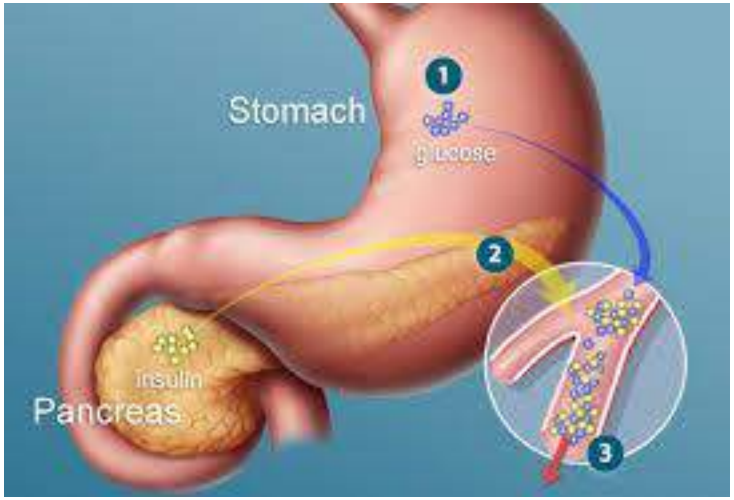
Glycolysis I



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Glucose as Energy Substrate

- To function properly, our cells are in need for energy which can be generated from the metabolism of various biomolecules such as carbohydrates, proteins and lipids
- Actually CHO particularly glucose is a major energy substrate in certain tissues like brain
- What are the metabolic pathways of glucose inside our cells?

Glycolysis

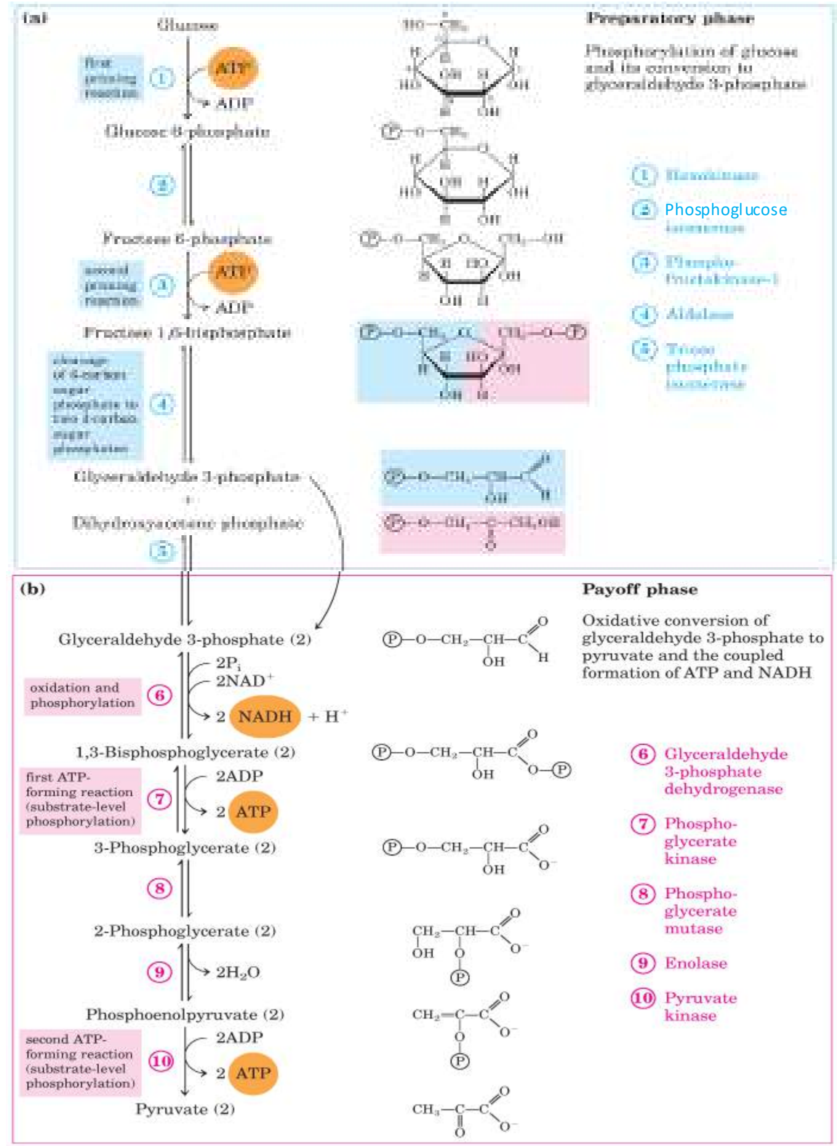
- Glycolysis is the metabolic pathway which converts glucose (6C) into 2 pyruvate molecules (3C)
- It occurs in the cell cytosol
- Glycolysis takes place in nearly all organisms both aerobic and anaerobic (i.e. microorganisms live in O_2 free environments)
- Glycolysis is a sequence of ten oxygen-independent and enzyme-catalyzed steps
- The intermediates either provide entry points to the cycle or themselves directly useful (biosynthetic intermediates)

Glycolysis

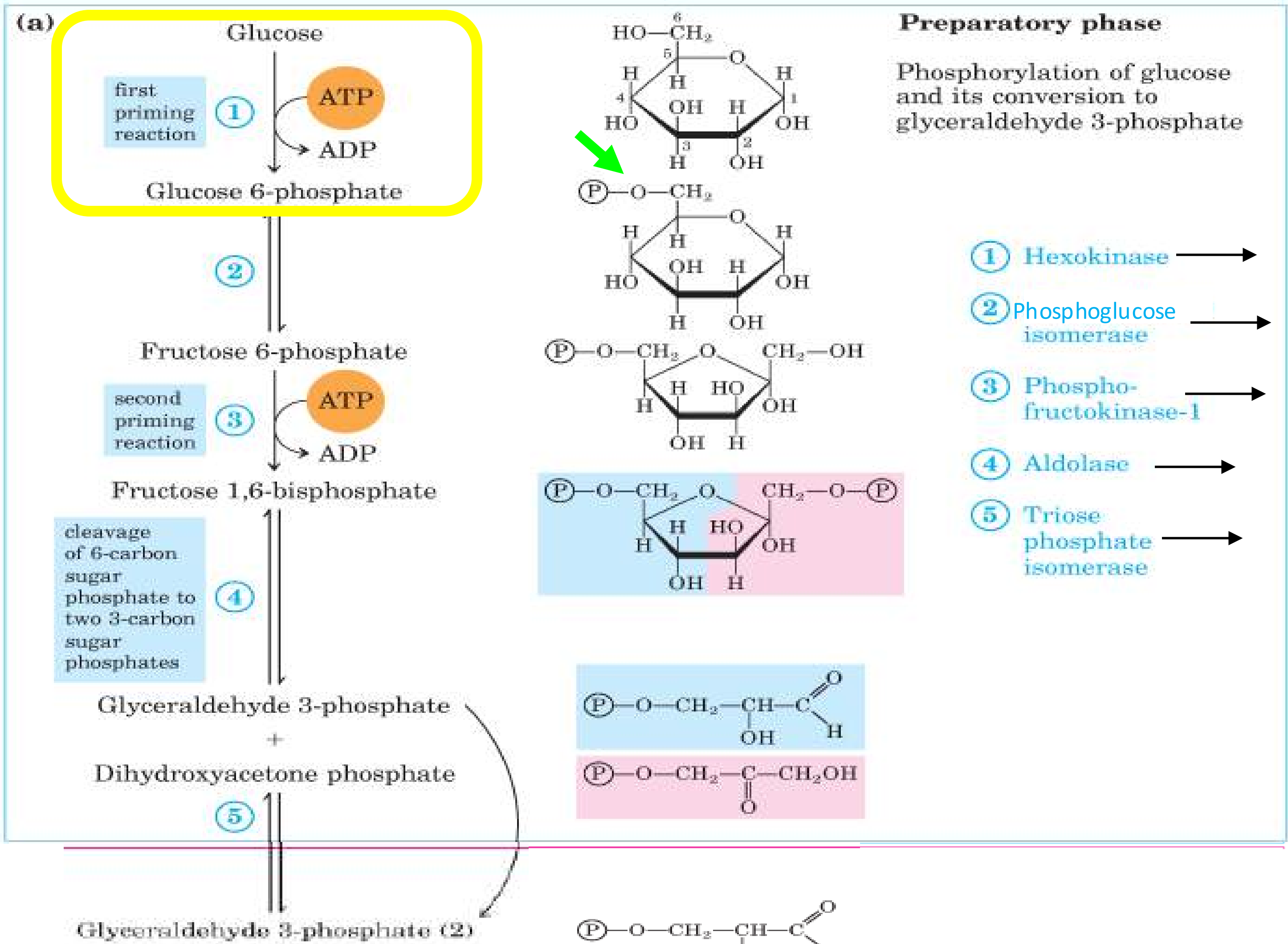
- The entire pathway is divided into two distinct phases:

A. Energy Investment Phase (Preparatory Phase)

B. Energy Generation Phase (Pay Off Phase)



A. Preparatory Phase



phosphorylation

isomerization

phosphorylation

cleavage

isomerization

A. Preparatory Phase

- **Step 1:** Hexokinases catalyze the ATP- dependent phosphorylation of glucose to produce glucose-6-phosphate (G6P)
- Hexokinase is a transferase enzyme which phosphorylates hexoses by transferring an inorganic phosphate from ATP usually to hydroxyl O at C6
- Irreversible reaction (another enzyme catalyzes the dephosphorylation, only found in specific tissues). Therefore, it is a target site for cycle regulation
- This first priming reaction is important to maintain the influx of glucose through glucose transporters (GLUTs) and at the same time to trap the transported glucose molecules inside the cell

