فريق طوفان الأقصى

METABOLISM

Modifide N.

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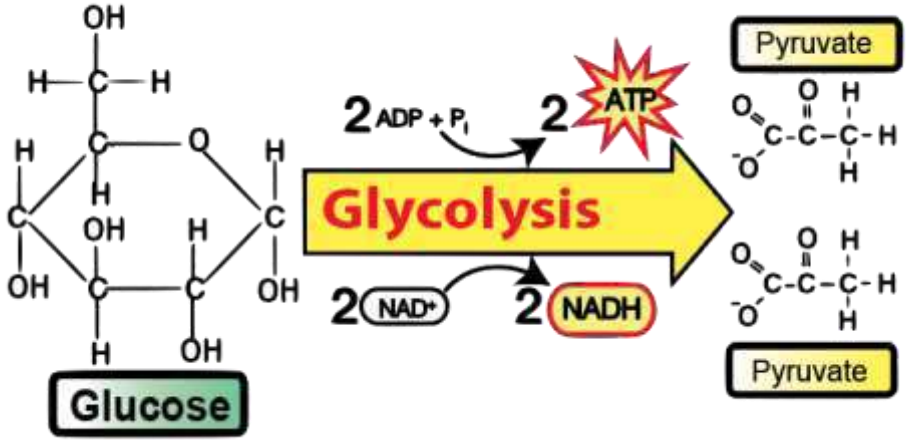
- **NOTE:** Glycolysis is the process of degradation of carbohydrates especially glucose to a smaller molecule (pyruvate) to get energy.
- Glycolysis is one of the carbohydrate metabolism pathways.It is composed of 10 steps (7 reversible steps, 3 Irreversible)



Glycolysis Reactions and Regulation

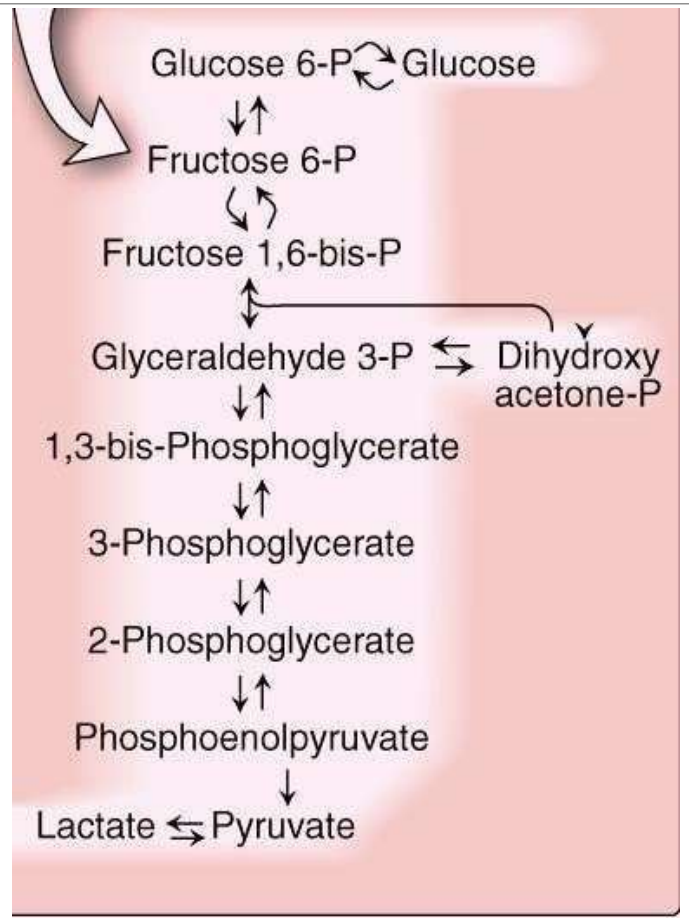
DR. DIALA ABU-HASSAN

SUGGESTED READING:

LIPPINCOTT'S ILLUSTRATED REVIEWS: BIOCHEMISTRY

sciencemusicvideos

Glycolysis is an example of metabolic pathway

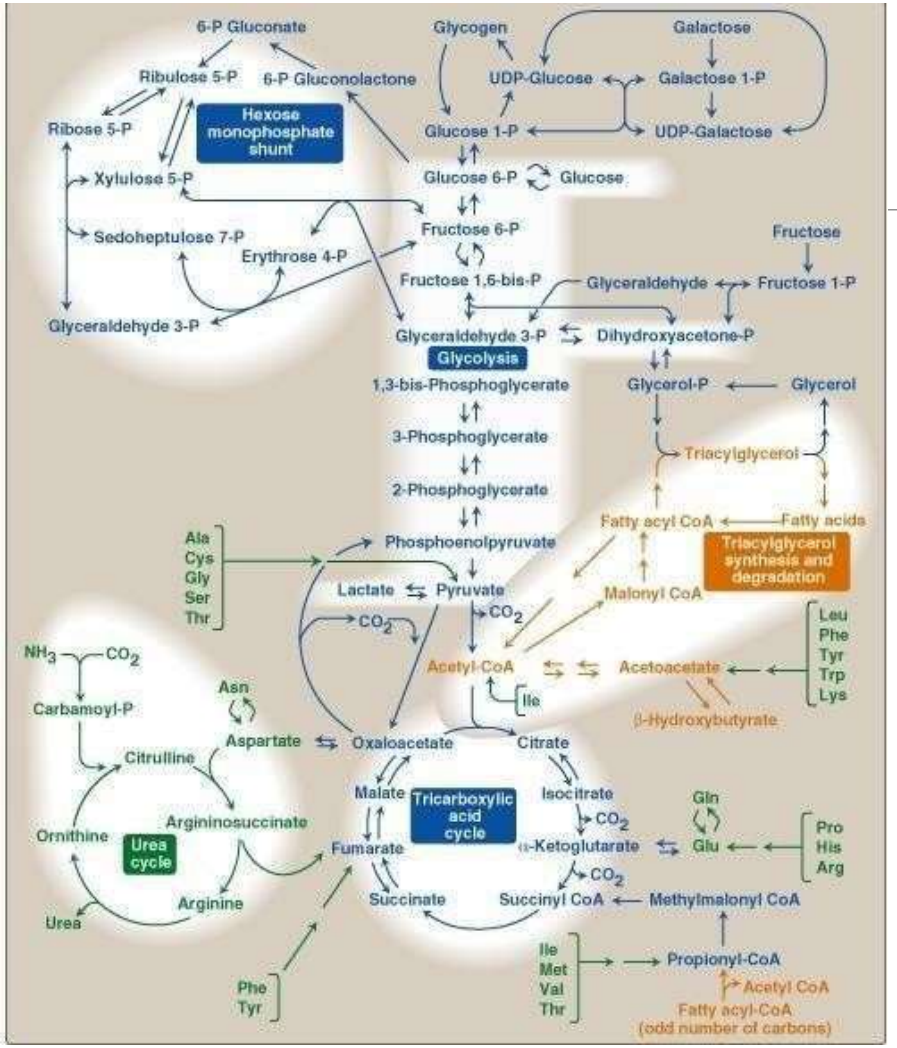


- **NOTE: Glycolysis is a general pathway (performed in so many cells).**
- **As long as you have sugar in your body , your body will use this sugar as a source of energy.**
- **Glycolysis is considered as a linear pathway (the product of the first step is going to be the reactant of the second step) ,unlike TCA cycle which is a cyclic pathway.**

