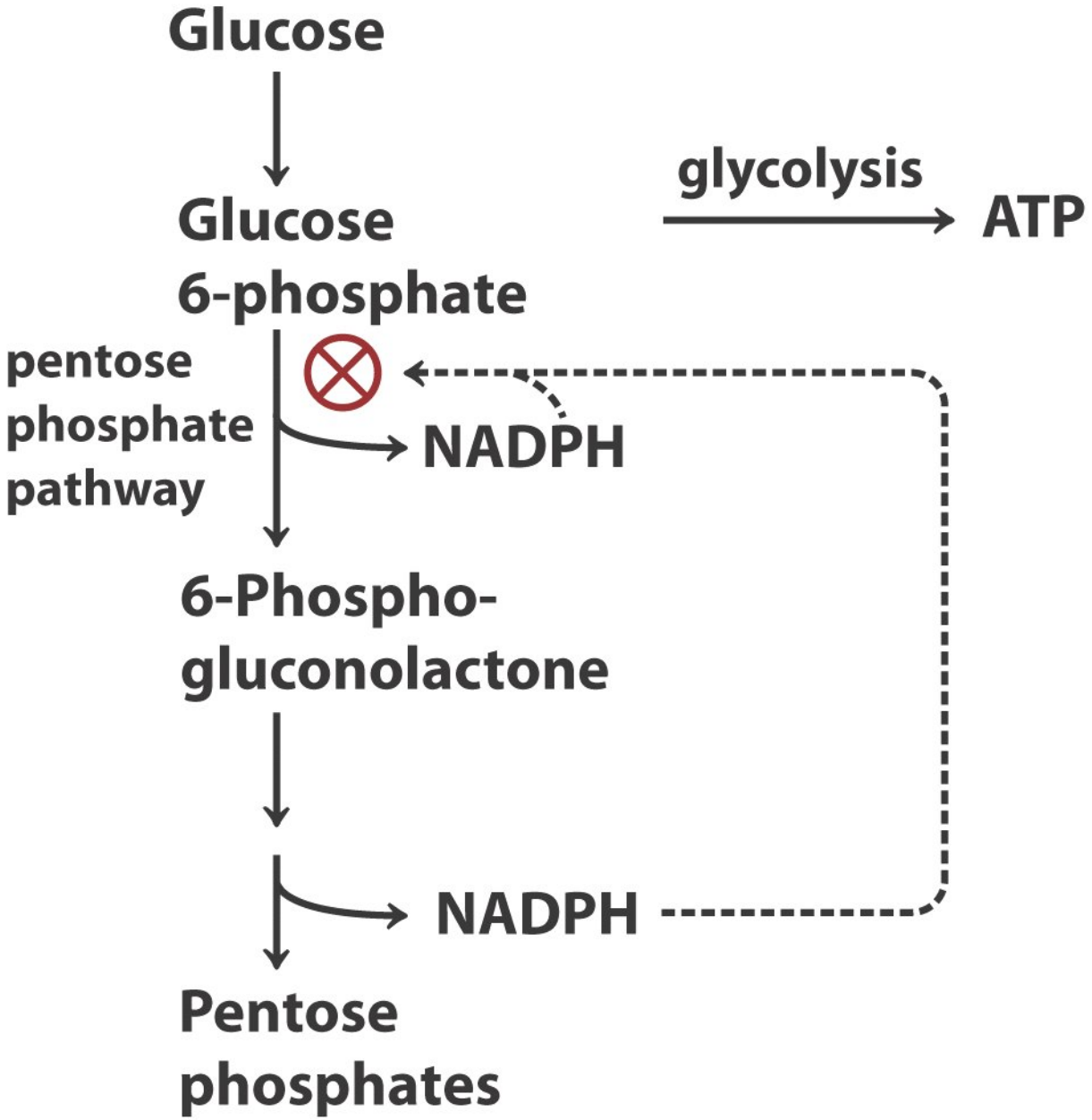
Thus the Pentose Phosphate Pathway serves as an **entry into Glycolysis** for both 5-carbon & 6-carbon sugars.

Regulation of pentose phosphate pathway

The major specific product of this pathway are *NADPH and Ribose 5P* which are used as reducing power for synthetic reactions and nucleic acid synthesis respectively.

Glucose 6 phosphate dehydrogenase (G6P DH) is the main enzyme that controls the flux (overall rate) of this pathway.