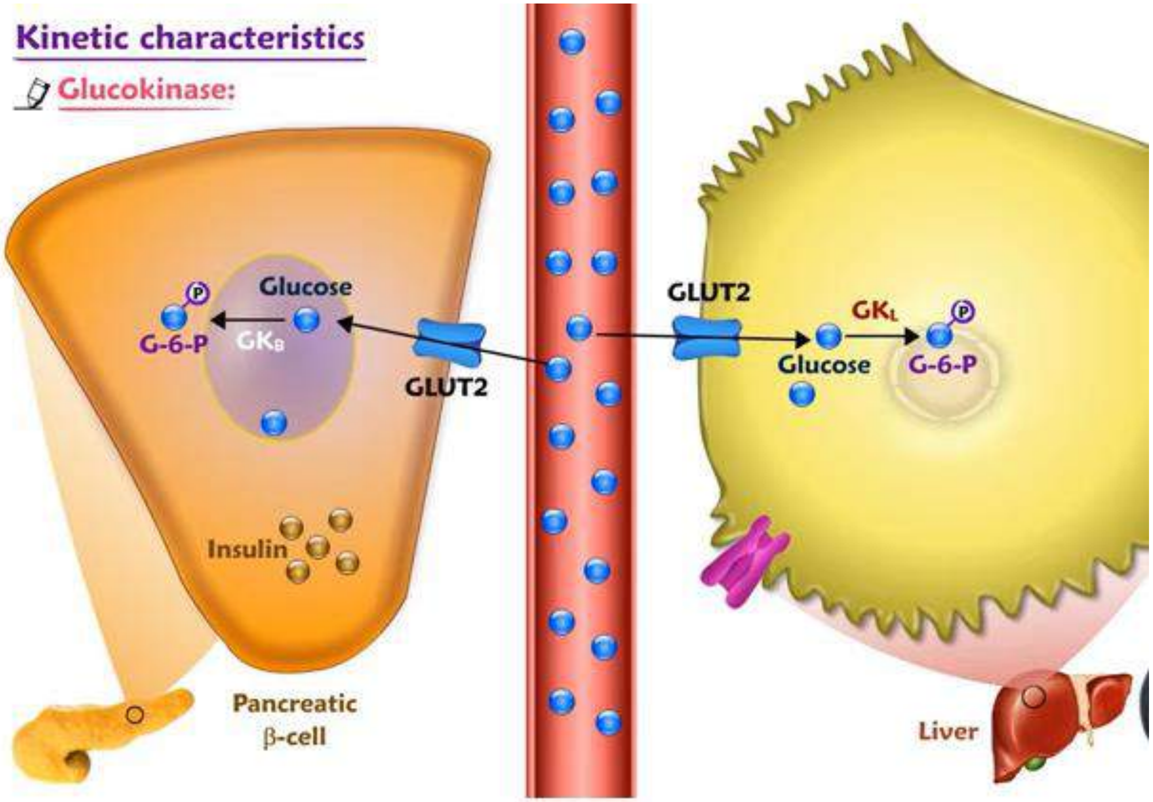
Hexokinases

- 4 isoforms (isozymes) of hexokinase (I, II, III & IV) which differ in their **location**, **catalysis** and **regulation** thereby, contributing to different pattern of glucose metabolism in different tissues
- Hexokinase I, II & III are nonspecific and can phosphorylate a variety of hexoses (e.g. glucose, fructose, mannose) but type I is involved in **catabolic pathways** like glycolysis whereas type II & III are involved in **anabolic pathways** like glycogenesis
- Hexokinase IV is called glucokinase expressed in liver and pancreatic β-cells. It is specific for D-glucose

Hexokinases

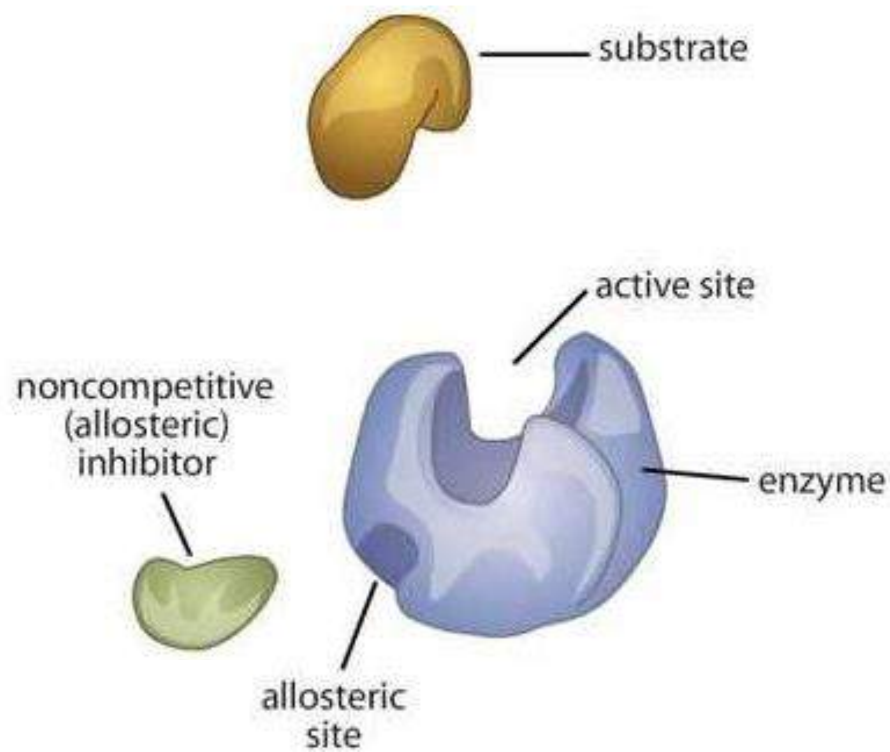
- Glucokinase has low affinity for glucose (high Km value) compared to others (low Km value)
- Therefore, glucokinase in **liver** is active only at high blood glucose level to accumulate G6P for glycogen synthesis but in the **pancreas** it acts as glucose sensor to control insulin release from beta cells
- Hexokinase isoforms (except isoform IV) are allosterically inhibited by G6P **only** at high level

Glucokinase

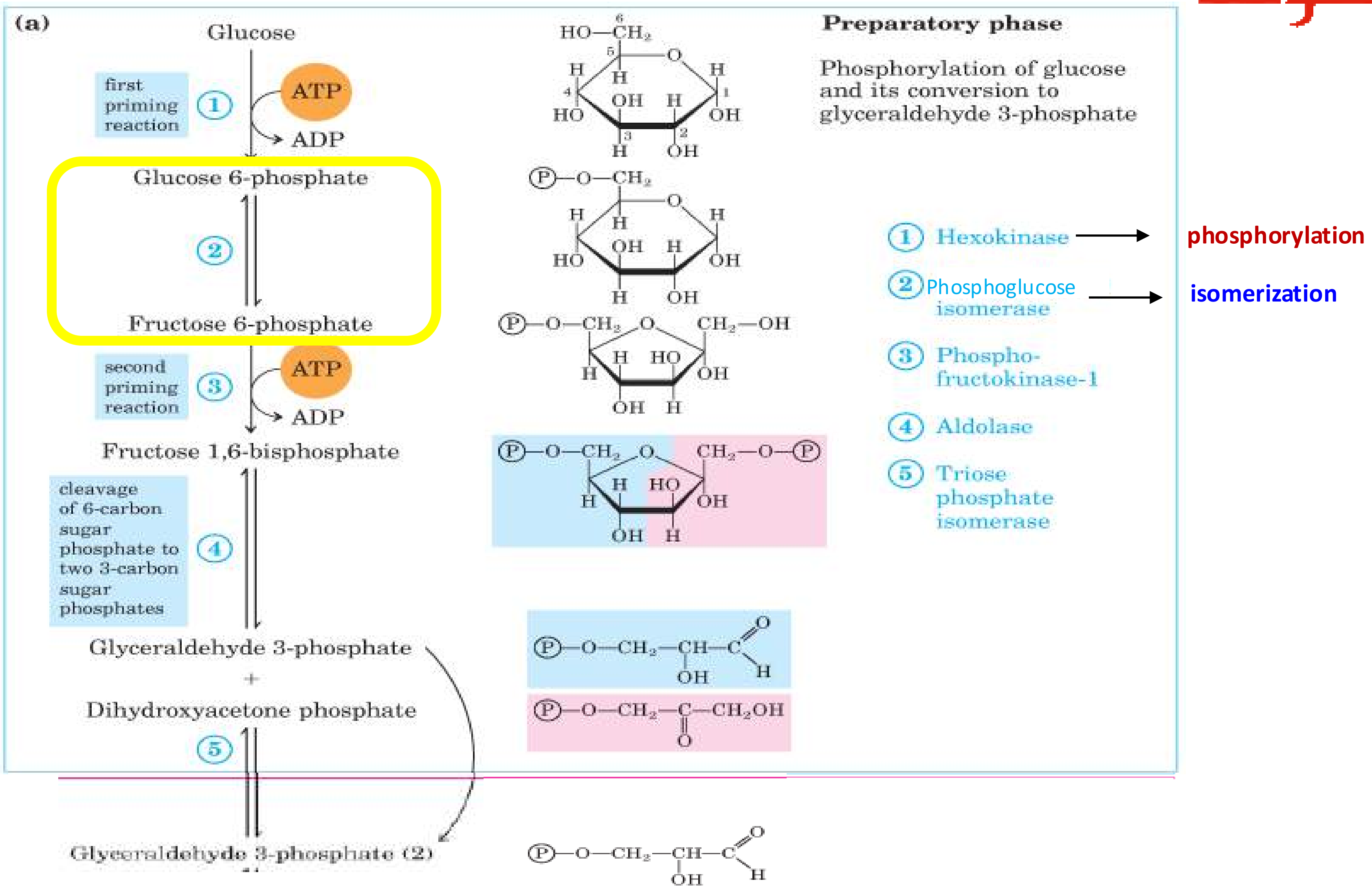


Hexokinases

- Hexokinase is an allosteric enzyme with two binding sites: catalytic site (binds substrate) and regulatory site (allosteric site binds effectors)

