

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

METABOLISM

MID – Lecture 11

GLYCOLYSIS



﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

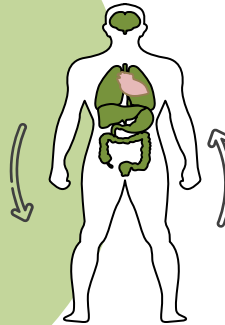
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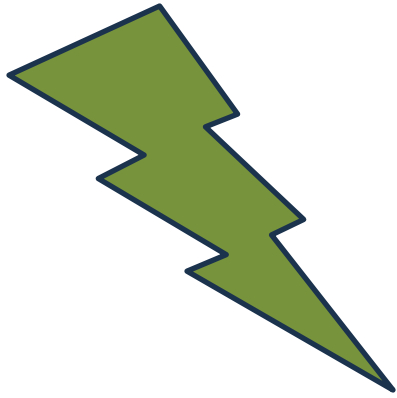
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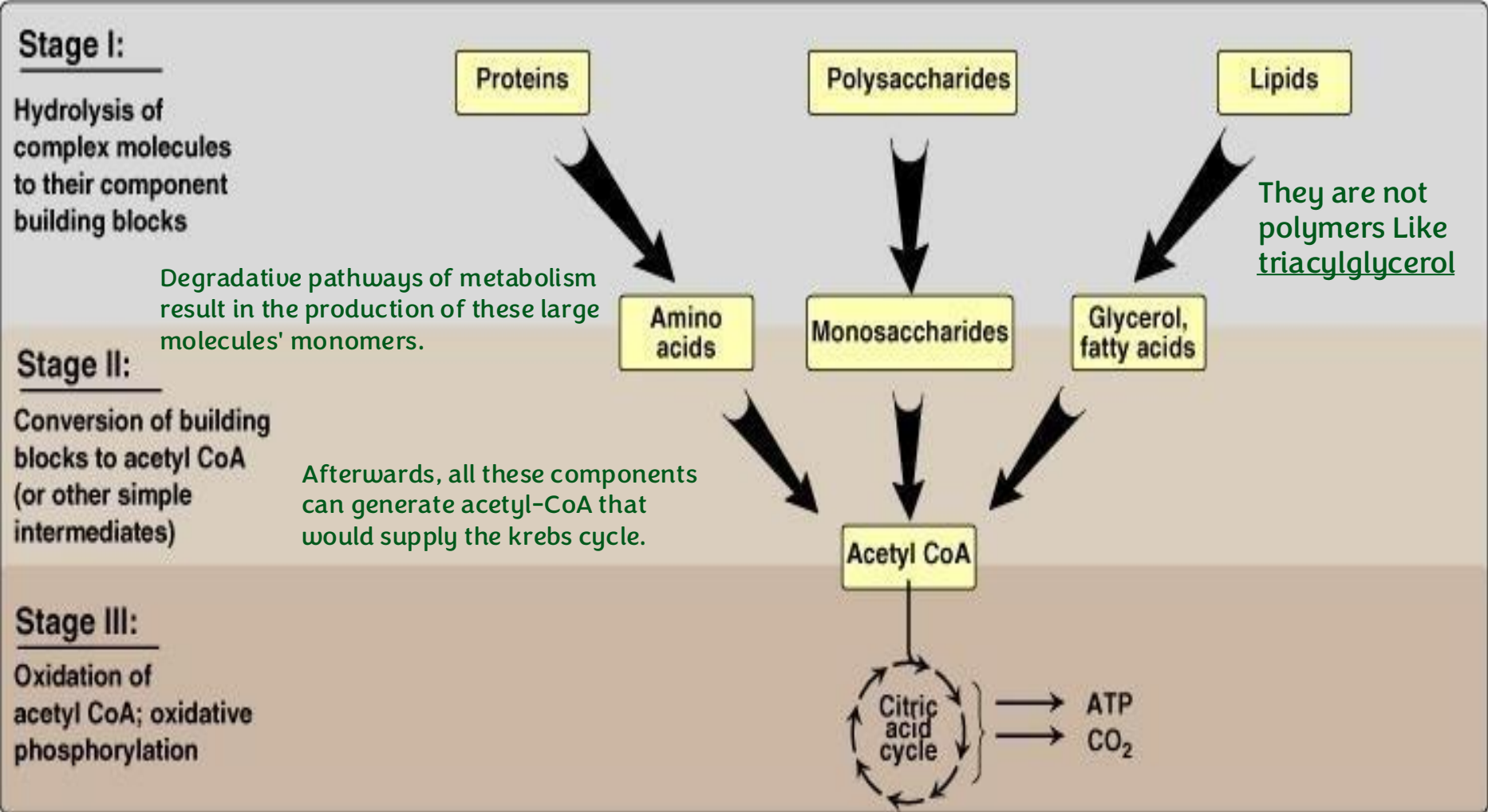
Quiz for the previous lecture

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General Stages of Metabolism

Before getting into the glycolytic pathway, note that the metabolic pathways in general specifically the catabolic pathways (pathways that include the degradation of molecules) start with large molecules.



So, what is the difference between digestion and metabolism?

Digestion: is the breakdown of food into smaller components that can be absorbed by the body.