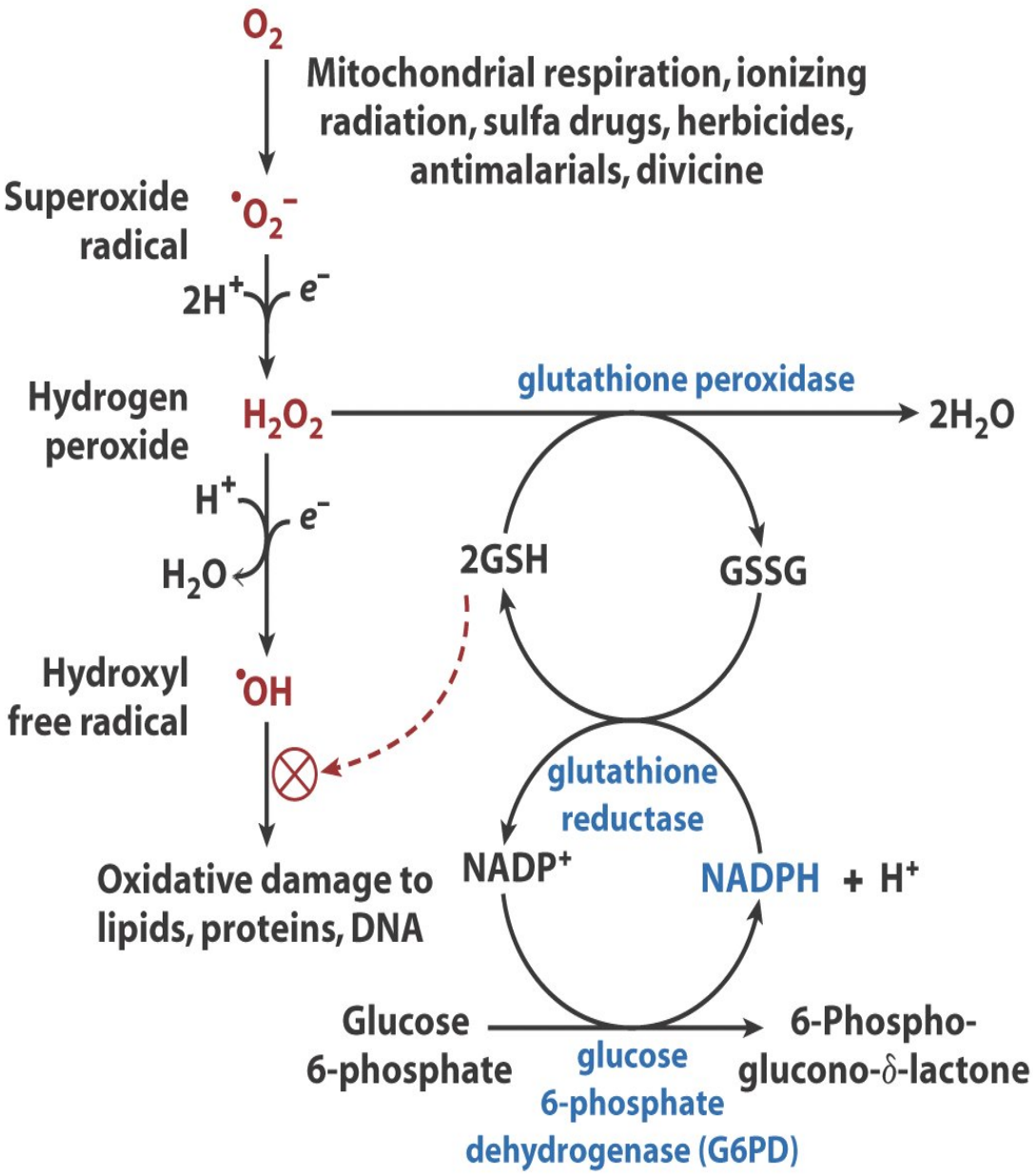
G6P-DH is strongly inhibited by NADPH. Thus if the NADPH concentration decreases, the G6PDH is activated.