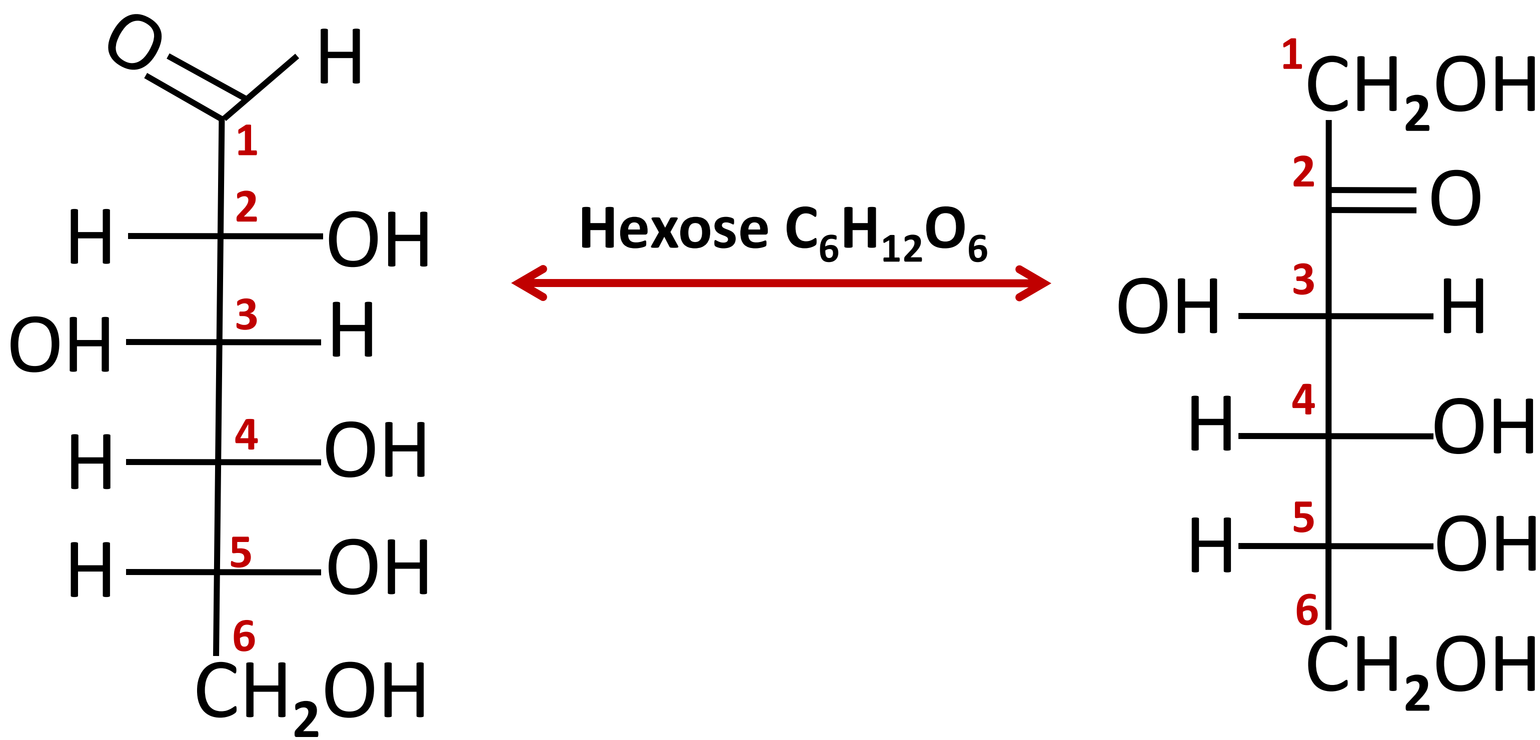


A. Preparatory Phase



A. Preparatory Phase

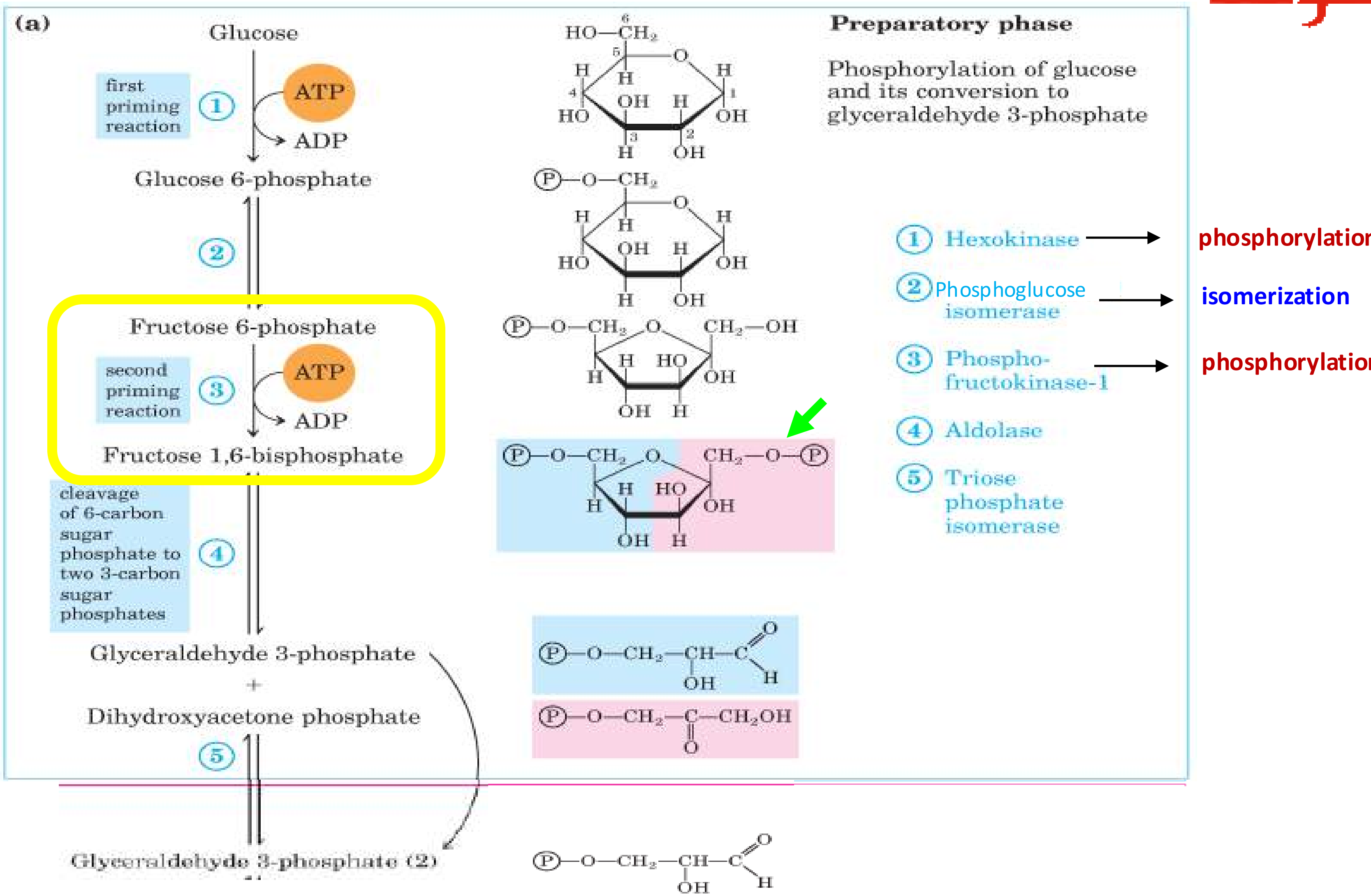
- **Step 2:** Phosphoglucose isomerase (PGI) interconverts G6P and F6P (reversible reaction).
- Indeed, **Mannose and Fructose** can enter the glycolysis pathway at this point