Metabolism: refers to all the reactions and pathways that occur within the cells, so the moment these nutrients enter the cells, they will become part of these metabolic reactions and pathways.

However, the breakdown of food into smaller components is not necessarily always digestion.

For example, proteins can be degraded into amino acids inside the cells rather than inside your digestive system. Same for polysaccharides, for example glycosaminoglycans in the ECM can be degraded into monosaccharides within the cell. So, similarities exist between digestion and metabolism, however the latter (metabolism) occurs inside the cell.

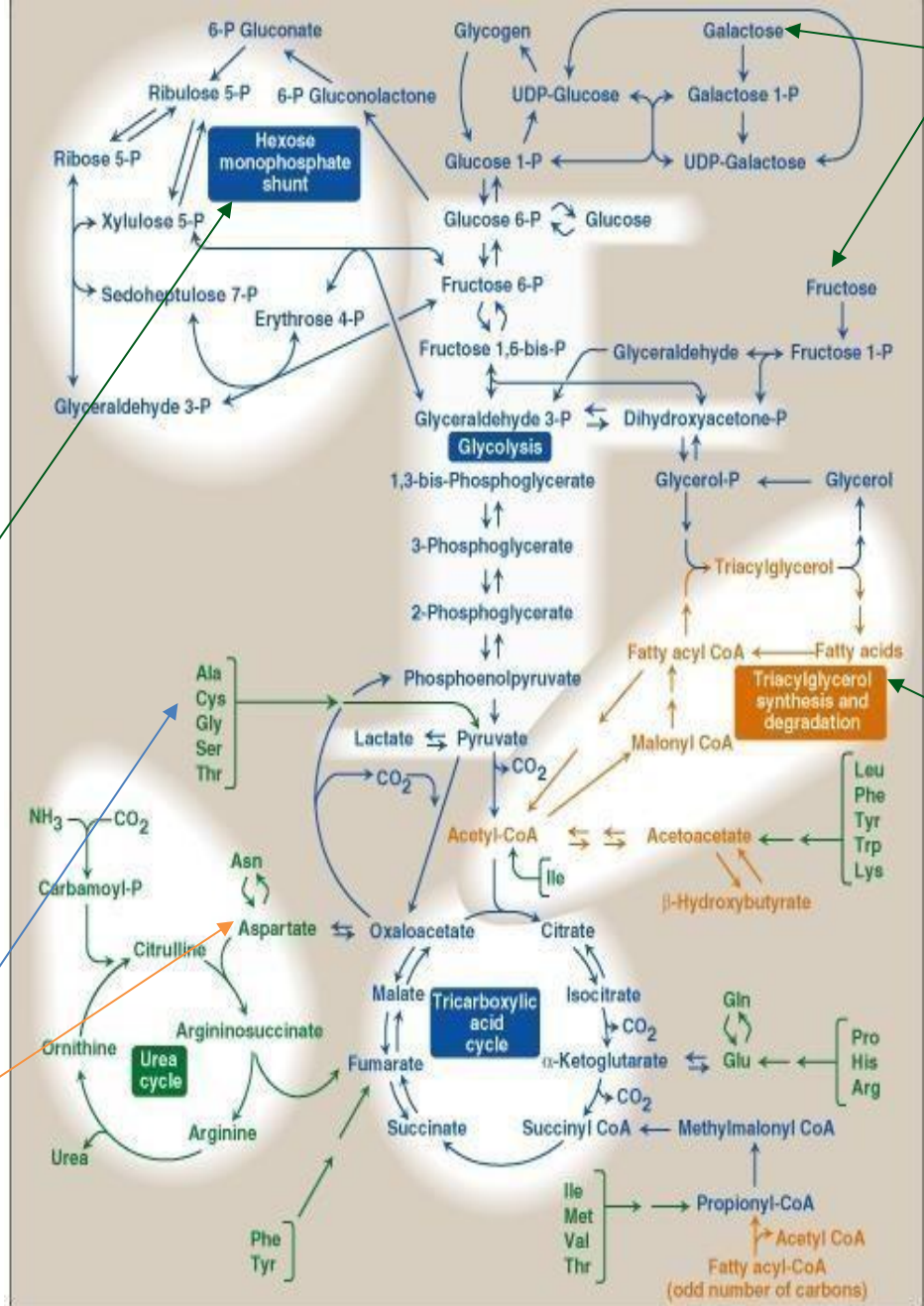
Metabolic pathways intersect to form network of chemical reactions

Metabolic pathways are highly interconnected -> they interact (crosstalk) with each other.

Glycolysis interacts with pathways that produce pentose sugars called "Hexose monophosphate shunt" or "pentose phosphate pathway".

Interaction with the degradative pathways of amino acids. Some amino acids will produce **pyruvate**, or **Acetyl-CoA**, or **other krebs cycle intermediates**. Amino acids release ammonia NH3 which its toxicity must be reduced by the conversion to urea through the urea cycle.

The urea cycle interacts with the krebs cycle.