The product of one reaction is the substrate of the next reaction

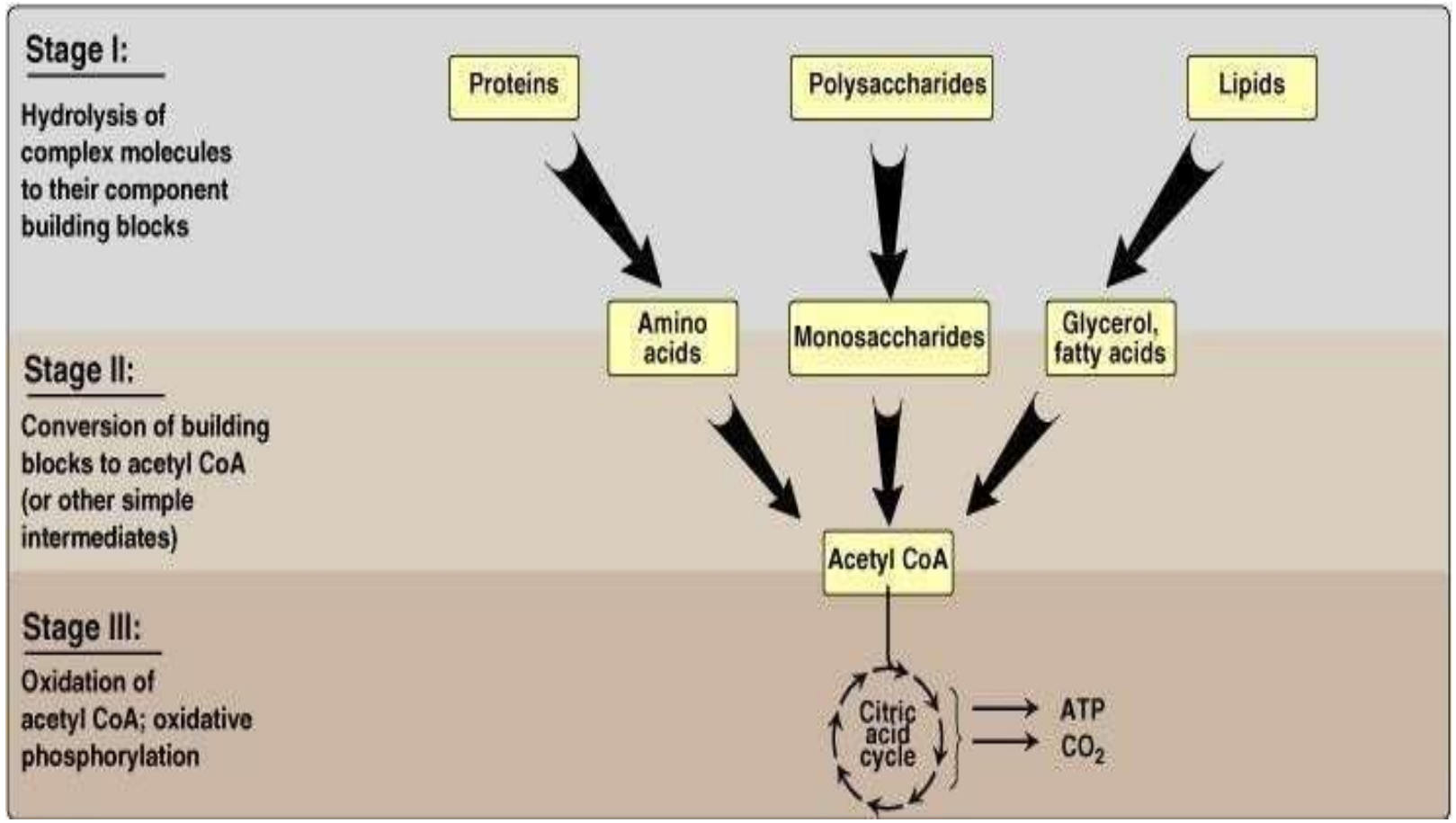
- **NOTE: there are many metabolic pathways in the human body, they always interact with each other in a cooperative way.**

Metabolic pathways intersect to form network of chemical reactions



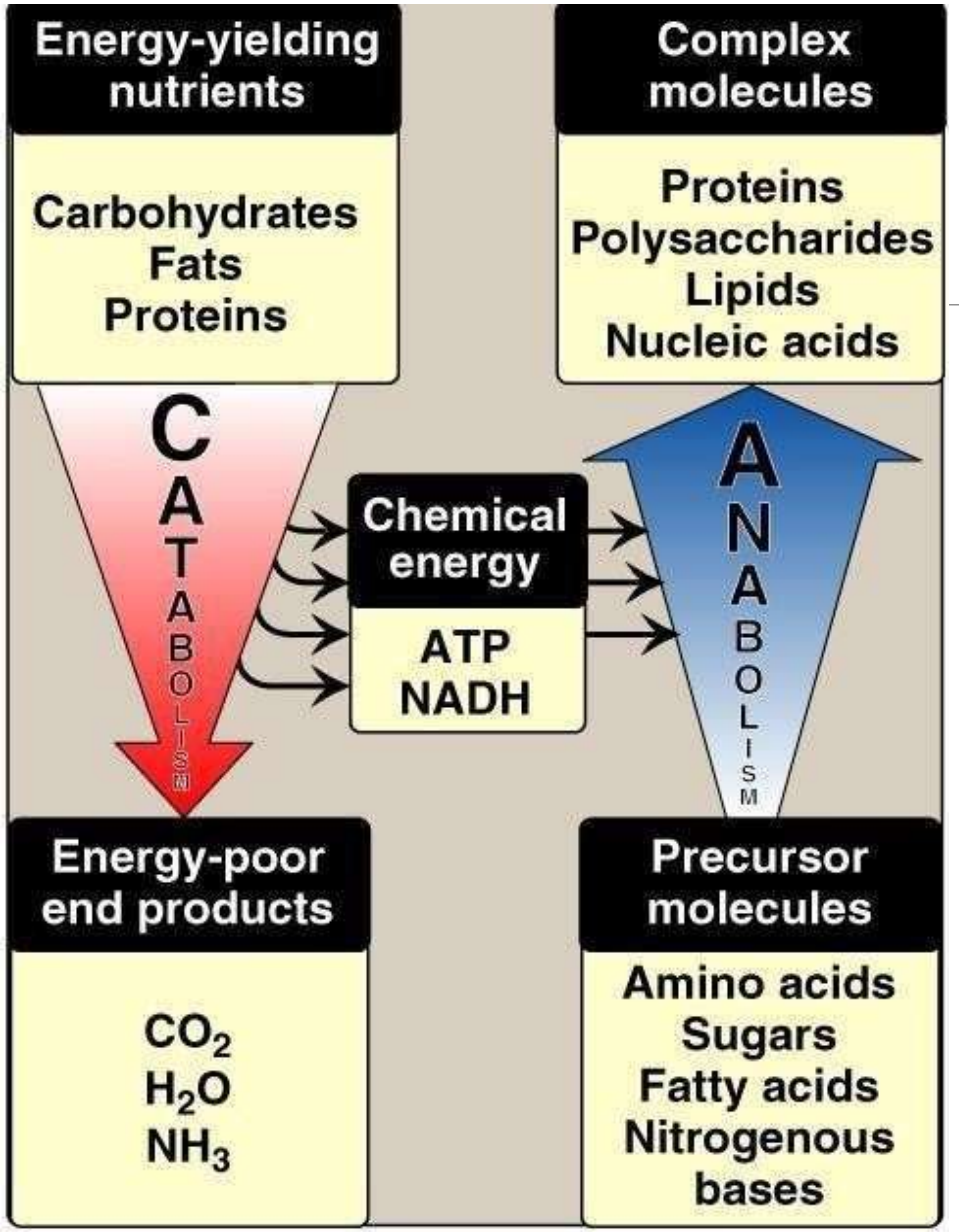
- **The complement in this slide:** This pathway (Glycolysis) is a complex metabolic pathway that is performed in our cells.
- **Examples as you see in the previous picture:** the beginning of glycolysis can be connected to glycogen metabolism or pentose phosphate pathway, it can also be connected to fructose and galactose metabolism, lipid (fatty acid) can interact with carbohydrates metabolism too.
- It is important to know that Krebs cycle **isn't only connected** to carbohydrates degradation, it involves other molecules such as fatty acids and amino acids.
- So, there **is a very complex network of pathways that interact with each other .**

General Stages of Metabolism



- **The complement in this slide:** Generally , In metabolism we start with a large molecule that gets simplified into a smaller one (polymers become monomers) and then get degraded even further to very small molecules that can supply the universal pathway of citric acid cycle (Krebs cycle).

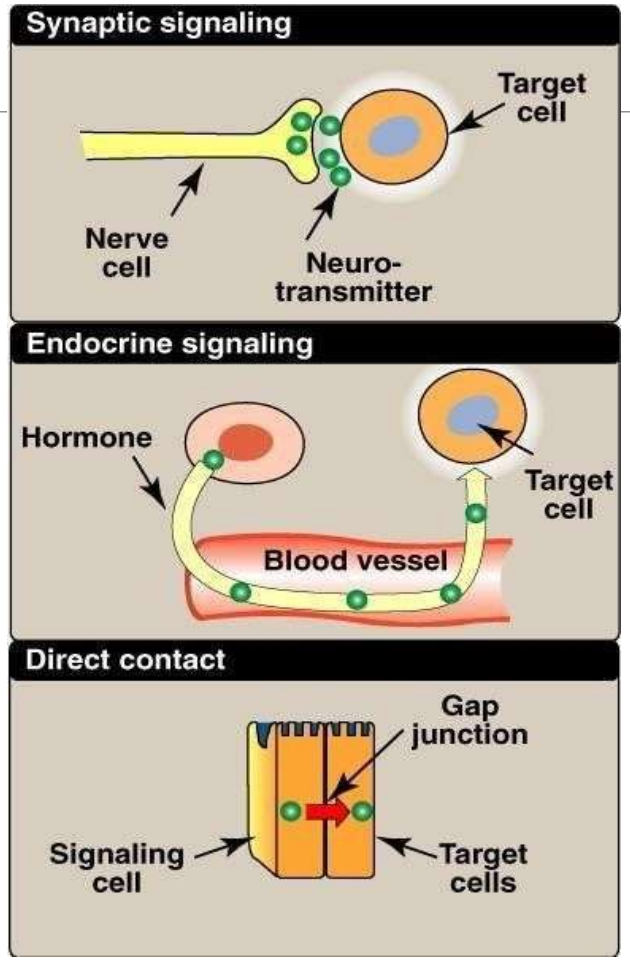
Types of Metabolic Pathways



- **NOTE:** Metabolism can be divided into two general pathwas: Anabolism (building complex molecules) and catabolism (breaking down complex molecules).
- **Catabolism** is the process of breaking down large molecules to smaller molecules that **can't be metabolised further** (in contrast of digestion) and they are energy producers.
- **Anabolism** start with precursor molecule or small molecules that can be used to build up large and sometimes complicated structures.
- Whenever we have high energy)well fed state(, we activate anabolic pathways.
- Catabolic pathways are activated whenever we need energy.