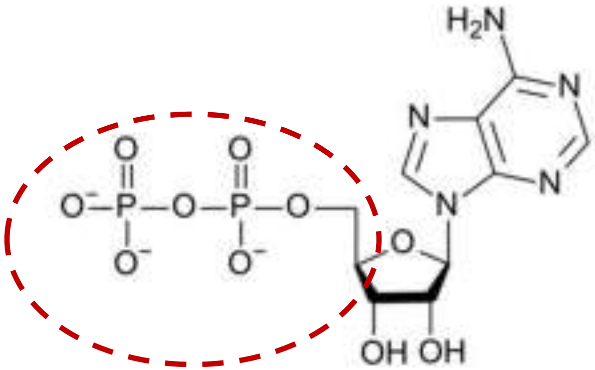
D-glucose

D-fructose

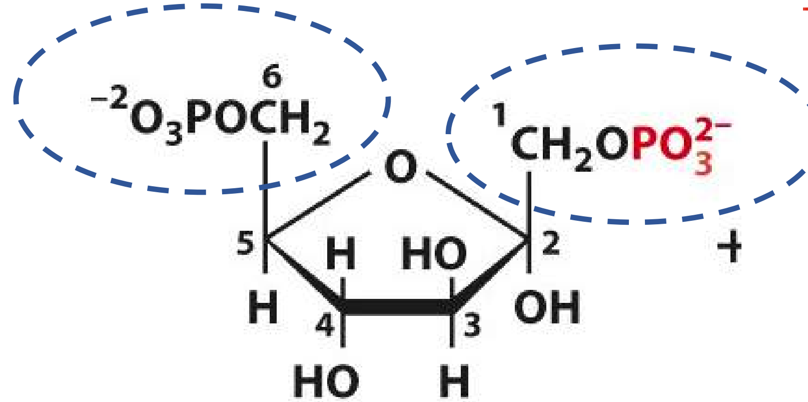
A. Preparatory Phase



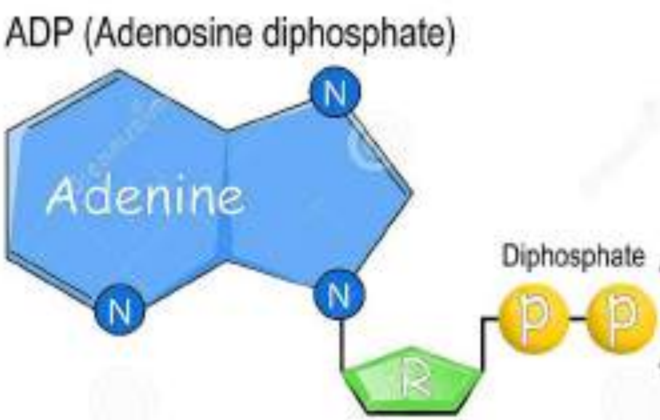
A. Preparatory Phase

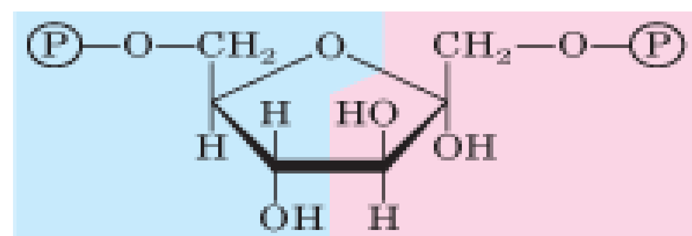


ADP



Fructose 1,6 bisphosphate

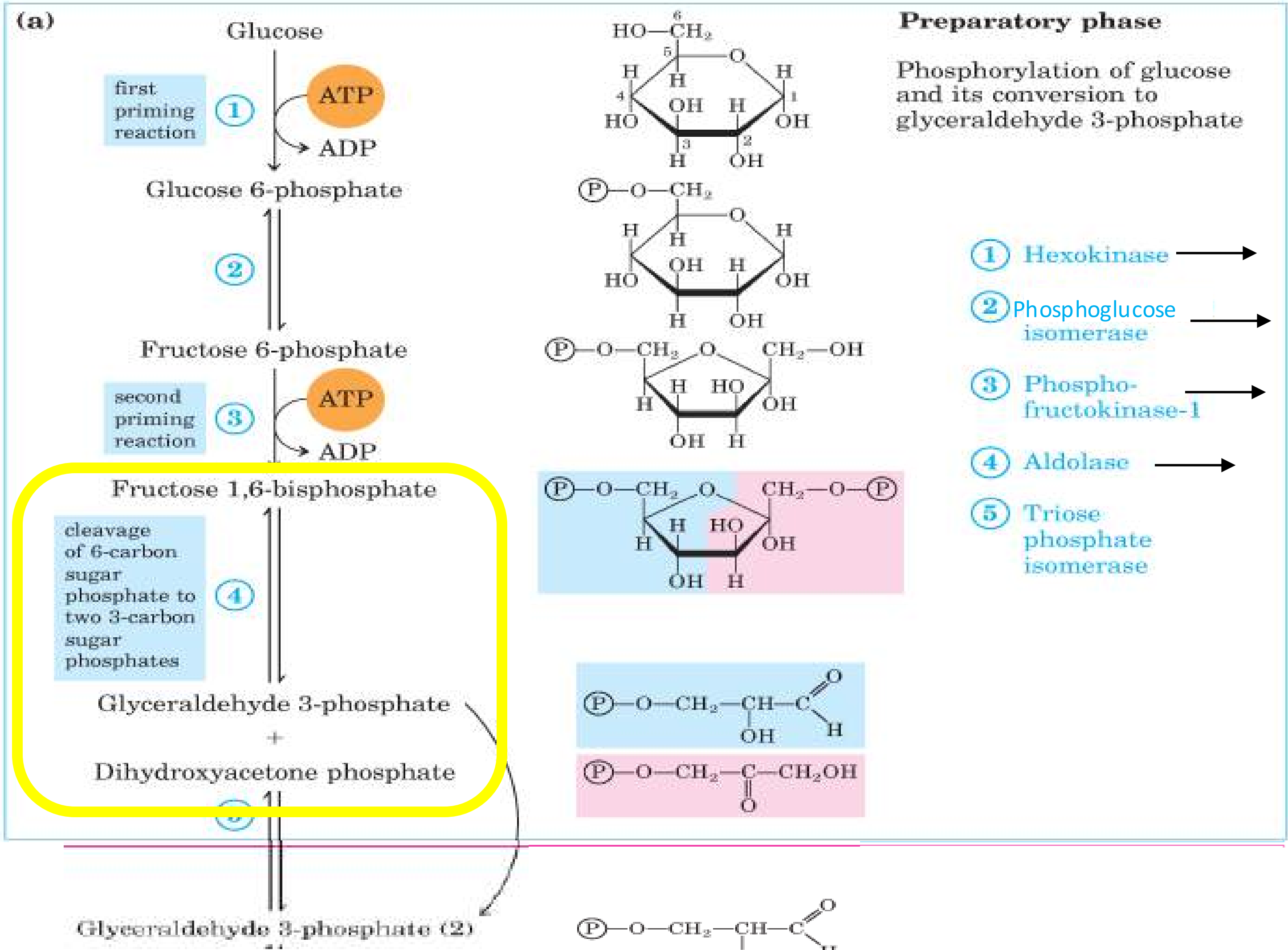




A. Preparatory Phase

- **Step 3:** This is the rate limiting or key regulatory step. The activity of phosphofructokinase-1 (PFK-1) enzyme can be controlled. PFK-1 catalyzes the phosphorylation of hydroxyl oxygen at C1 to produce **fructose-1,6-bisphosphate**
- **Step 4:** Aldolase enzyme catalyzes the cleavage to two triose phosphates: DHAP (dihydroxyacetone phosphate) and GAP (glyceraldehyde-3-phosphate)
- The addition of the second phosphate group on C1 from the previous step destabilizes the hexose ring and facilitates the cleavage reaction