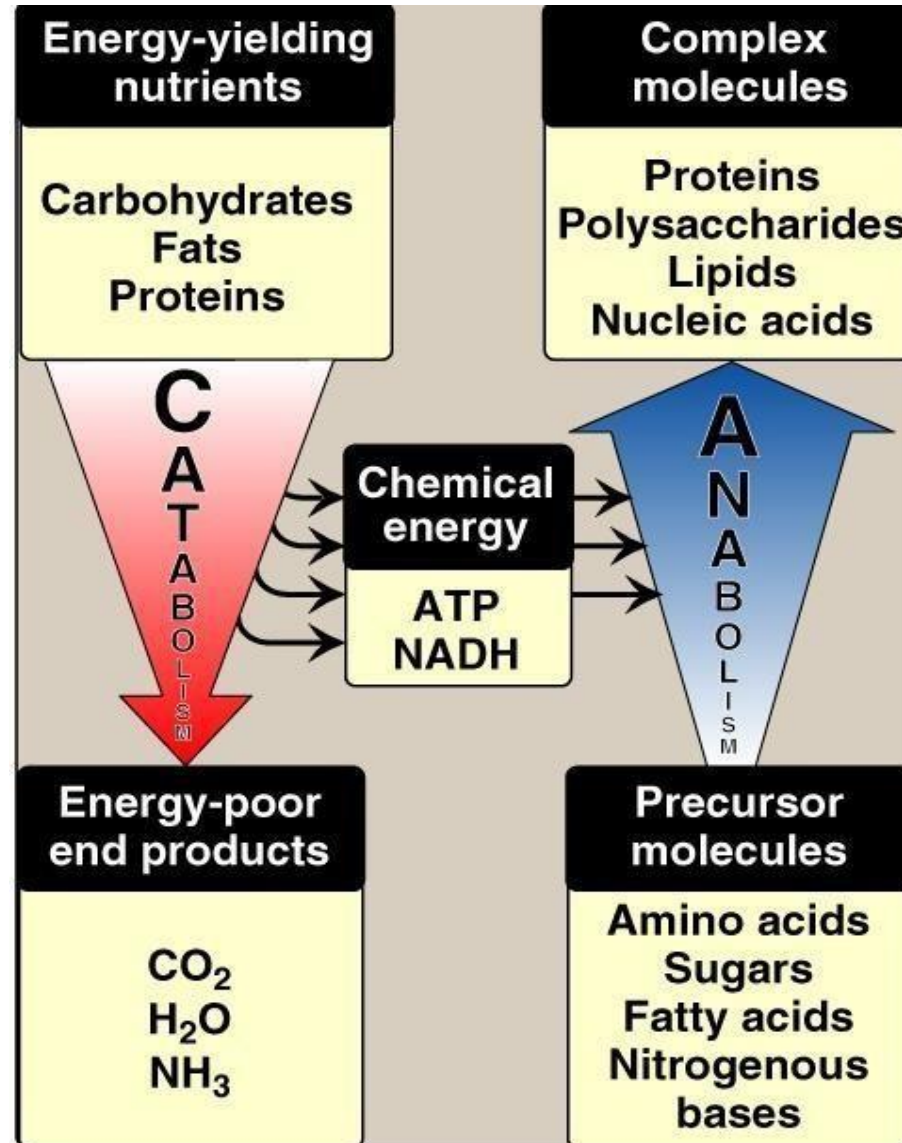


The glycolytic pathway interacts with the metabolism of other monosaccharides including Galactose & Fructose.

Glycolysis produces pyruvate which will be used to make Acetyl-CoA that supply Krebs cycle.

Krebs cycle interacts with the degradative pathways of lipids which results in the production of fatty acids + glycerols from triacylglycerols. This in turn generates acetyl-CoA which activates the krebs cycle.

Types of Metabolic Pathways



Degradation (catabolism) outcomes:

1. Produce energy.
2. Oxidize main substrates and reduce the coenzymes.

The final products of the degradative pathways cannot be further degraded.

Anabolism outcomes:

1. Requires energy.
2. Reduce main substrate and oxidize the coenzyme.

Note: Anabolism & catabolism do not happen together at the same time.