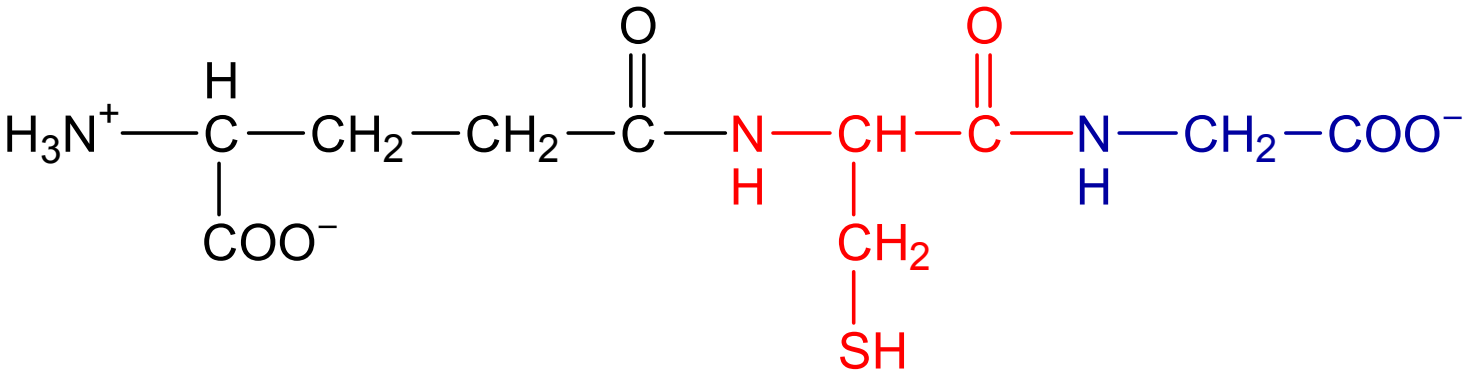

Glucose
Glucose
6-phosphate
glycolysis
ATP
pentose
phosphate
pathway
NADPH
6-Phospho-
gluconolactone
NADPH
Pentose
phosphates

NADPH is used to generate reduced glutathione (GSH). GSH plays a critical role in quenching the oxyradicals in the cells.

If NADPH generation is inhibited due to the mutations in G6PDH, cells become susceptible to oxidative damage.

Malaria parasite is very sensitive to Oxy-radicals, and people with G6PDH defect are resistant to malaria as the parasite is killed by oxy-radicals.





γ-glutamyl-cysteinyl-glycine

Glutathione

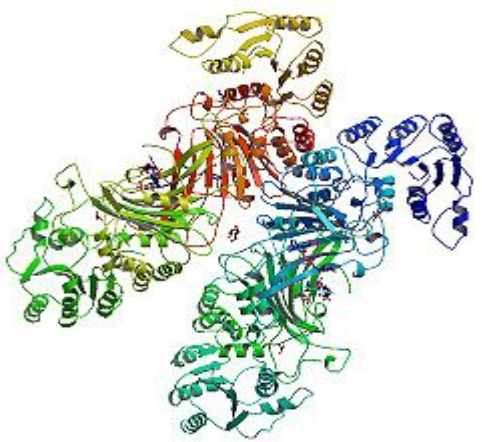
Glutathione is a tripeptide that includes a Glu linked by an isopeptide bond involving the side-chain carbonyl group.

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.

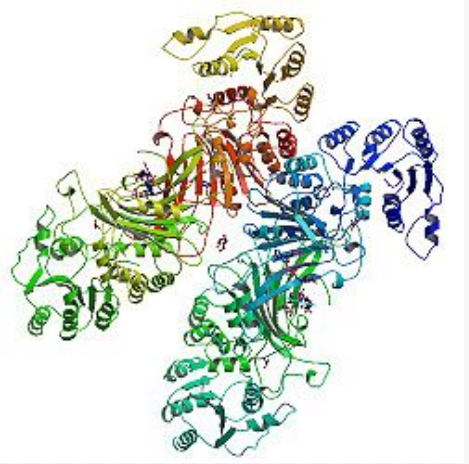
Hydroperoxides can react with double bonds in fatty acids of membrane lipids, making membranes leaky.