A. Preparatory Phase



phosphorylation

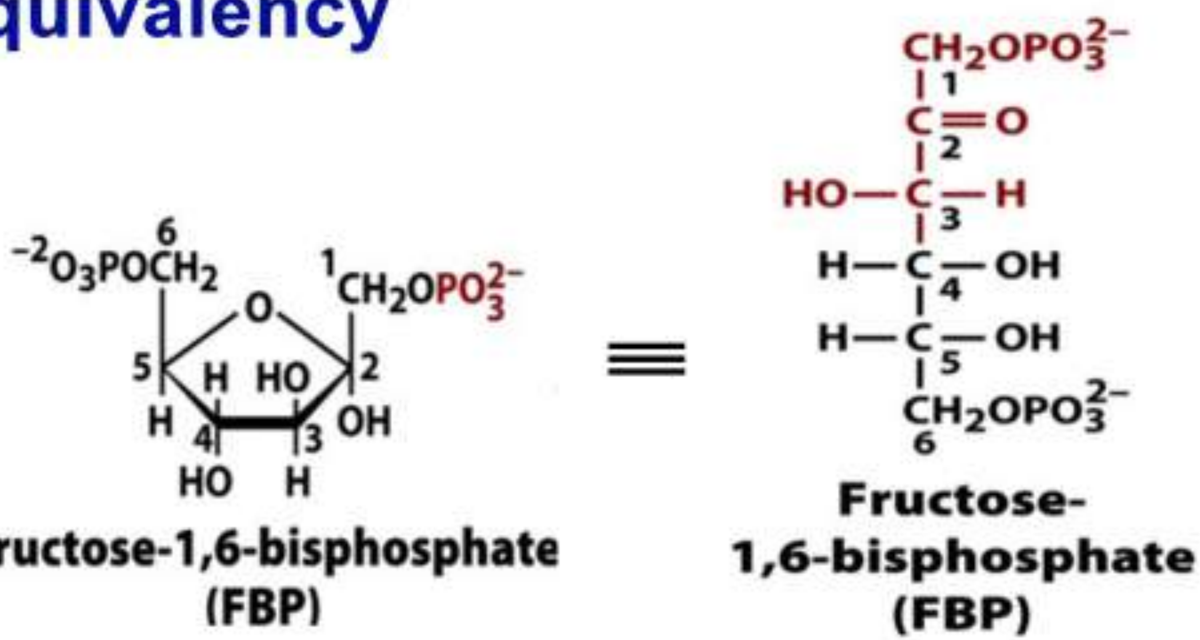
isomerization

phosphorylation

cleavage

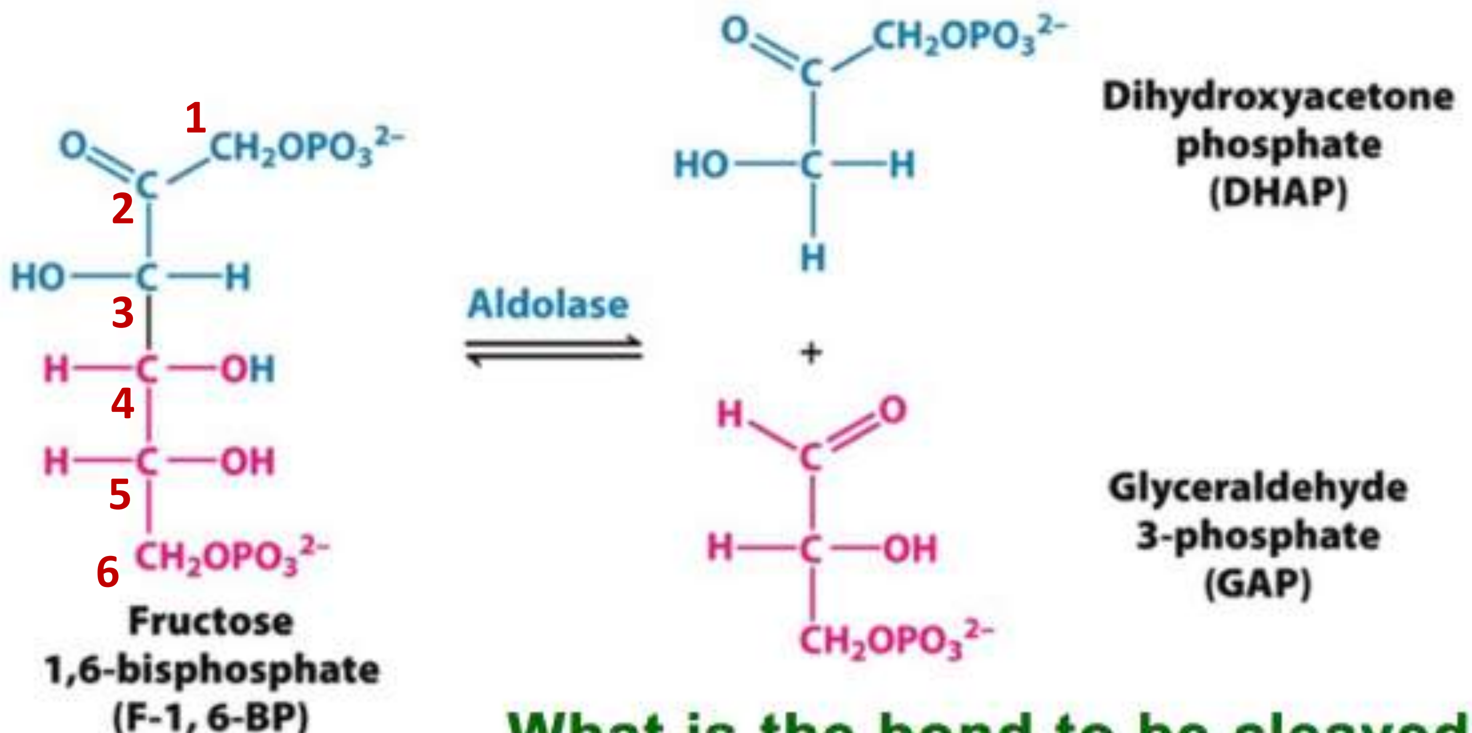
Aldolase Mechanism of Action

Haworth and Fischer Projections Equivalency



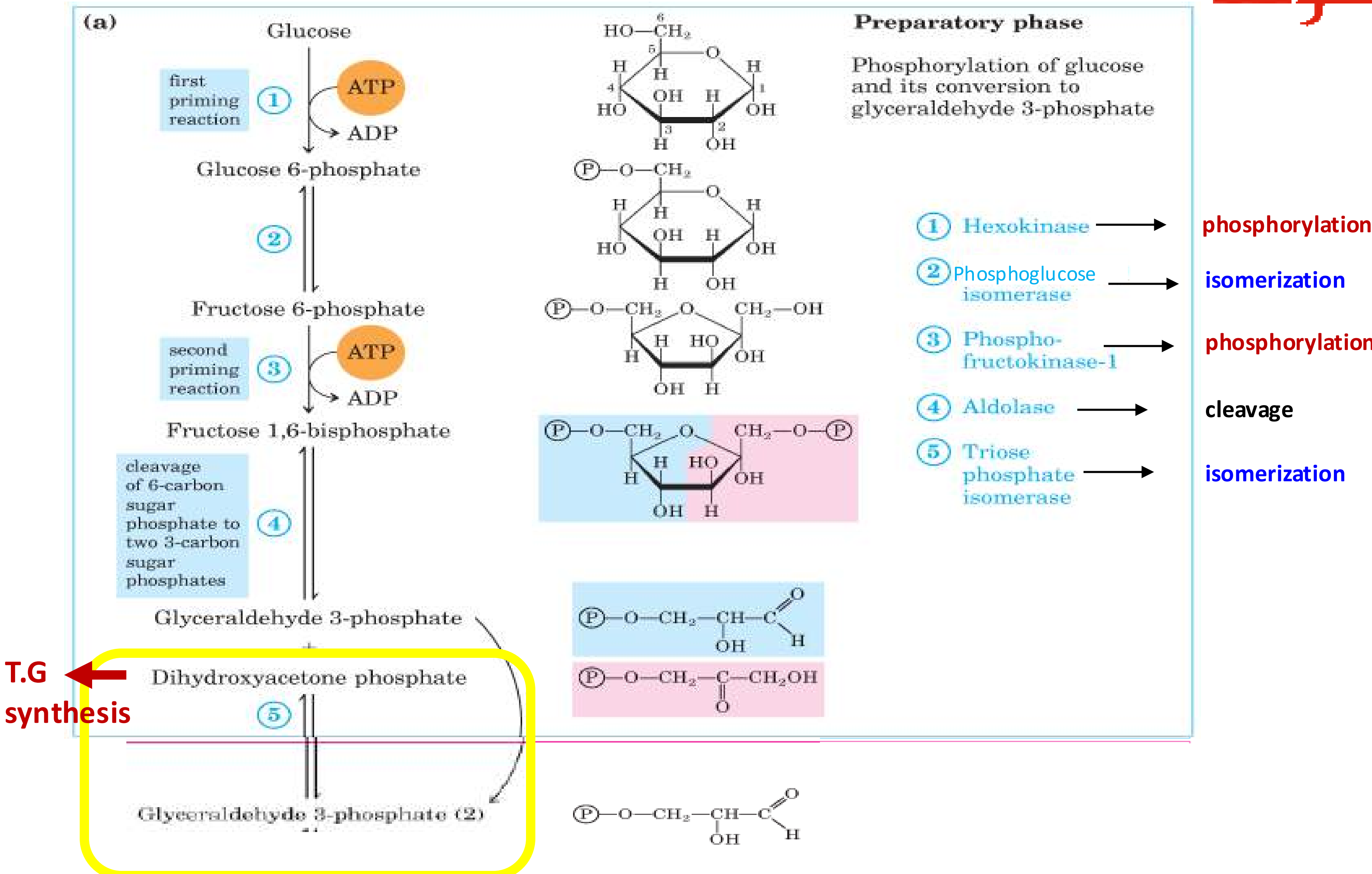
Aldolase Mechanism of Action

Six Carbon Sugar Cleaved to Two Three Carbon Units



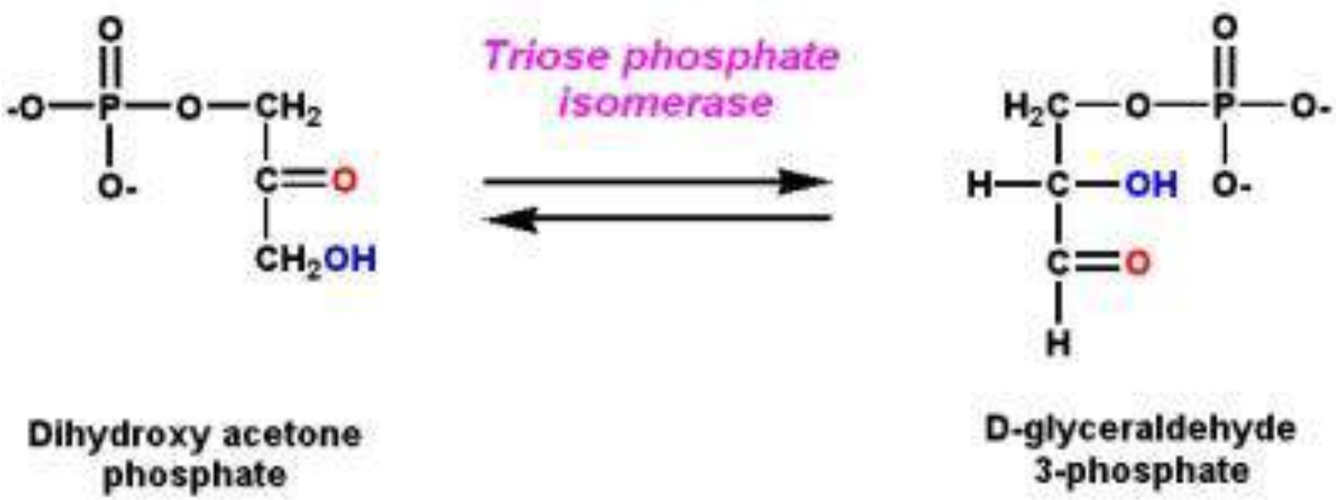
What is the bond to be cleaved?