Regulation of Metabolism

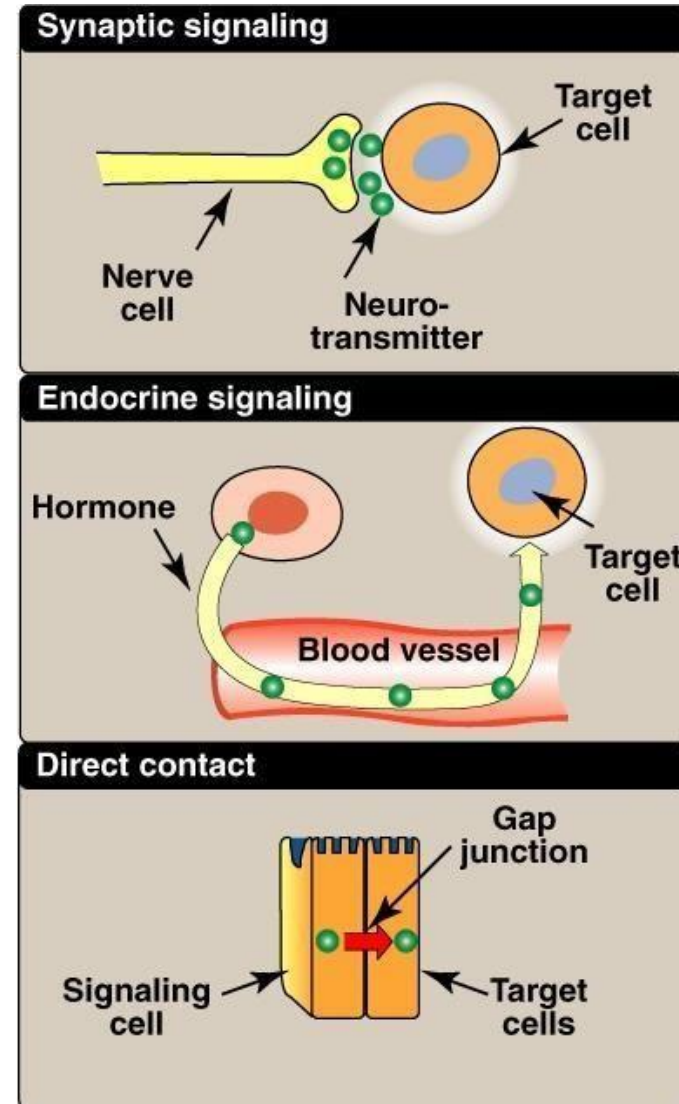
Metabolic pathways must be tightly regulated to maintain the harmony inside the cell (for example to prevent catabolic & anabolic pathways of the same molecule working together).

Regulation can occur within the cell itself or from outside signals.

The following points refer to general principles of metabolic regulation (not specific to glycolysis):

- **Signals from within the cell**
 - Substrate availability, product inhibition, allosteric regulators, con. of enzymes, con. of receptors.
- **Compartmentalization whether substrates or enzymes.**

Compartmentalization is a spatial relation that involves separating processes especially those opposite to each other into distinct places.
- **Rapid response because this regulation comes from within the cell, moment to moment**



Commonly used mechanisms of communication between cells

- Communication between cells (intercellular)

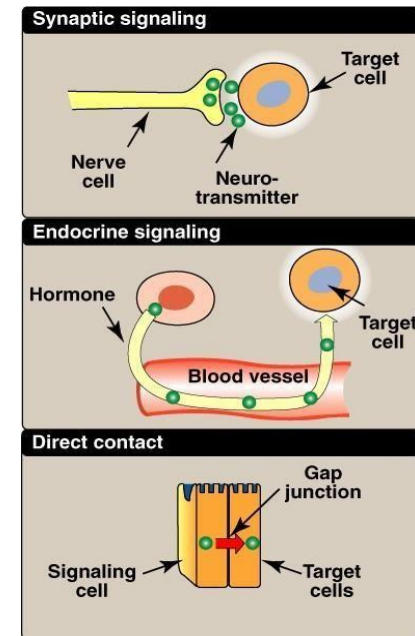
1. Slower response -> Since stimulus came from outside (neighboring cell or distant cell) it will take time to reach the site of action.

- 1 For example, a hormone by endocrine signaling will be secreted under a certain condition, travel through the bloodstream, and then reach the target tissue where it binds to its receptor and induces a certain response.
- 2 Paracrine (similar to synapses): a neighboring cell will secrete compounds (NT, hormone, growth factors) that bind to a receptor on the target cell, which induces a response.
- 3 Gap junctions: openings between the cells. However, they're limited due to allowing the entry of molecules less than 1 kDa (so limited to only small molecules < 1 kDa).

In terms of faster response:

Signal from within the cell > Paracrine > Endocrine

, longer range integration.



- Second messenger

Endocrine signaling: firstly the hormone got released from the endocrine gland, travel through the bloodstream, and then bind to the receptor on the cell surface. To induce a certain effect in response to this signal within the cell, the message must be transmitted from the membrane to inside the cell.

Hormone → first messenger. It brought a certain signal, but this signal will remain on the surface of the membrane (not transmitted inside the cell).

This transmission inside is done by a second messenger.