Regulation of Metabolism

- Signals from within the cell
  - Substrate availability, product inhibition, allosteric
  - Rapid response, moment to moment
- Communication between cells (intercellular)
  - Slower response, longer range integration
- Second messenger
  - Ca^{2+} / phosphatidylinositol system
  - Adenylcyclase system



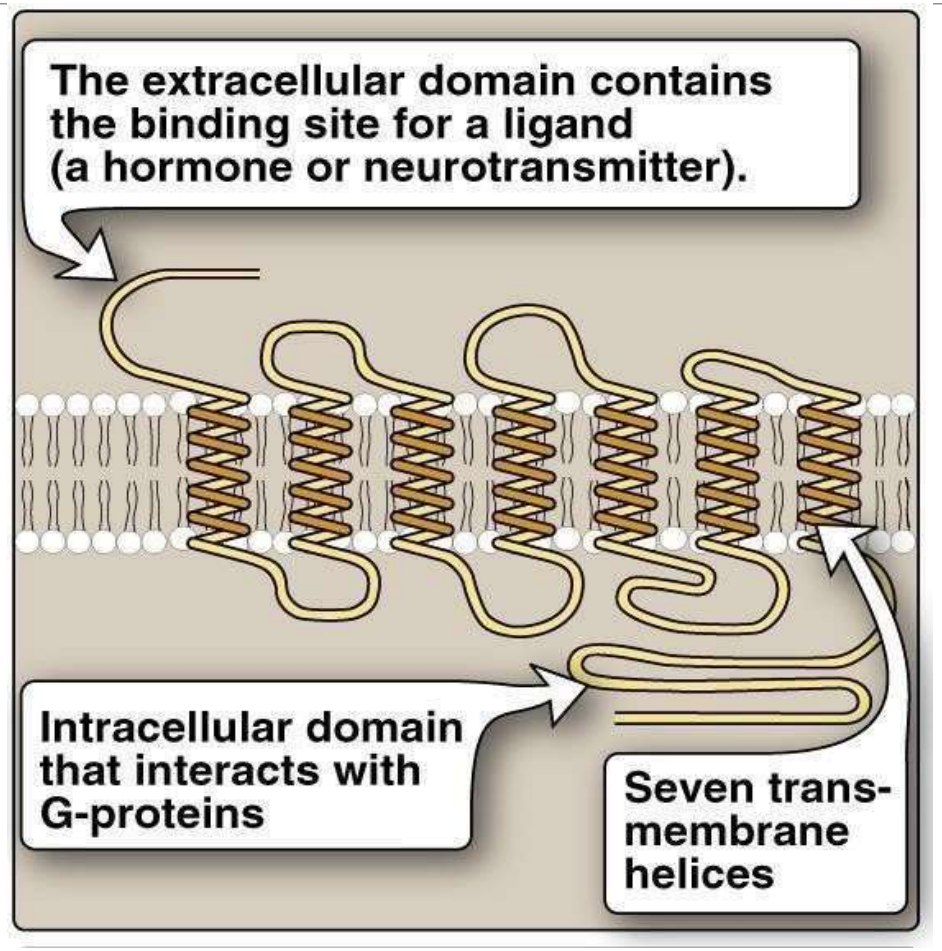
Commonly used mechanisms of communication between cells

- **The complement in this slide:** Regulation of metabolism can occur by three ways
- The first way is **signalling from within the cells:** by regulation of **substrate availability,** or by **allosteric regulators** whether they are inhibitors or stimulators , and regulation of the **feedback inhibition** loops (product of a certain reaction inhibits the substrate that forms it) and this is a **very rabid way** because the regulation is coming from the cell itself (no need for hormone or something else from other cells).
- The second way is **communication between cells (intercellular)** by **signalling** (releasing compounds from one cell and binding on receptors of the target cell to induce certain response).
- Signaling doesn't produce a single response , the signal will be amplified leading to a diversity of responses.

- **The complement in this slide:Communication between cells (signaling) can be divided into three type: synaptic,endocrine and direct contact.**
- **Synaptic signaling** considered as a paracrine effect (calls are close to each other).
- **Endocrine signaling** (cells are far away from each other) they can be affected by each other. hormones are released from endocrine gland and released into the blood stream and reach the target where it can induce it's effect. It takes time**(long range)** so it's a **slow response** way to regulate metabolism.
- **Direct contact** (especially in epithelial cells) which have a Gap junction that transports molecules between cells, but that way is limited to small molecules since the gap is small (usually less than 1KD).
- The third way is through **second messenger** (It is the first molecule that will dissociate (separate) from the membrane receptors going into the cell to induce certain effect such as cAMP, Ca+2 and others .

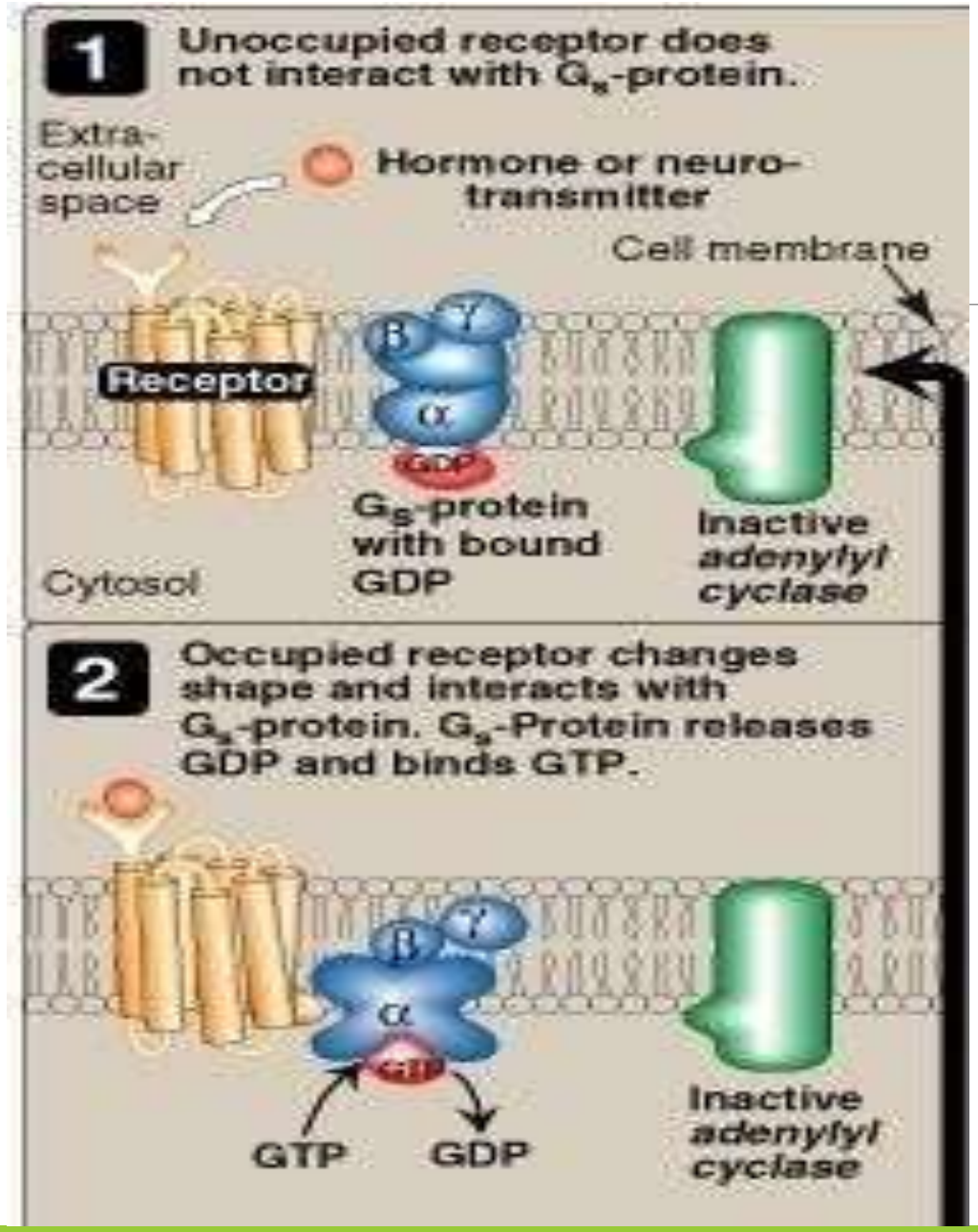
Communication between Cells through Receptors- GPCR