A. Preparatory Phase



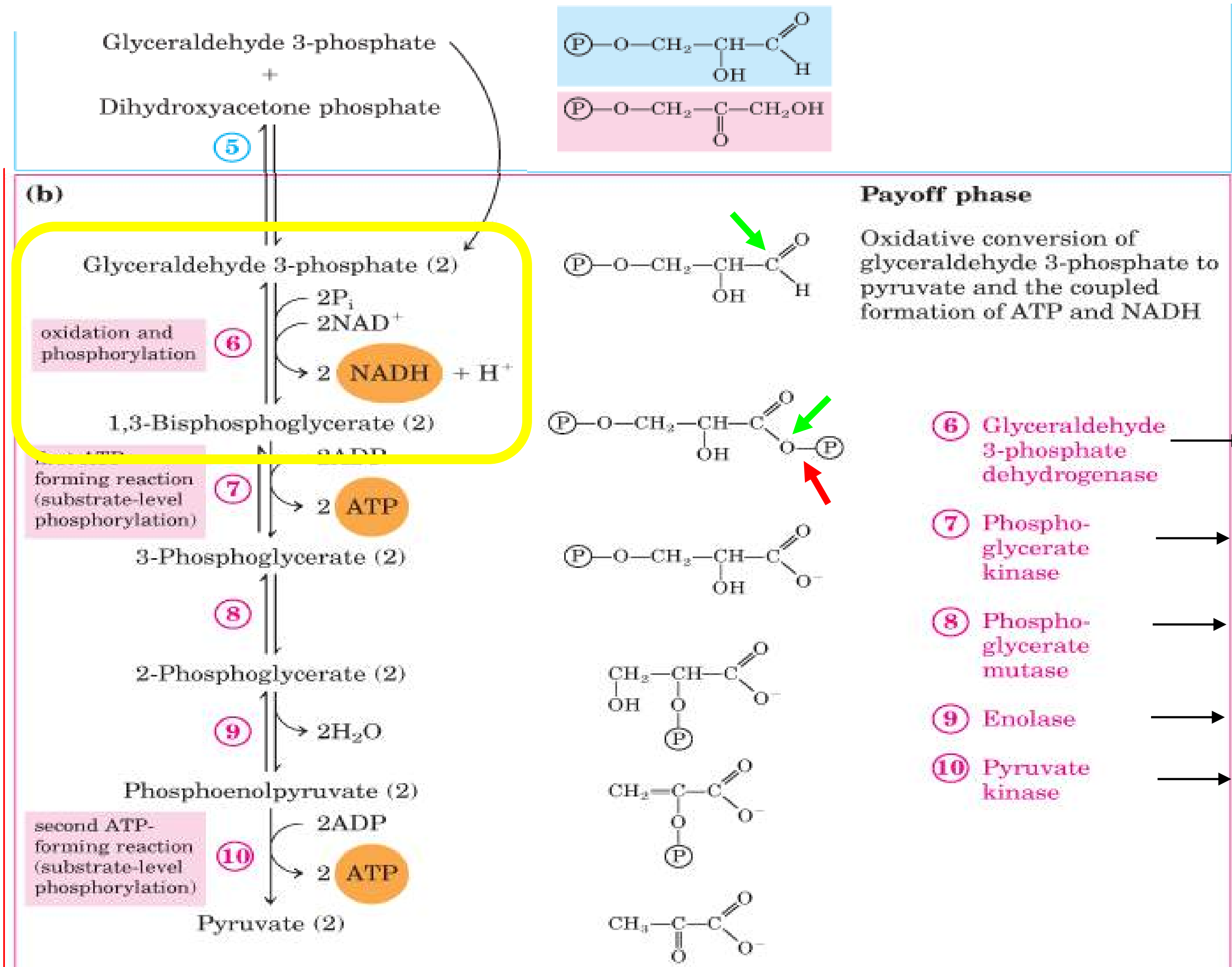
A. Preparatory Phase

- **Step 5:** Isomerization of **DHAP** by triose phosphate isomerase (TPI) to **GAP** to proceed further in glycolysis as GAP is the substrate for the next reaction. This reaction is reversible