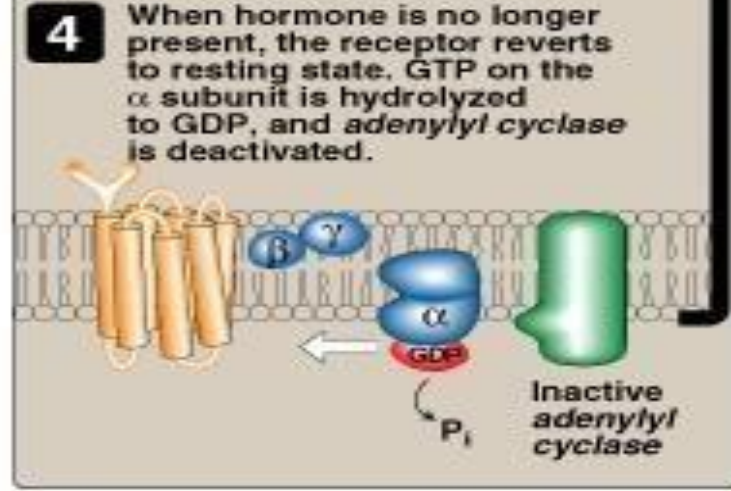
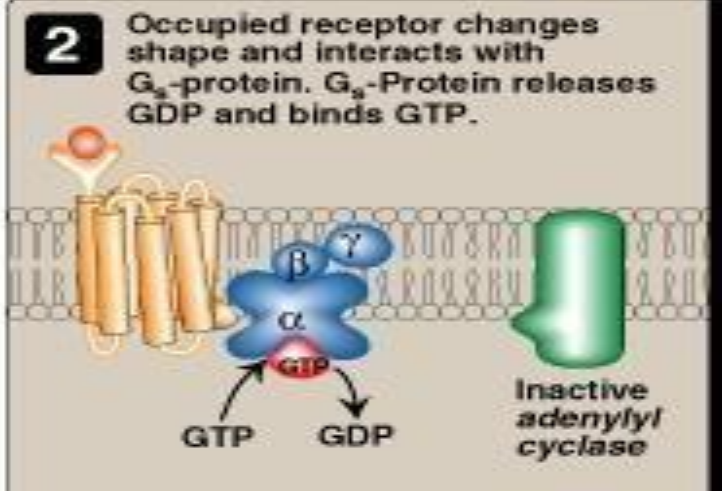
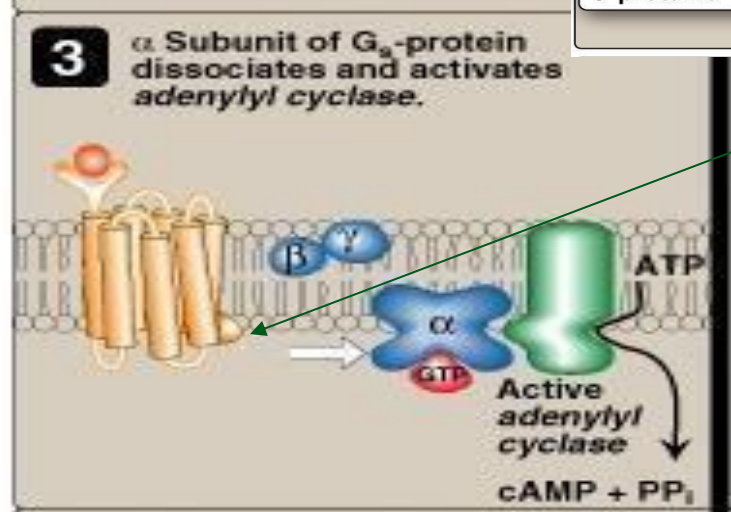
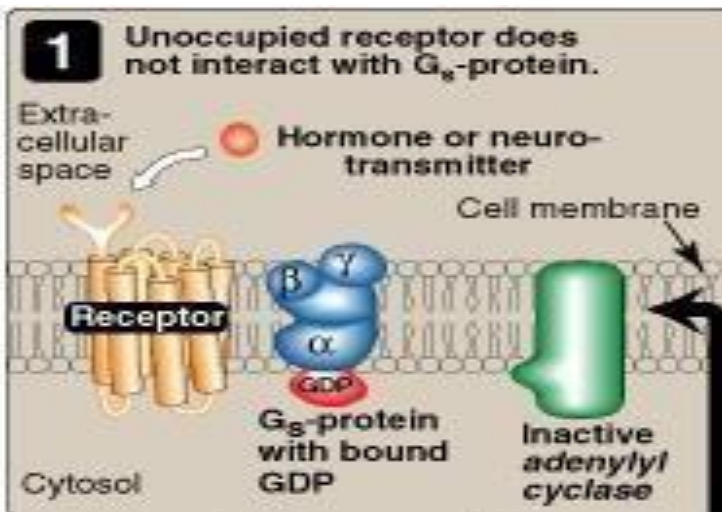
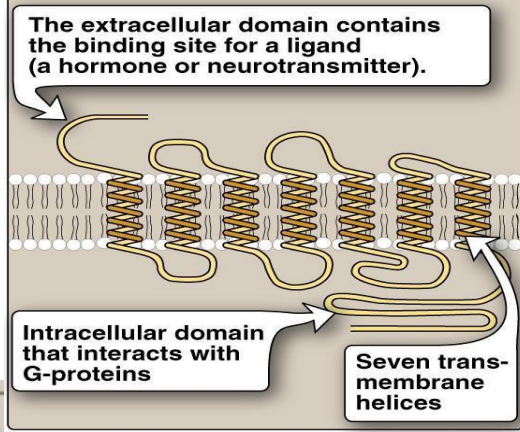
Second messenger: is a molecule not attached to the membrane, and transmits the message from the membrane to inside the cell (to the organelles, nucleus, ect...).

Examples on second messengers:

Cyclic AMP: activated by GPCRs.

- Ca^{2+} / phosphatidylinositol system
- Adenylcyclase system

Communication between cells through **G**-Protein **C**oupled **R**eceptors (GPCRs)



Binding of a ligand cause a conformational change. (Explained in next page)

Notes on the previous slide:

GPCRs are composed of 7 transmembrane helices → ECM domains bind to ligands while intracellular domains interact with downstream effectors in the signaling pathway. They get inactivated once unbound from their ligands, which also inactivates the G-protein attached to it.