G6PDH Deficiency and Hemolytic Anemia

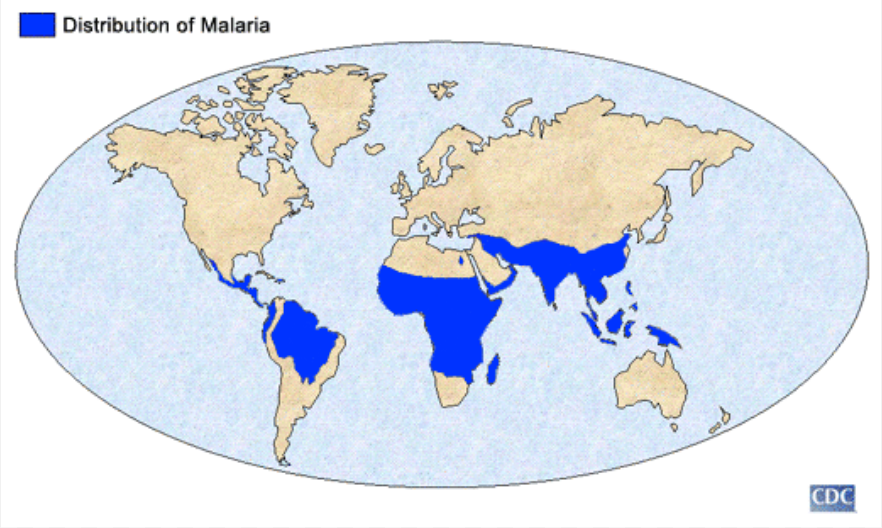


- Most common genetic enzymopathy
  - 400 variants of G6PDH deficiency
  - Mediterranean, Asian, African descent
    - 400 million people affected worldwide
    - 50% of Kurdish men
    - 10-14% of African-American men with G6PD deficiency

G6PD Deficiency



- Distribution of G6PD deficiency coincides prevalence of malaria



- G6PD deficiency may impart some degree of malaria resistance
  - Also sickle cell anemia

Genetics

- Recessive sex-linked mutation
  - X-chromosome
  - Rare in females (two X-chromosomes)
- Homozygous mutation:
  - high hemolysis and anemia
- Heterozygous mutation:
  - Normally asymptomatic
    - unless exposed to drugs (primaquine, anti-malarial drug) or compounds (fava bean) that produce superoxide or hydrogen peroxide

- **G6PDH deficiency is an X-linked recessive ereditary disease characterised by abnormally low levels of G6PDH**

- **People with G6PDH deficiency are at risk of hemolytic anemia in states of oxidative stress. Oxidative stress can result from infection and from chemical exposure to medication and certain foods. Broad beans, contain high levels of vicine, divicine, convicine and isouramil, all of which are oxidants.**

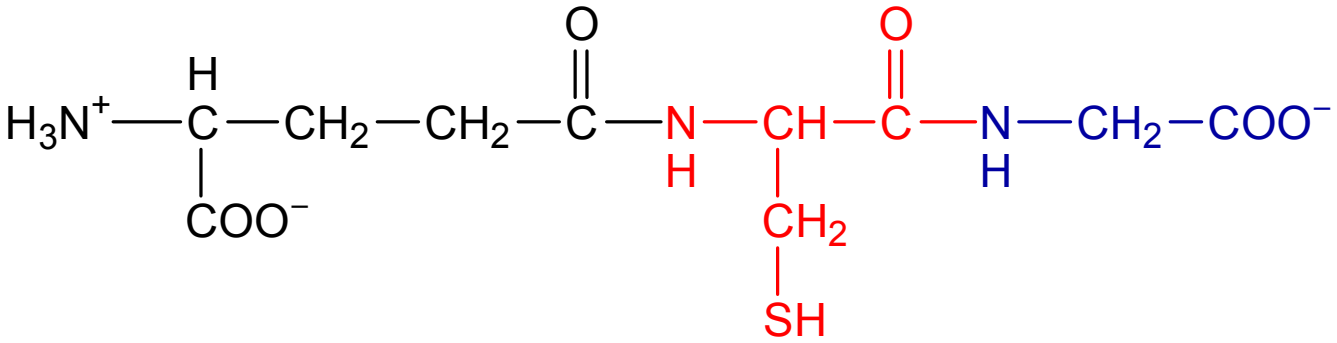
- When all remaining reduced glutathione is consumed, enzymes and other proteins (including Hb) are subsequently damaged by the oxidants, leading to electrolyte imbalance, and protein deposition in the red cell membranes.
- Damaged red cells are phagocytosed and sequestered (taken out of circulation) in the spleen. The Hb is metabolized to bilirubin. The red cells rarely disintegrate in the circulation, so hemoglobin is rarely excreted directly by the kidney, but this can occur in severe cases, causing acute renal failure .

Defective G6PDH

- Results in enzyme with unstable structure
  - Patient with 10% of normal activity
  - Enough to generate NADPH under normal condition
- Newly made RBCs have normal 6PDH activity
  - Patients recover quickly (8 days)

Broad Beans

- Grown worldwide
  - Important in Middle East
  - High in protein
  - Frost resistant perennial
- Genetically modified broad bean being developed
  - Low in vicine and isouramil
- Favism



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.

$2\,GSH + ROOH \rightarrow GSSG + ROH + H_2O$

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**).