DHAP **GAP**

B. Pay Off Phase



Oxidative phosphorylation

Substrate-level Phosphorylation

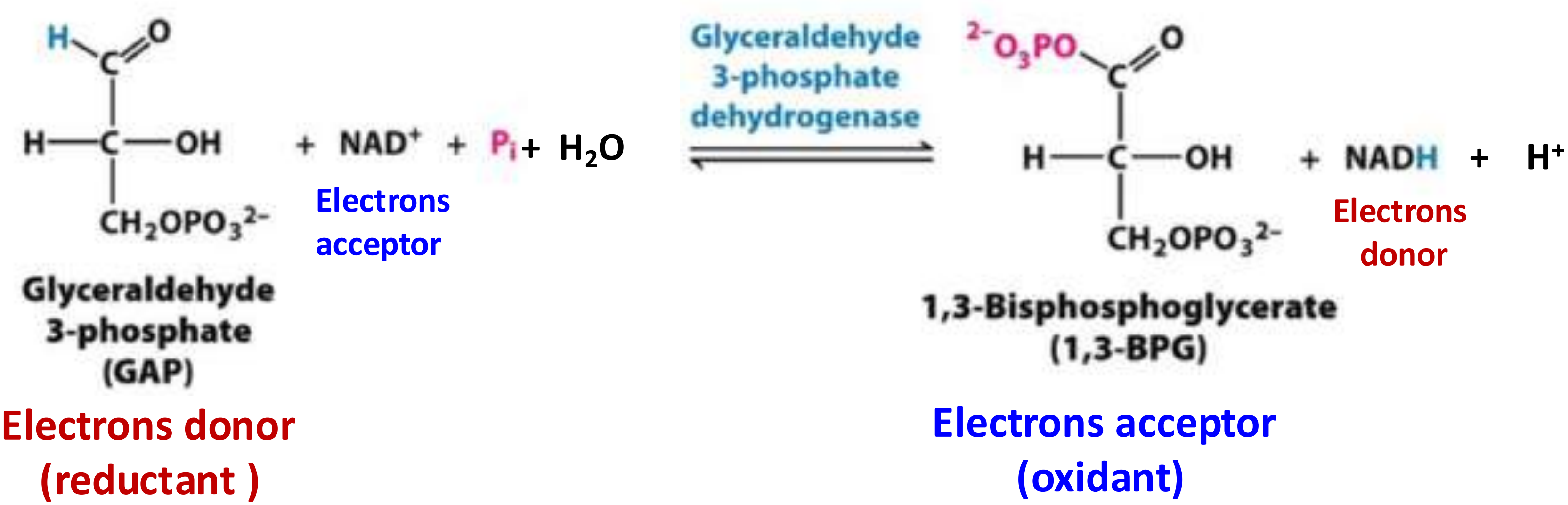
isomerization

Dehydration

Substrate-level Phosphorylation

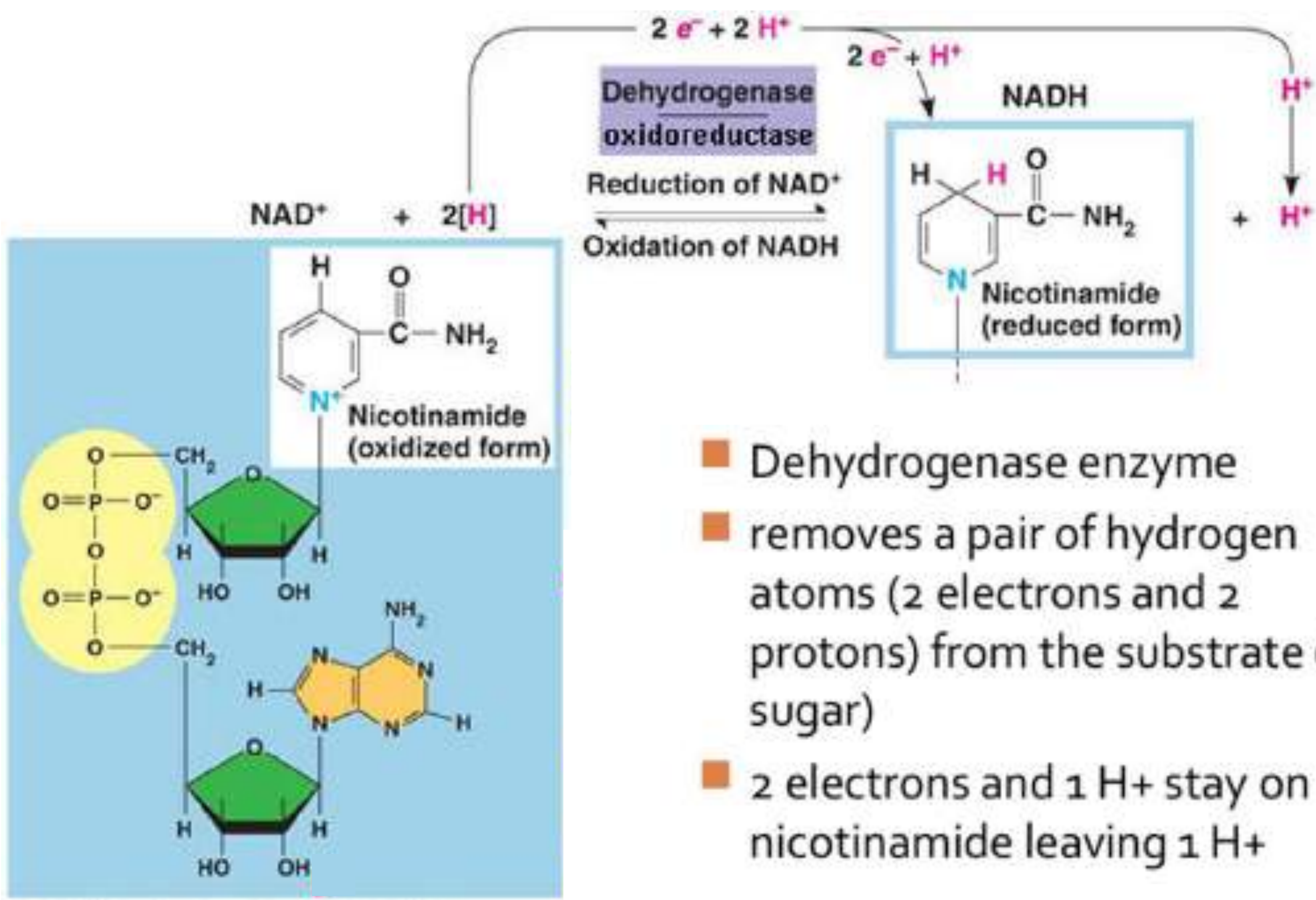
B. Pay Off Phase

- **Step 6**: GAP dehydrogenase enzyme catalyzes the oxidative phosphorylation of GAP (electron donor) into super-high-energy compound **(1,3-BPG)** and the transfer of electrons into the coenzyme NAD^+(electron acceptor) forming **NADH**
- Dehydrogenases are named as electrons donor substrate -dehydrogenase



Nicotinamide Adenine Dinucleotide

- **NAD (Nicotinamide adenine dinucleotide)** is a coenzyme which exists in two forms: NADH (the reduced form) and NAD^+ (the oxidized form)

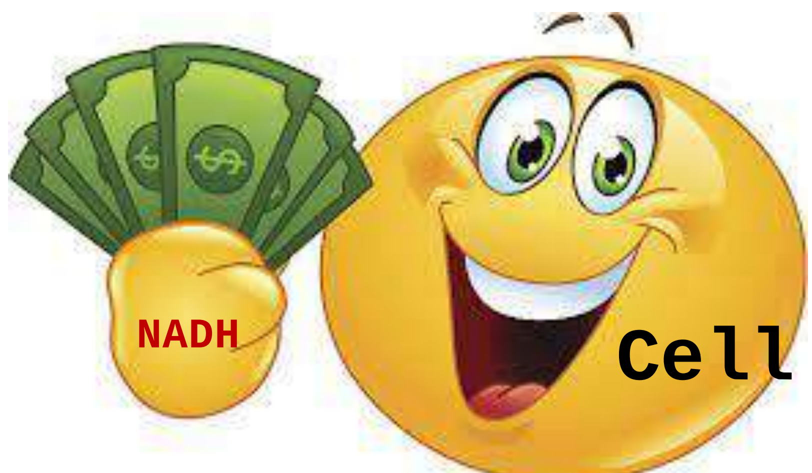


- Dehydrogenase enzyme
- removes a pair of hydrogen atoms (2 electrons and 2 protons) from the substrate (eg. sugar)
- 2 electrons and 1 H+ stay on nicotinamide leaving 1 H+

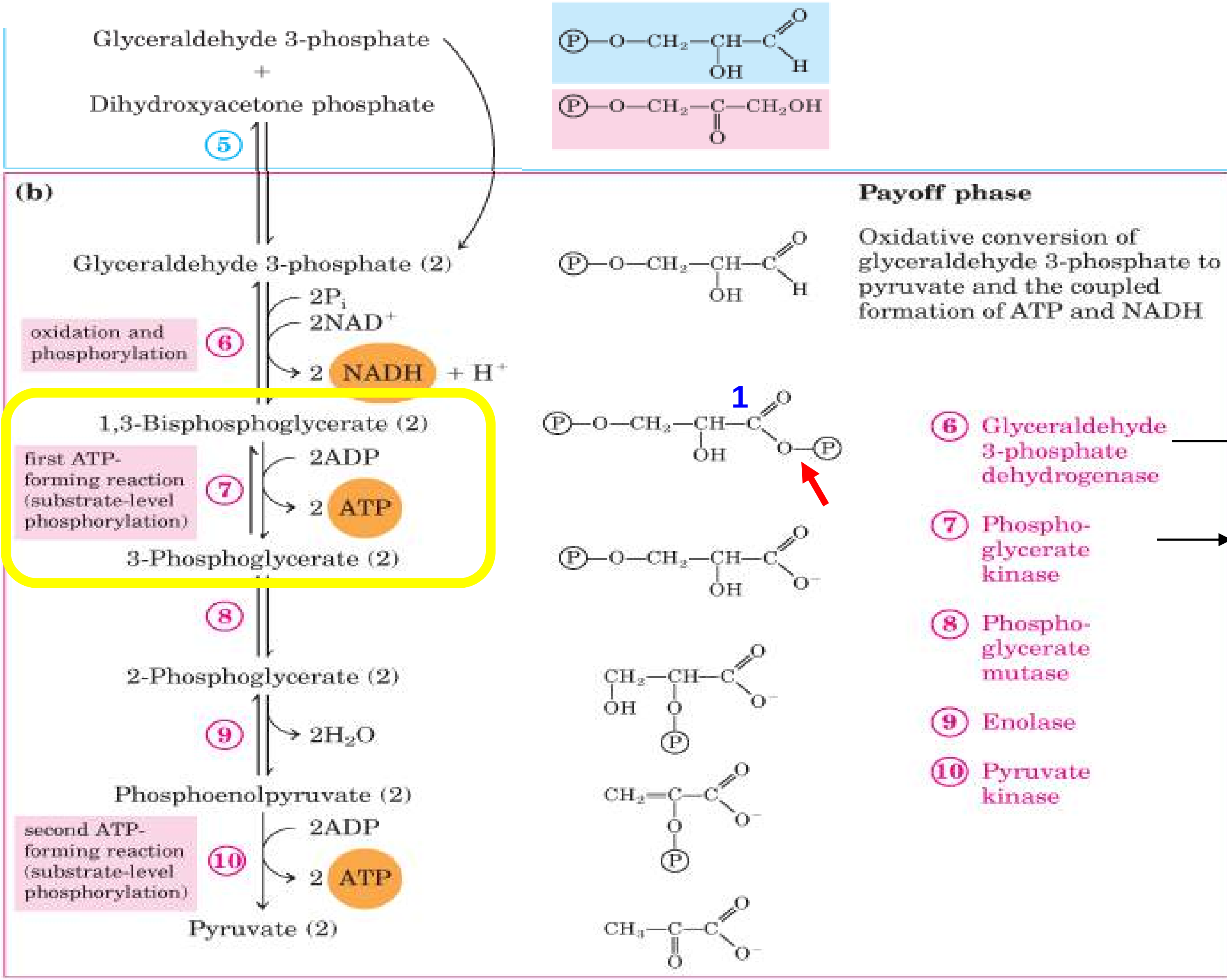
Nicotinamide Adenine Dinucleotide

- **NAD (Nicotinamide adenine dinucleotide)** is a coenzyme of dehydrogenases
- The reduced form NADH is electrons carrier and it is called **energy rich molecule**. It is an indirect form of energy