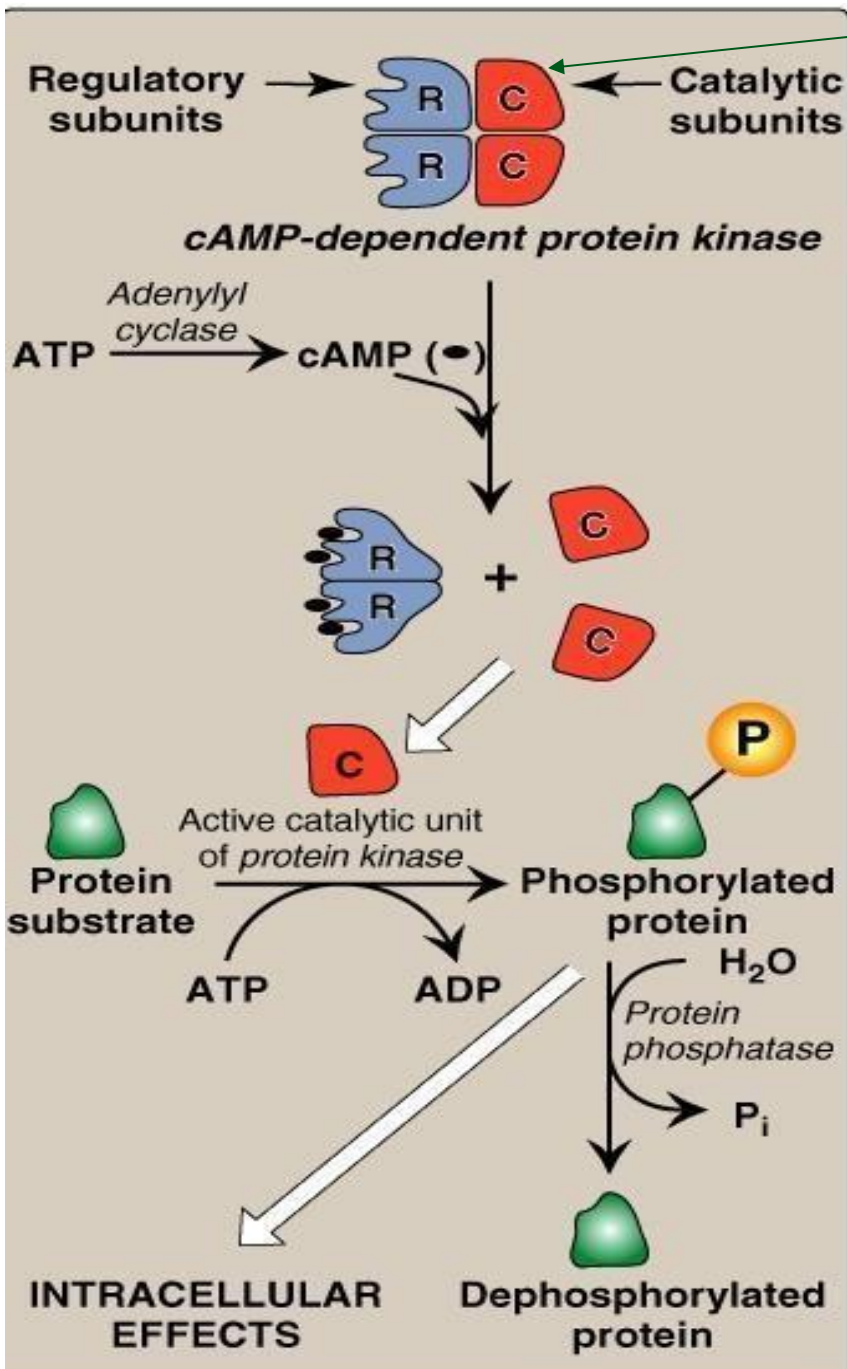
G-protein is composed of 3 subunits (alpha, beta, & gamma) → they form a complex together.

1. The alpha subunit is bound to a GDP molecule.
2. Once the hormone or neurotransmitter binds as a ligand to its binding site on the GCPR, it will induce a conformational change.
3. This conformational change pushed or "kicks" the G-protein.
4. G-protein exchanges GDP to GTP. Once GTP is bound to the alpha subunit, conformational changes occur because GTP (with its additional phosphate group "negative charge") is larger in size than GDP thus detachment of alpha subunit from beta & gamma occurs. Notice that (beta & gamma) remain attached to each other.
5. Alpha subunit activates the subsequent component of the signaling pathway (Adenylyl cyclase in this case).
6. Adenylyl cyclase will use the ATP to make cyclic AMP (second messenger).

In cell signaling pathways involving cAMP, everything that occurs before the production of cAMP generally happens at the cell membrane, hence cAMP called a second messenger. cAMP is the first to leave the membrane and enter into the cell.