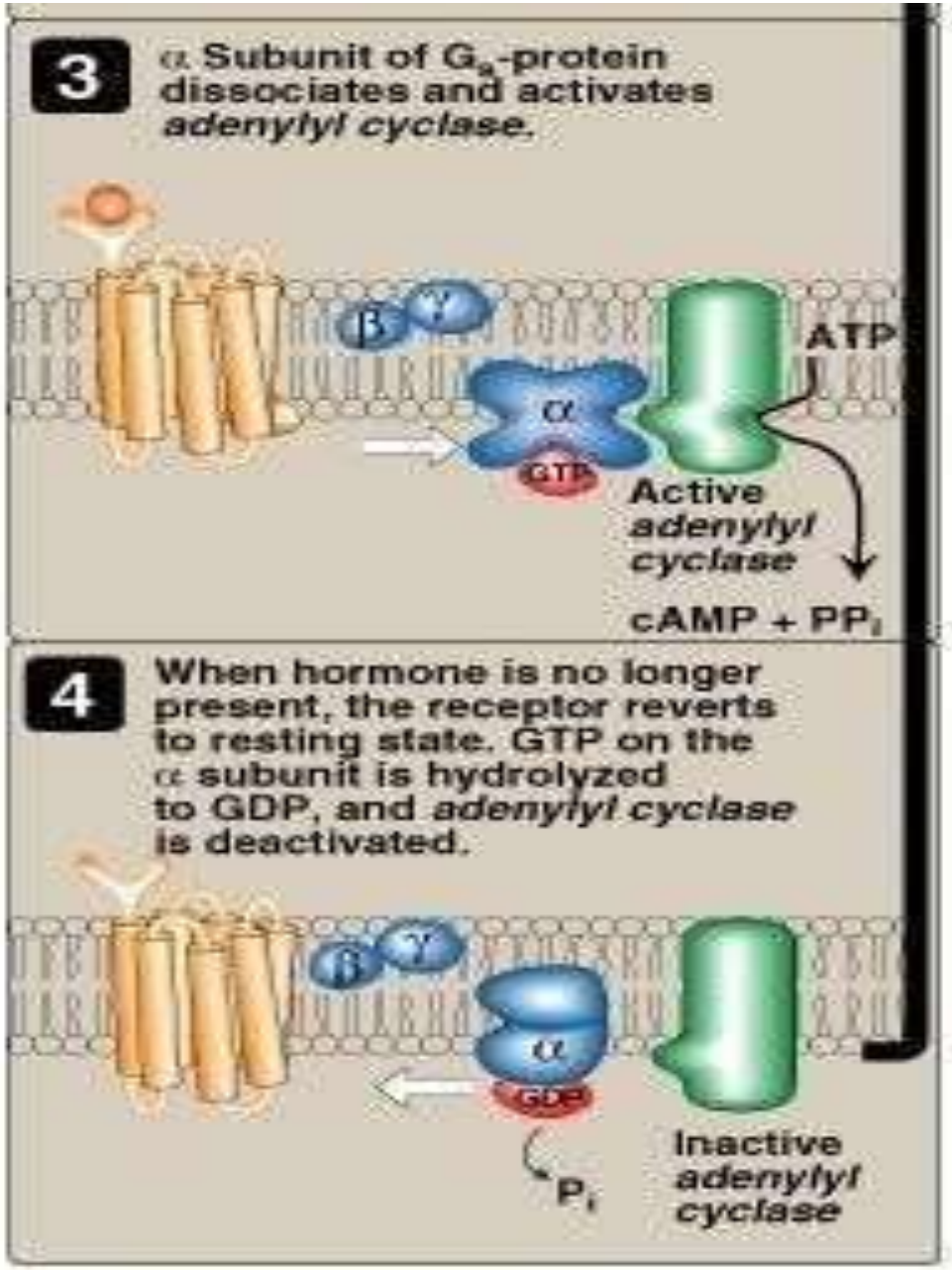
G protein-coupled receptor of plasma membrane



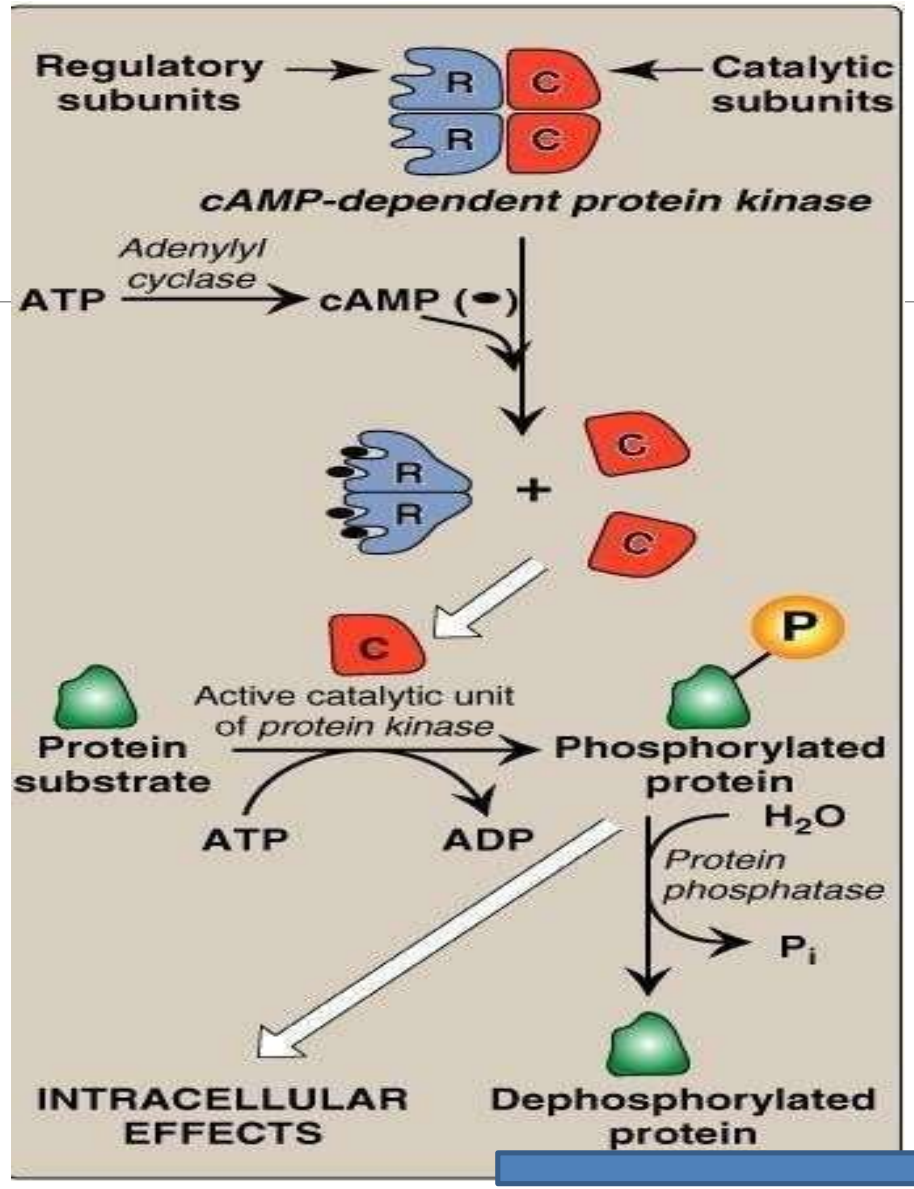
- **NOTE:** GPCR is the most common membrane receptor

- **NOTE:** GPCR have differences in ligand binding domain so it a can bind different ligands examples(hormones ,neurotransmitters)….etc.


1
Unoccupied receptor does not interact with G_s-protein.
Extra-cellular space
Hormone or neuro-transmitter
Cell membrane
Receptor
β
γ
α
GDP
G_s-protein with bound GDP
Inactive adenylyl cyclase
Cytosol
2
Occupied receptor changes shape and interacts with G_s-protein. G_s-Protein releases GDP and binds GTP.
GTP
GDP
Inactive adenylyl cyclase


3
α Subunit of G_s-protein dissociates and activates adenylyl cyclase.
ATP
GTP
Active adenylyl cyclase
cAMP + PP_i
4
When hormone is no longer present, the receptor reverts to resting state. GTP on the α subunit is hydrolyzed to GDP, and adenylyl cyclase is deactivated.
GDP
P_i
Inactive adenylyl cyclase

- **The complement in this slide:** Once ligand binds to GPCR , GPCR can change its conformation to an active conformation and binds to G protein (alpha , beta , gamma subunits attach to each other in G protein)…once it binds to G protein ,GDP is exchanged to GTP, Alpha subunit is activated and thus changes its conformation, separating away from (beta/gamma) subunit…..the active alpha subunit will bind to adenylyl Cyclase and activate it….once it becomes activated, it starts the formation of the second messenger (cAMP).
- Once the ligand is no longer present (dissociated from the receptor)…we must stop the whole signaling pathway by inactivating the alpha subunit and that occurs by hydrolysis of GTP to GDP.