1 NADH = 2.5 ATP



B. Pay Off Phase



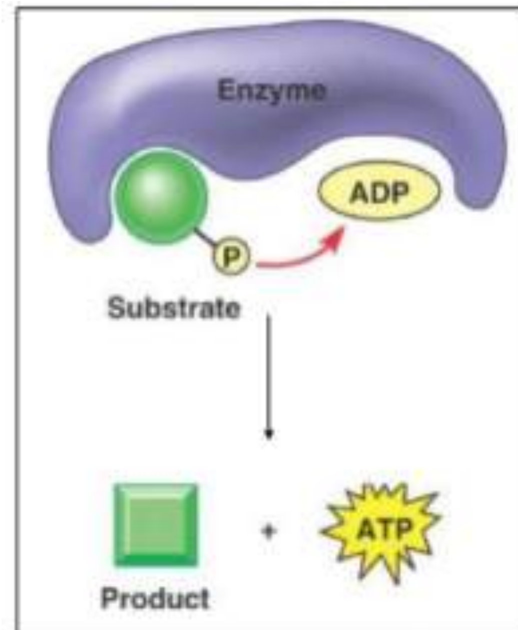
Oxidative phosphorylation

Substrate-level Phosphorylation

B. Pay Off Phase

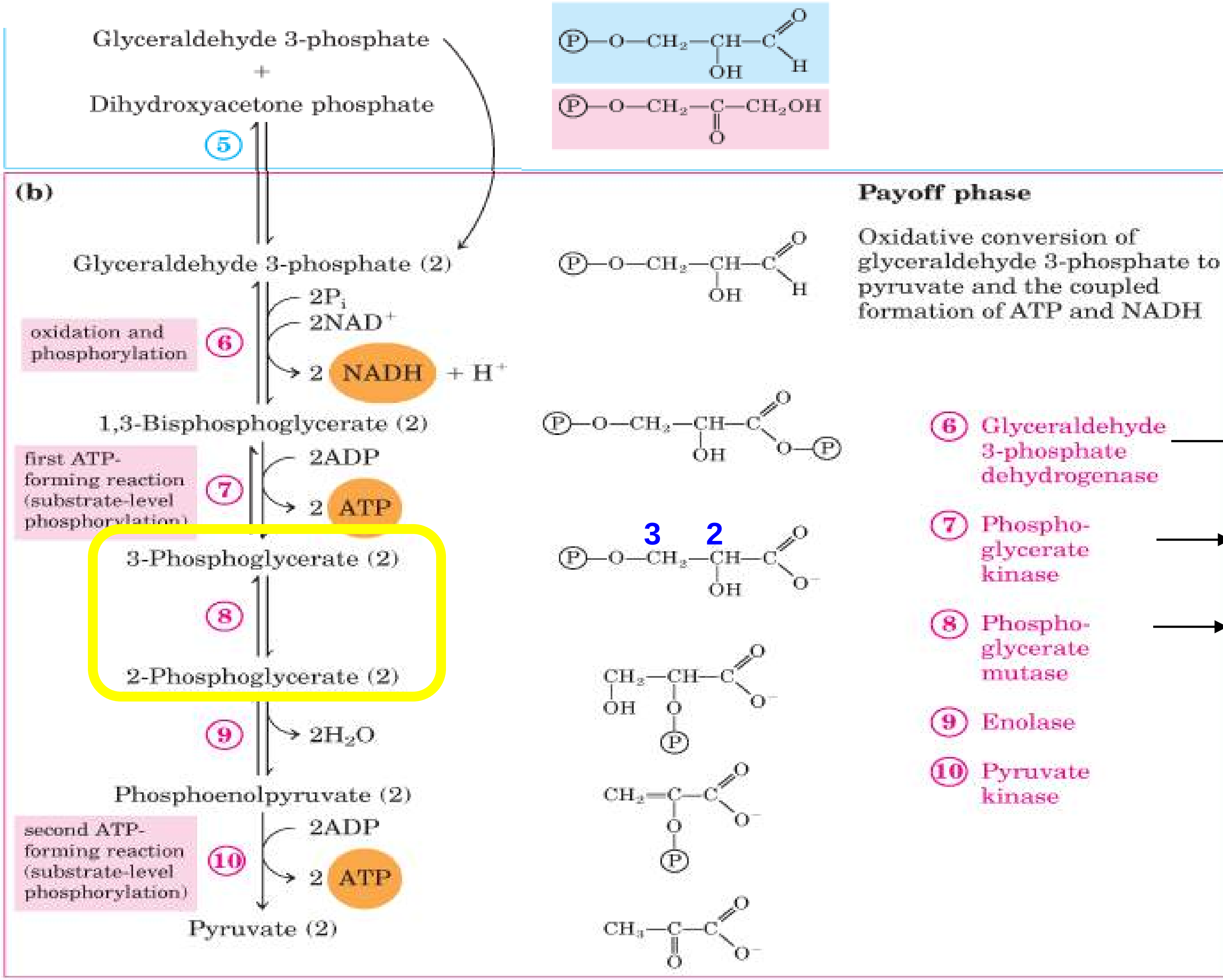
- **Step 7:** The **first ATP** molecule is generated by the substrate-level phosphorylation process catalyzed by phosphoglycerate kinase (PGK)
- 2 ATP molecules will be generated in this step
- Methods of ATP synthesis:

1. Substrate-level phosphorylation: it is a direct method of ATP synthesis by an enzyme which catalyzes the transfer of phosphate group from substrate to ADP
2. Oxidative phosphorylation: indirect method of ATP synthesis in which the oxidation of NADH/$FADH_2$ and the subsequently transferred electrons indirectly drive ATP synthesis from ADP



An enzyme transfers phosphate from substrate to ADP

B. Pay Off Phase



Oxidative phosphorylation

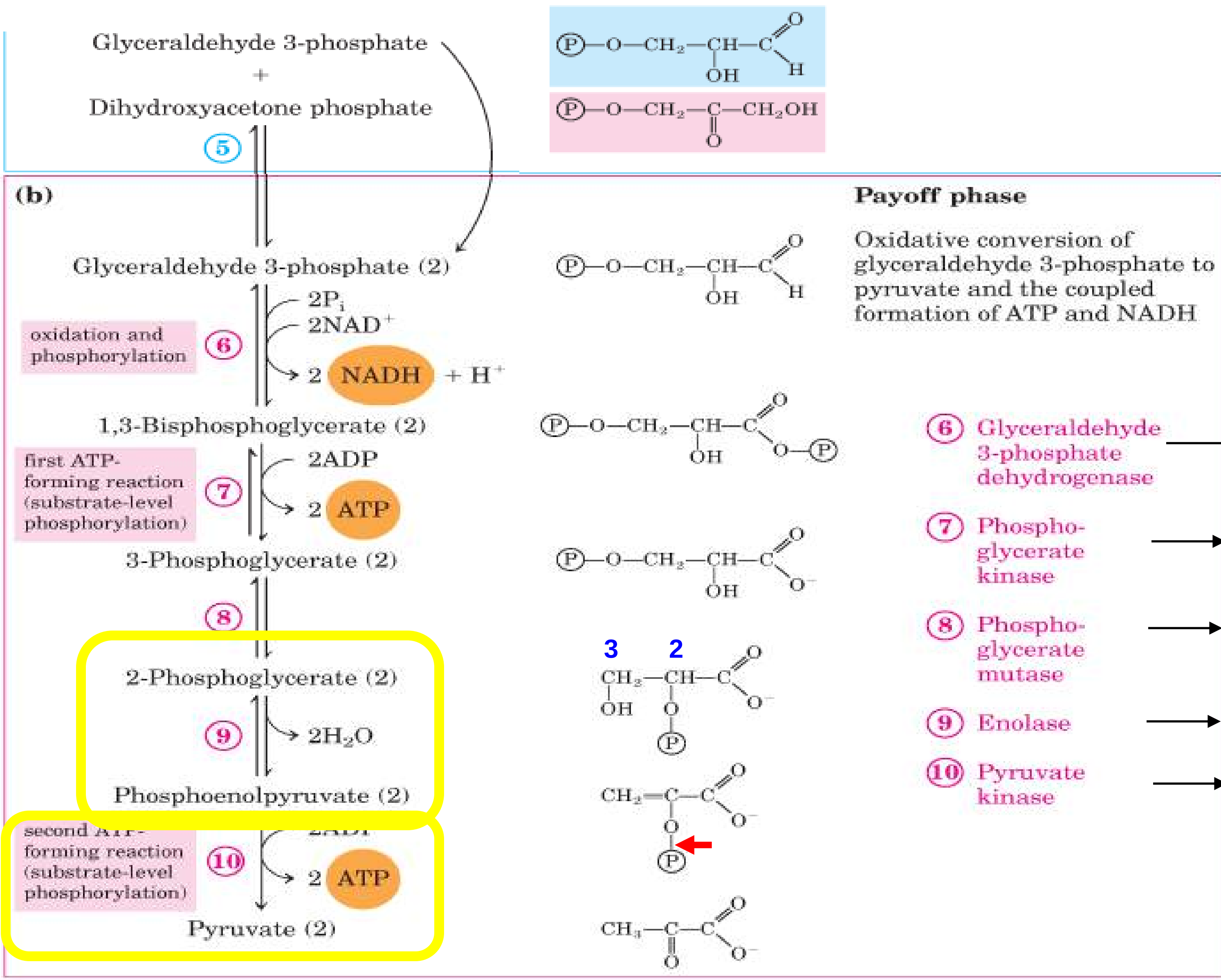
Substrate-level Phosphorylation

isomerization

B. Pay Off Phase

- **Step 8:** Phosphoglycerate mutase (PGM) is an isomerase which catalyzes the isomerization of 3-phosphoglycerate to 2-phosphoglycerate
- It is actually an **internal shifting of P** group from C3 to C2 within the same molecule
- The main purpose of this step is the activation of the phosphate group to prepare for the generation of the second ATP in the next reactions
- **Step 9:** The synthesis of the second super-high-energy compound **phosphoenolpyruvate (PEP)** in a simple dehydration reaction catalyzed by enolase enzyme
- Thus, phosphate group on C2 is locked into unfavored (unstable) enol configuration. The aim of this step is to increase the energy stored in the phosphate bond

B. Pay Off Phase



- Glyceraldehyde 3-phosphate dehydrogenase → Oxidative phosphorylation
- Phosphoglycerate kinase → **Substrate-level Phosphorylation**
- Phosphoglycerate mutase → isomerization
- Enolase → Dehydration
- Pyruvate kinase → **Substrate-level Phosphorylation**

B. Pay Off Phase

- **Step 10:** The **second ATP** molecule is generated by the substrate-level phosphorylation process catalyzed by pyruvate kinase (PK). Pyruvate is the final product of glycolysis
- The activity of pyruvate kinase can be controlled (irreversible reaction) so this reaction is regulatory step
- The net result of glycolysis is the formation of:

  2 pyruvate

  2 ATP

  2 NADH