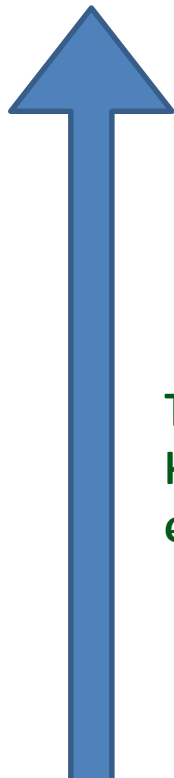
Defective turn-off of the receptor may lead to cancer. Acquired mutations that affect certain receptors result in keeping those receptors 'on' all the time (as if they're bound continuously, even when they are not). These mutations change the shape of the receptor, which affects its activity, keeping it in the active state all the time. Treatments include designing inhibitors for these overactivated GPCRs.

Process of stopping the signal: starts with the hydrolysis of GTP to GDP. This changes the conformation of alpha subunit to the inactive state, so it can complex with beta & gamma. So, the hormone or neurotransmitter detaches from its binding site to inactivate this signaling pathway.



Inactive state of the enzyme before binding to cAMP.

INTRACELLULAR EFFECTS



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

There are many targets of protein Kinase A that will be discussed in each pathway of this course.

Notes on the previous slide:

What happens to cAMP inside the cell?

1. After the cAMP is formed by Adenylyl cyclase, it will bind to protein kinase A. It is called Kinase (A) because it links to c(A)MP. {Another example, protein Kinase (G) is so named because it links to c(G)MP}.
2. Protein kinase A is composed of 4 subunits = 2 regulatory + 2 catalytic. It has 2 binding sites per unit.
3. CAMP binds to the regulatory subunits inducing conformational changes. This will result in detaching the catalytic subunits.
4. Once the catalytic sites are free, they start to catalyze phosphorylation.

There are so many targets of protein kinase A.

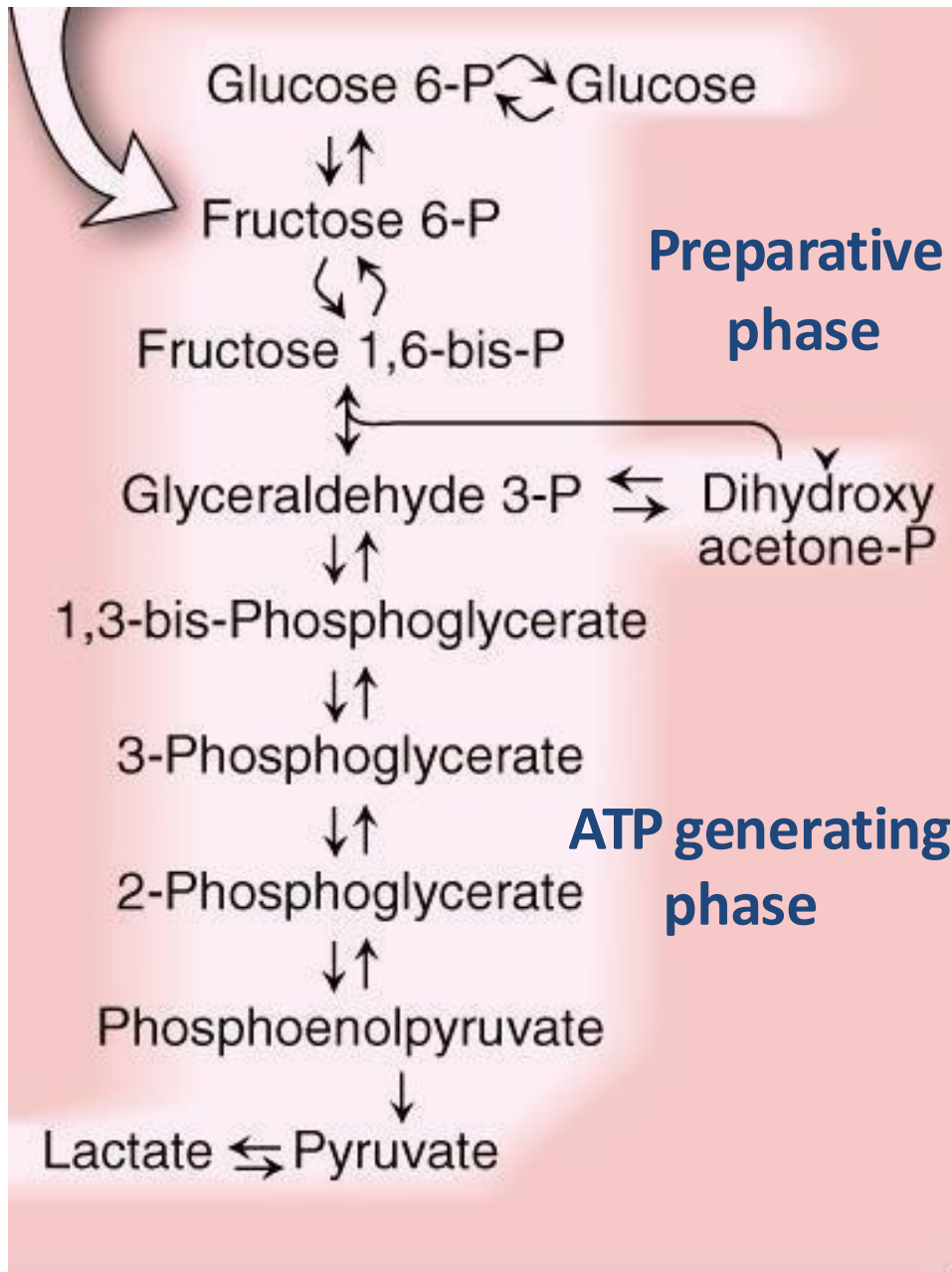
These targets are proteins (so not a small molecule) that get phosphorylated on certain sites (example their structure). Once phosphorylated they can either get activated or deactivated.