INTRACELLULAR EFEECTS

- ✓ Activated enzymes
- ✓ Inhibited Enzymes
- ✓ Cell's ion channels
- ✓ Bind to promoter

- **The complement in this slide:** cAMP binds to PK(protein kinase A)…it's called (A) because it binds to c(A)MP.
- cAMP act as a regulator for PKA
- Once cAMP bind to PKA , it can induce conformational changes in the regulatory subunits allowing the dissociation of the catalytic subunits and thus become active.
- The active catalytic subunits of PKA can act on certain substrates and phosphorylate them, either activating or inactivating them depending on the enzyme phosphorylated….result in certain response .
- We can stop the action (response) of that substrate by de-phosphorylating it .
- The importance of this covalent modification (phosphorylation) is to:
- **1)** Turn on or off in opposite direction for opposite pathway (it's not efficient to degrade molecule X and synthesizing it in the same time , so we must turn on or turn off either)
- **2)** activation of gene expression
- **And other beneficial result as in the slides**

GLYCOLYSIS

✓ **Breakdown of glucose to pyruvate Pathway characteristics**

- **Universal Pathway: In all cell types**
- **Generation of ATP**
- **With or without O_2**
- **Anabolic Pathway:**

  → **biosynthetic precursors**

- **The complement in this slide:**

-Glycolysis is the breakdown of glucose into 2 molecules of pyruvate.

-composistion: glucose has 6 carbons, pyruvate has 3 carbons.

يعني قسمنا الجلوكوز من النص

-Based on this, you'll see how the reactions are designed to create out of the glucose molecule a degradeable molecule that can be cleaved into almost 2 identical molecules.

يعني راح نعدل على الجلوكوز لحد ما نوصله لشكل قابل للانقسام إلى جزئيتين متماثلين تقريبًا

The complement in this slide:

-After that, we would have two molecules (3C,3C) that we are going to extract energy from. Note that in the first steps of glycolysis, we didn’t extract energy since all we are doing is rearrangement.

- Also, glycolysis can occur in **ALL** conditions (it doesn’t have to be aerobic). On the other hand, processes like **Krebs cycle** and **oxidative phosphorylation** require oxygen.

- glycolysis is a catabolic pathway for glucose, but it's an anabolic one for see other molecules. It can generate some precursors for the synthesis of other molecules.

The complement in this slide:

It's splitted into two phases;

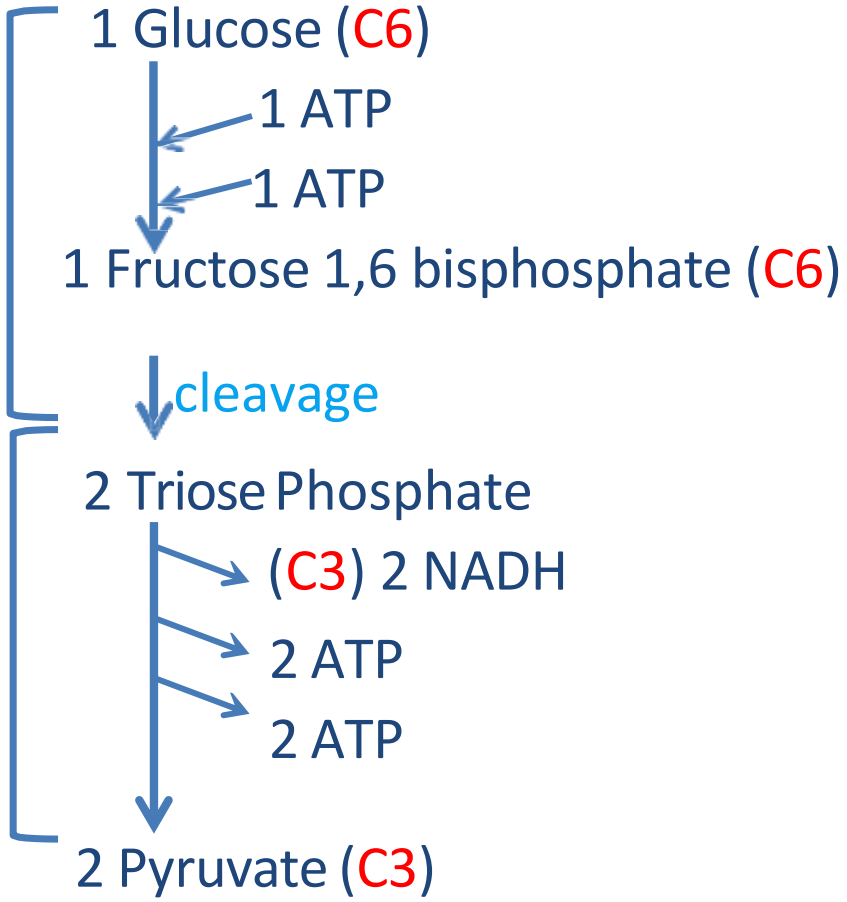
1 "preperative phase" is on which we are changing the shape of glucose molecule to become prepared to be cleaved into two identical molecules

2 the second phase is the glucose reactions in which we start to generate pyruvate (3C), once we get a 3C molecule we start to modify it to extract energy. BUT **we don't lose carbons**.

The Two Phases of the glycolytic Pathway

1- Preparative Phase

2- ATP-generating Phase



NOTE: we use ATP in the 1st phase

- **NOTE:** in the 2nd phase, the reducing equivalent is NADH

NOTE: overall, this process produces energy.

Types of Glycolytic Reactions

- Phosphoryl transfer
- Isomerization
- Cleavage
- Oxidation reduction
- Phosphoryl shift
- Dehydration

NOTE:

Eg on cleavage enzymes: lyases.

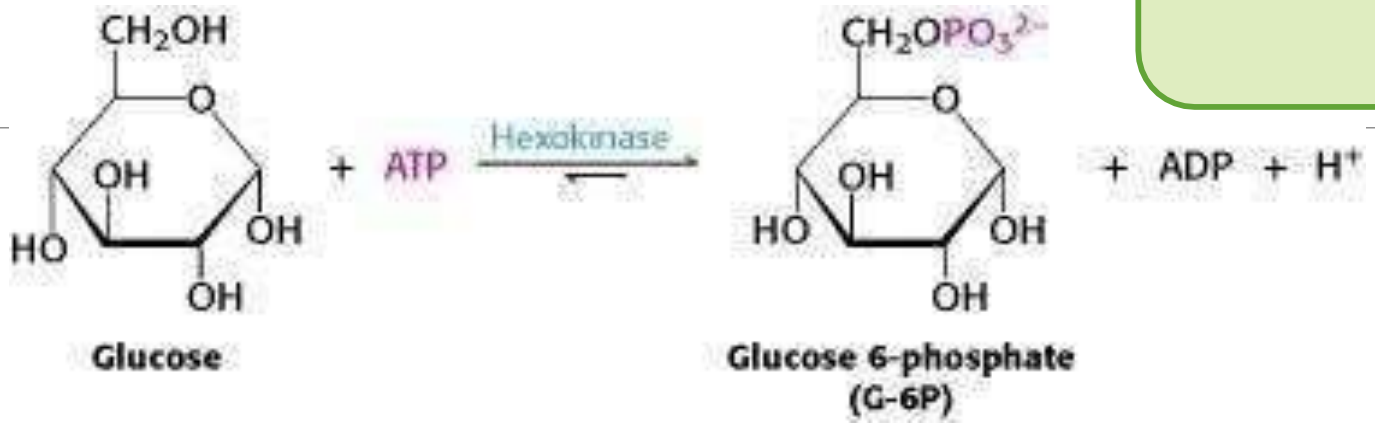
Steps of Glycolysis

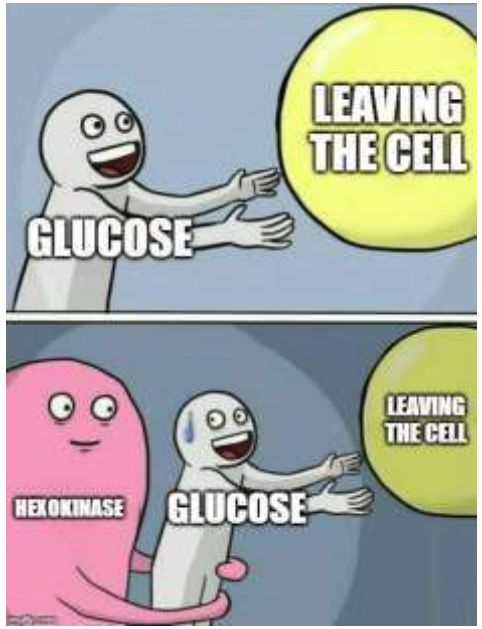
Very simple!!

عليهم... خذلك نفس ويلا

Step 1

NOTE: no need to memorize Km values





	Hexokinase	Glucokinase
Occurrence	In all tissues	In liver
Km	< 0.02 mM	10-20 mM
Specificity	Gal Man, Fruc,Glc.,	Glc.
induction	Not induced	↑ insulin, Glc
Function	At any glucose level	Only > 100 mg/dl

The complement in this slide:

- We mentioned in the previous lecture that GLUTs are bidirectional. they will bind to glucose and they would spontaneously get it out of the cell (since the glucose conc. outside < inside) BUT this doesn't happen because we trap them in the cell by phosphorylation, **we use ATP**.
- Phosphorylation occurs in C-6, producing **Glucose-6-phosphate.** this happens by one of two enzymes:

a) Hexokinase: can phohosphorylate different hexoses (glucose, fructose, galactose, mannose, etc) not just glucose. So its non-specific.
b) Glucokinase.

- G6P can't exit the cell, because the transporter is specific for glucose. Additionally, the P-group is big and negatively charged, which makes it uneasy to get out

The complement in this slide:

- G6P doesn’t necessarily go into glycolysis, it could go to other pathways.
  ولكنها من ضمن خطوات ال glycolysis, لذلك تعتبر هاي الخطوة خطوة مَوزعة
  some go to glycolysis, some go to be used in glycogen synthesis, some go to pentose phosphate pathway, etc.
- Hexokinase works as soon as we have glucose, no need for a very high conc. It's active all the time, it has a **wide tissue distribution**, more generalized that glucokinase.
- So it has low Km since its active all the time (high affinity)

The complement in this slide:

- Whereas glucokinase is limited in tissue expression, not expressed in all tissues (major expression site for glucokinase: **liver**).
- It gets activated whenever we have a VERY high conc. of glucose. While hexokinase is already working, glucokinase supports it in specific tissues like the liver. Especially after absorption, the sugars are directed to the liver in cases of big amounts, since it's not going to be fully used, it'll get stored. And this step is shared with other factories and that’s why phosphorylation in hepatocytes is important.
- Higher Km, so lower affinity than hexokinase.
- **Specific to glucose**, can only bind to it and phosphorylate it.
- It indicates that there's a high conc of insulin. So, it's activated by insulin.

The complement in this slide:

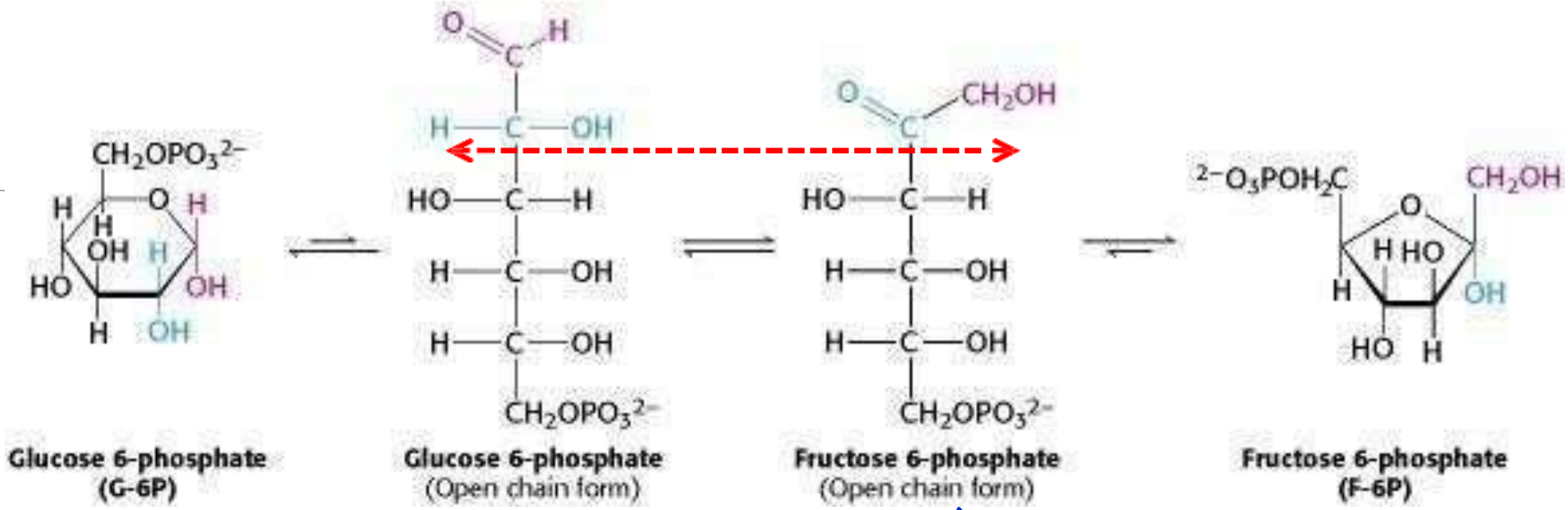
In metabolism there are two types of steps

*Reversible: can be reversed using the same enzyme.

*Irreversible: can be reversed by a different enzyme.

- The previous rxn is an irreversible step
- It's not a committed step because this product (G6P) may go into glycolysis, but may go to other pathways. So there's no guarantee that this molecule will go into the production of pyruvate (glycolysis).

Step 2


Glucose 6-phosphate
(G-6P)
Glucose 6-phosphate
(Open chain form)
Fructose 6-phosphate
(Open chain form)
Fructose 6-phosphate
(F-6P)
Phosphoglucose
Isomerase

The complement in this slide:

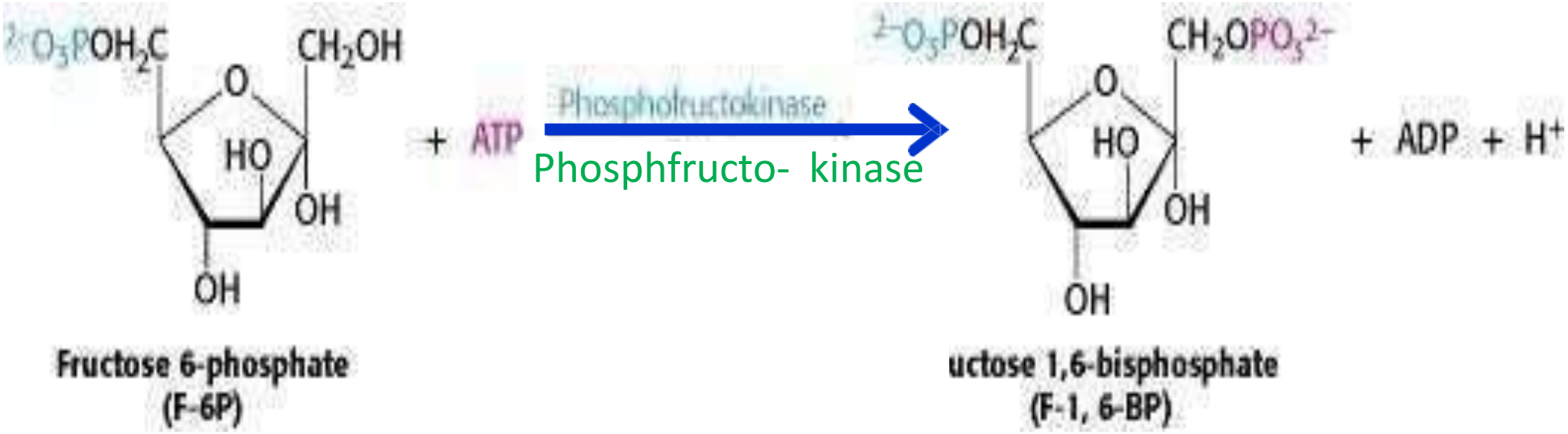
- Remember that the purpose of the 1st phase is to cleave glucose into 2 almost identical molecules
- So if you look at glucose or G6P,can it be cleavable into 2 almost identical molecules? NO!! Because of the phosphate group and the external C-6.

عشان هيك بدنا فعليًّا نطلع كربونة لبرا ونحط عليها Phosphate

That's why this step is going to be isomerization.
(Aldose--->ketose)
In other words: (glucose-6-p ---> fructose-6-p) using a phosphoglucose isomerase

- This is a **reversible step** (F6P--->G6P by the same enzyme).
- In order for the isomerization rxn to happen, we must open the chain. rearrange the electrons, transfer carbonyl to C-2 instead of C-1 and Finally, formation of the ring structure.

Step 3


$^{2-}O_3POH_2C$
CH_2OH
O
HO
OH
OH
+ ATP
Phospholructokinase
Phosphfructo- kinase
$^{2-}O_3POH_2C$
$CH_2OPO_3^{2-}$
O
HO
OH
OH
+ ADP + H^+
Fructose 6-phosphate
(F-6P)
uctose 1,6-bisphosphate
(F-1, 6-BP)

The complement in this slide:

- After formation of F6P we need to add another phosphate to get to the cleavable shape.

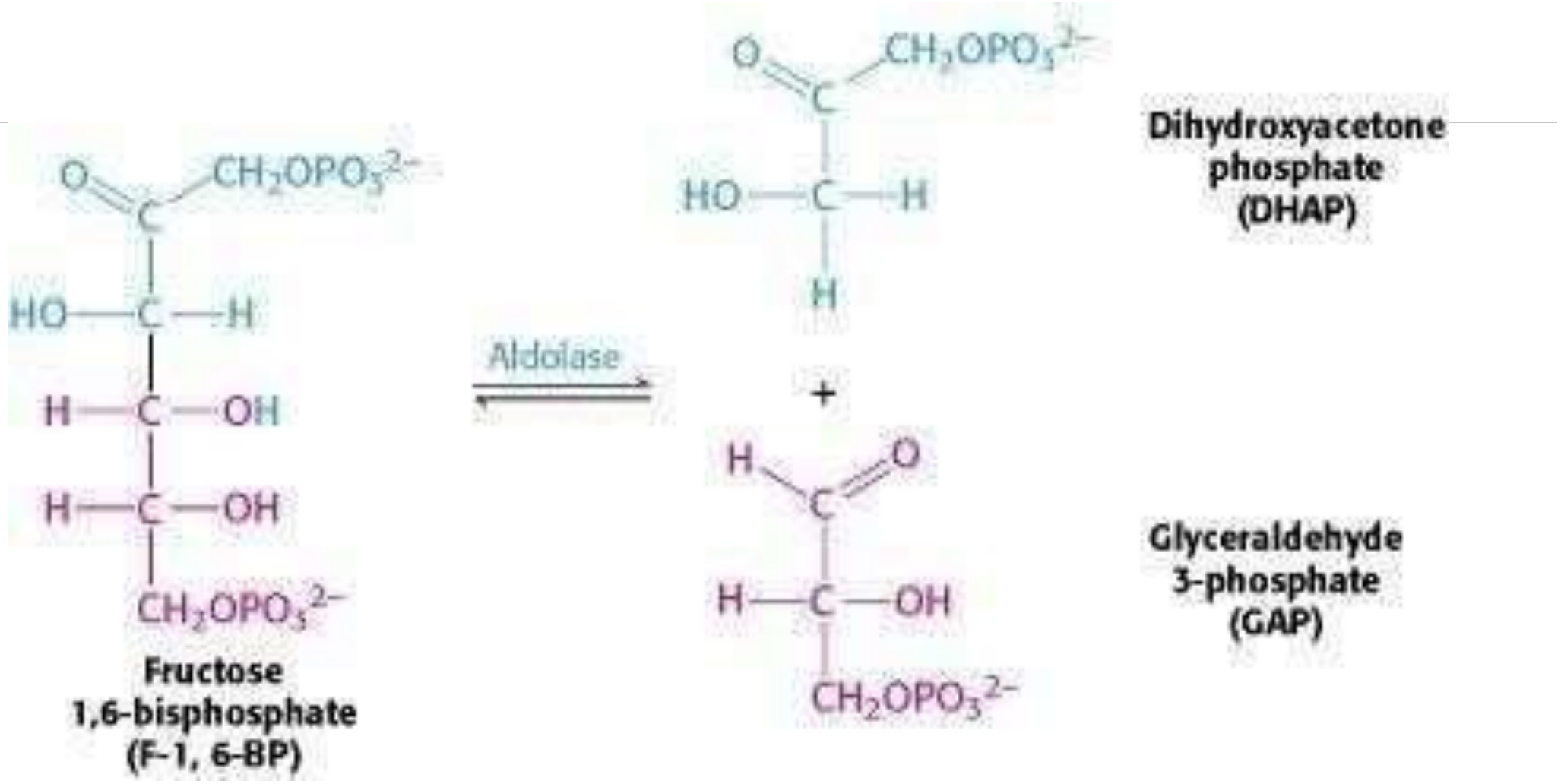
So F6P is phosphorylated into Fructose-1,6-bisphosphate

- We used an ATP molecule to get the 2nd P
- Enzyme: phosphofructokinase no.1 (no.2 has a different function).
- Till now, we used 2 ATP molecules; in the 1st step and in the 3rd step.
- Now the molecule/product is ready for cleavage.

(if we draw a line, the molecule will be almost symmetrical)

- In this case committed=rate-determining step which is step #3 (the slowest) and if it gets done successfully, glycolysis will last/continue. But if it doesn't, F6P might follow another pathway.

Step 4


Fructose
1,6-bisphosphate
(F-1, 6-BP)
Aldolase
+
Dihydroxyacetone
phosphate
(DHAP)
Glyceraldehyde
3-phosphate
(GAP)

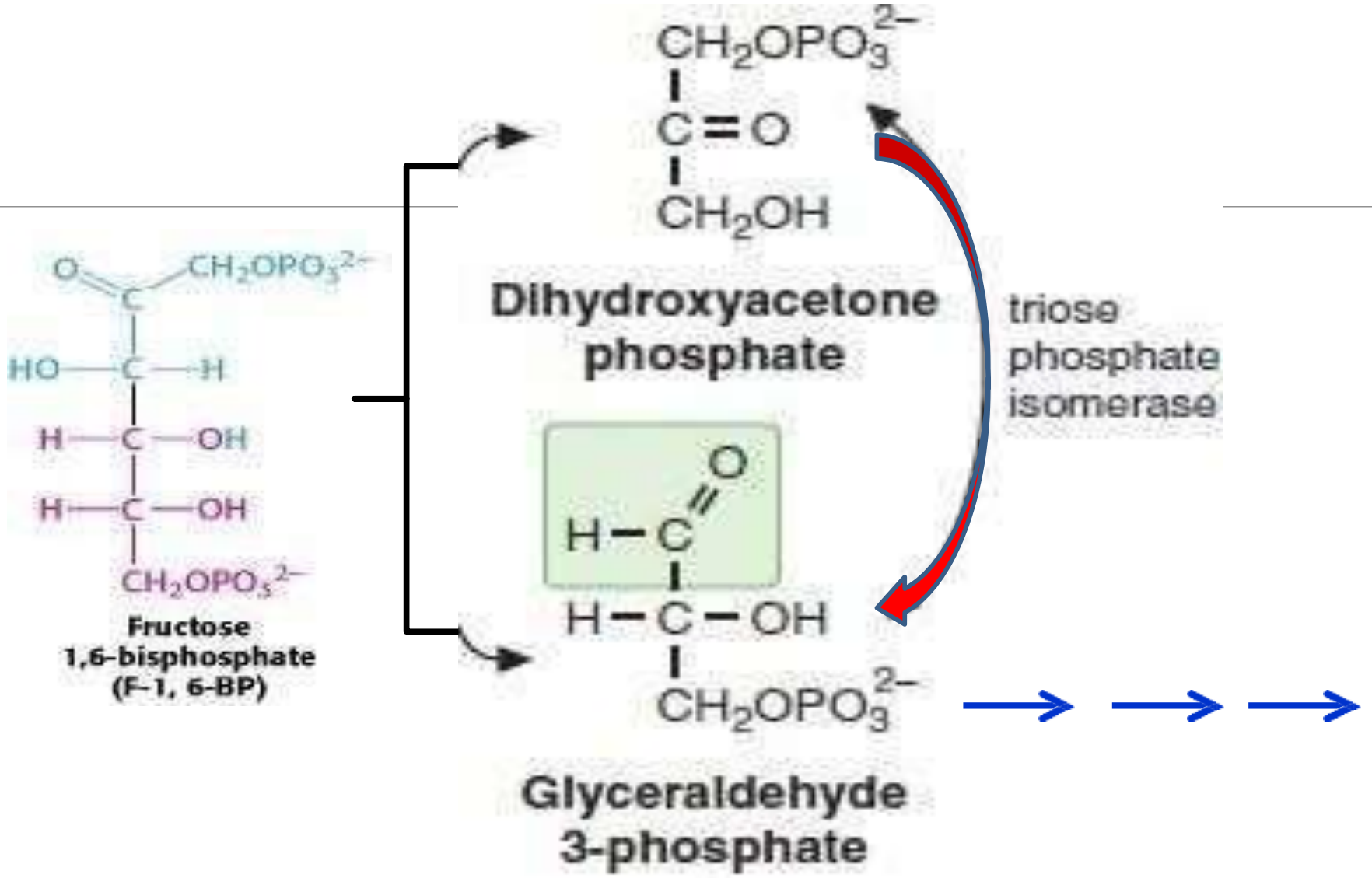
The complement in this slide:

- Cleavage by aldolase enzyme
- Reversible step
- 1st & 3rd: irreversible, 2nd & 4th: reversible
- **DHAP can't continue** to the next step, it has to be isomerized into glycrealdehyde-3-p (GAP) by a **triose phosphate isomerase.** This enzyme can work both ways, thus it can return GAP to DHAP. But it has to maintain equilibrium and since we will consume GAP in the next steps, the rxn will be driven towards the production of GAP.

الاشي الي بينسحب منه, بيتعوض بداله ...

العبارة فيها تفاؤل او اشي ;)

Step 5


Fructose
1,6-bisphosphate
(F-1, 6-BP)
Dihydroxyacetone
phosphate
triose
phosphate
isomerase
Glyceraldehyde
3-phosphate

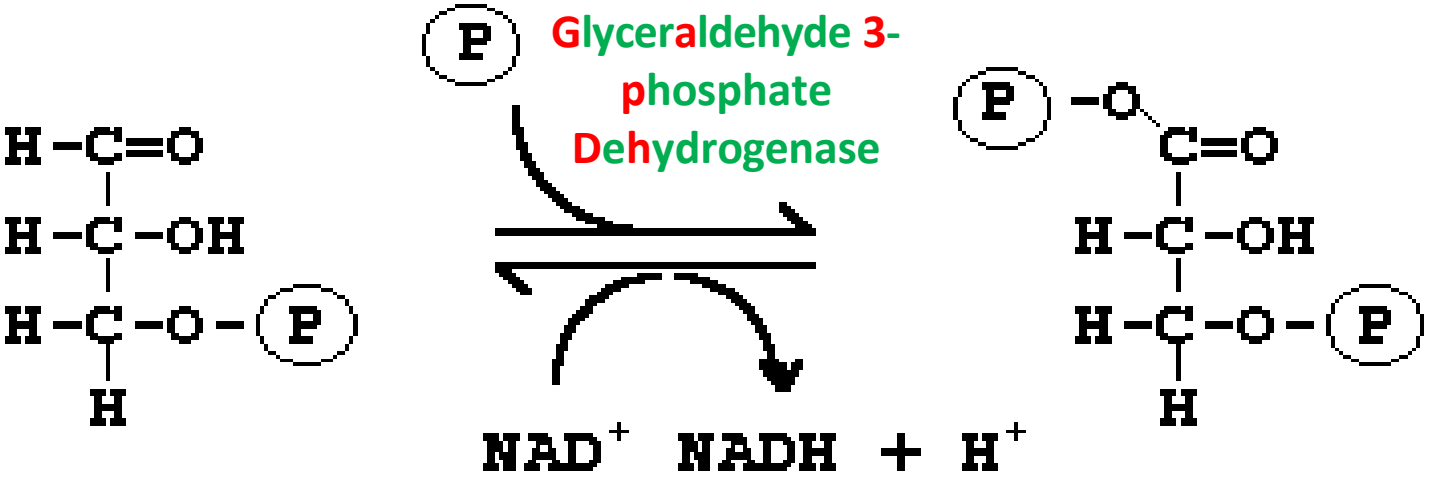
The complement in this slide:

So now we have 2 molecules of GAP

الخطوات الجاي حتنعاد مرتين بنفس الوقت يعني

بكل مرة لجزيء

Step 6



Glyceraldehyde 3-phosphate 1,3-Bisphosphoglycerate

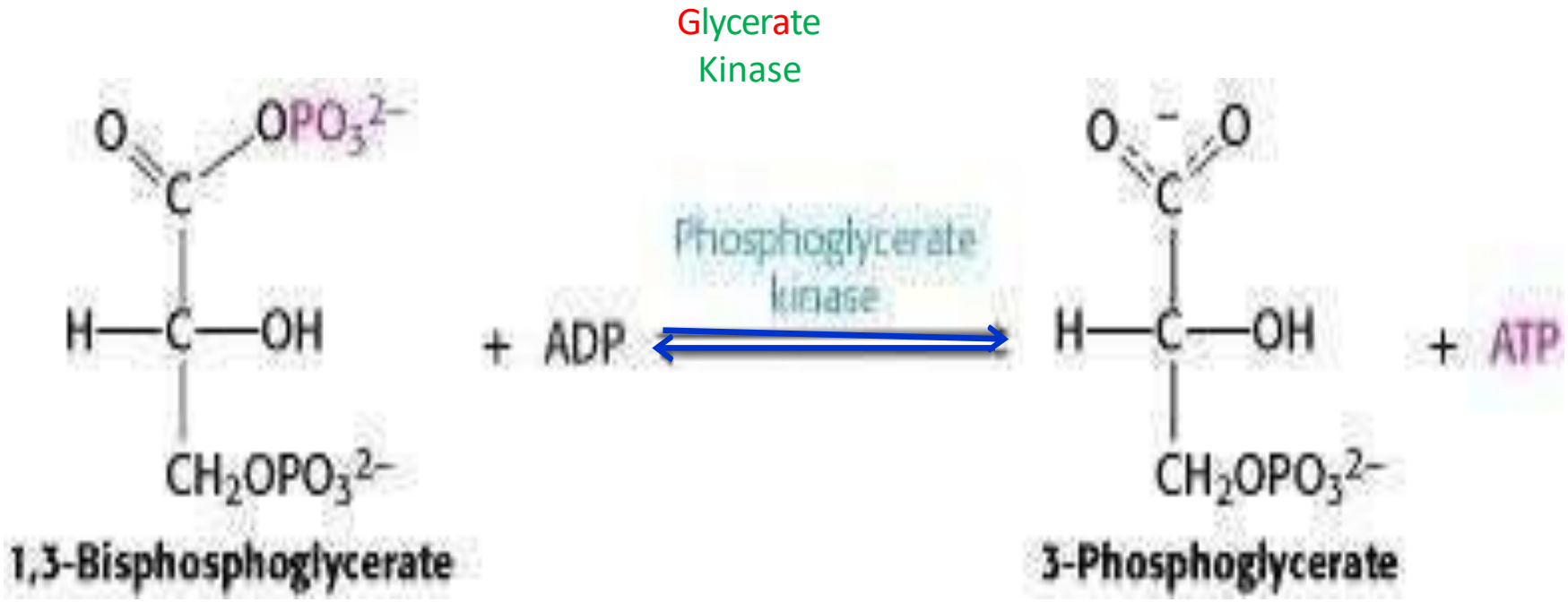
The complement in this slide:

- GAP is oxidized by glyceraldehyde 3-phosphate dehydrogenase
- NAD+ is reduced to NADH
- Notice the addition of inorganic P, which is free. So we didn't use energy
- Inorganic P is added to C-1, forming: 1,3-bisphosphoglycerate
- **Reversible step.**

Step 7

NOTE:

بالخطوة 6:
ربطنا الفسفور ببلاش من دون ما نستعمل طاقة
والآن بالخطوة 7:
رح نكسر الرابطة ونطلع الطاقة لأن الرابطة الي بتعملها
عالية الطاقة بتكون الفوسفات مع المركبات



The complement in this slide:

- We use the P on C-1 to phosphorylate an ADP molecule to produce THE FIRST ATP molecule in the pathway.

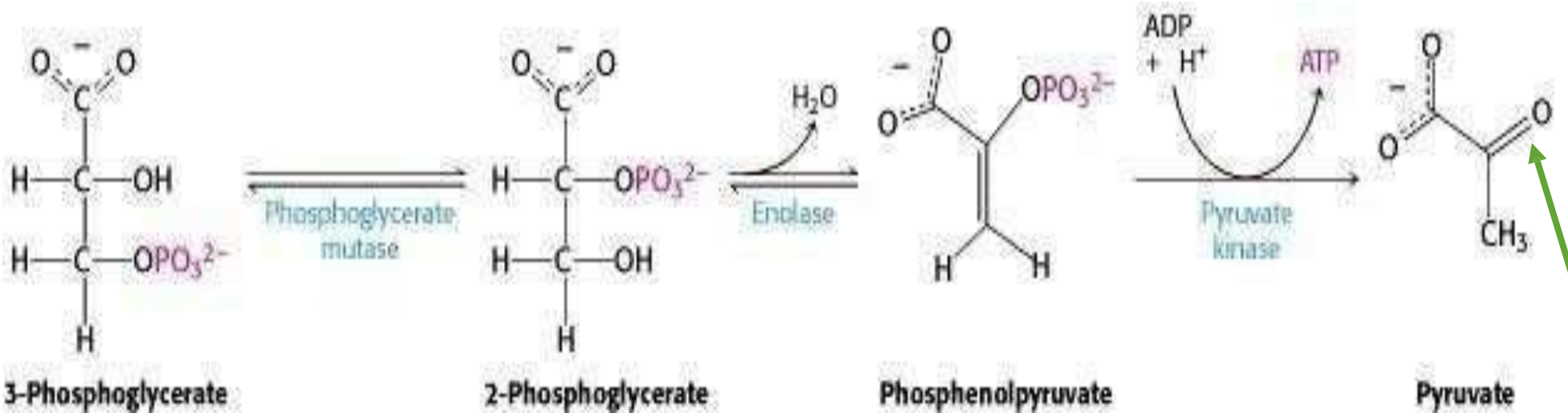
دخلناها ببلاش وطلعناها على شكل طاقة

.. It's a winning deal!!!

- product: 3-phosphoglycerate
- Reversible step
- Catalyzed by glycerate kinase. It didn’t phosphorylate 1,3-bisphosphoglycerate, it phosphorylated ADP (the 2nd substrate of this enzyme).

Step 8-10

8 9 10



NOTE: double bond formed to saturate O

The complement in this slide:

- Isomerization of 3-phosphoglycerate to 2-phosphoglycerate. We transported P from C-3 to C-2 in a reversible step catalyzed by phosphoglycerate mutase.
- Why mutase not ismoerase? Because we changed the location of a phospahte
- Then we perform a dehydration rxn (by enolase) on 2-phosphoglycerate to form a double bond. The product is: phosphophenolpyruvate.
- Notice how we are approaching the final product!! :))

The complement in this slide:

- the last step, **irreversible.**
- What happens in this step? Simply; we form a double bond to saturate O, add H so it becomes CH3, extract the phosphate group to form the double bond.
- So, we performed this isomerization and removal of P to move the double bond from C-1 to C-3, resulting in **pyruvate**
- Pyruvate: Methyl group ✓ Carbonyl ✓ Carboxyl ✓
- The irreversible steps are: 1, 3, 10 (they are all catalyzed by **kinases**).

V2: Slide 11
Intercellular instead of intracellular