To end the state of inhibition or activation of this phosphorylated protein, the phosphate is removed by another enzyme called protein phosphatase that converts it back to the dephosphorylated state.

Those many targets result in the amplification and diverse effect of the signal. However, in metabolic pathways there is one effect either production or degradation of a molecule. So metabolic pathways might be effected by these signaling pathways or might be part of the response to the activation or inhibition of the signaling pathway.

For example, once a protein is phosphorylated, it will activate a certain metabolic pathway or inhibit it, or opening/closing of some ion channels, or activation of transcription factors (gene expression) -> so diverse effect.

GLYCOLYSIS



✓ Breakdown of glucose to pyruvate Pathway characteristics

- Universal Pathway: In all cell types
- Generation of ATP
- With or without O_2 Glycolysis is independent from O_2 . Remember, krebs cycle indirectly depends on O_2 .

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

Notes on the previous slide:

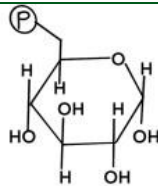
Glycolysis:

- The degradation of sugars (specifically glucose).
- A universal pathway. Present in all cells even those with no mitochondria like RBCs, because it occurs in the cytosol.
- Generation of a small amount of energy.
- Composed of 10 steps = 7 reversible + 3 irreversible
- The 3 irreversible steps are the highly regulated steps.
- Linear pathway: the product of one reaction is the reactant of the next reaction.
- Starting with 6 carbons, the end products are 2 pyruvate molecules, each containing 3 carbons. Therefore, there is no loss of carbon; nothing is emitted as CO₂ for example.
- Composed of 2 phases. The first phase (preparative phase): from glucose to Fructose 1,6-bis-P. The second phase (ATP generating phase).
- The preparative phase also called investment phase consumes energy. While the second phase generates ATP.
- The net (for the whole pathway) will be production of energy.

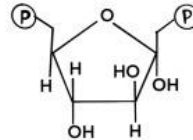
Glucose gets phosphorylated upon entry to the cell in order to prevent its exit by hexokinase or glucokinase, because they can enter and exit through transporters that increases the gradient. Although the 1st step in glycolysis is irreversible, it is not the committed step because this glucose-6-P can enter other pathways.

In the preparative phase, modification occurs to the shape of glucose-6-P until it ends up as a molecule that is cleavable to two identical molecules (Remember because end products of glycolysis are two identical pyruvates).

Modification include phosphorylation and isomerization.
Isomerization: reversible.
Phosphorylation: irreversible.



Glucose-6-Phosphate



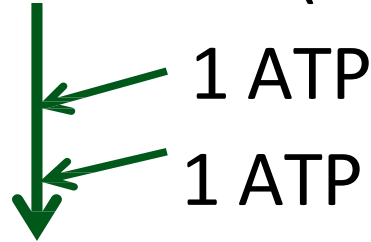
Fructose 1,6-Bis Phosphate

The Two Phases of the glycolytic Pathway

Preparative Phase

We use/invest ATP in this phase for the phosphorylations

1 Glucose (**C6**)

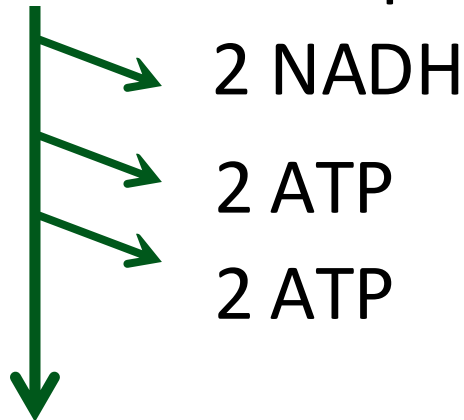


1 Fructose 1,6 biphosphate (C6)

ATP-generating Phase

We release/produce ATP in this phase

2 Triose Phosphate (**C3**)



2 Pyruvate (**C3**)

So , the NET is going to be positive towards production of ATP

Steps of Glycolysis

The FIRST Step

✓ This step includes the phosphorylation of glucose to glucose-6-phosphate



✓ This step is an **IRREVERSIBLE** step of glycolysis. The small reversed arrow represents that the step can be reversed by a single step, by different enzyme.

✓ **REVERSIBLE REACTIONS** should be catalyzed by the same enzyme

- We start with an irreversible step to prevent the exit of glucose residues outside the cells.
- Although it is an irreversible step, it has a positive delta G value. So we have to couple it with ATP hydrolysis reaction.
- The role of ATP in this reaction :
 - Providing the phosphate group
 - Providing energy

✓ The main role of ATP hydrolysis reaction is to provide energy. Providing a phosphate group is a secondary role as we can get any inorganic phosphate from the surroundings. This applied to any other phosphorylation reactions. If they need phosphate group only without energy, there is no need for ATP hydrolysis

✓ This step is catalyzed by **HEXOKINASE** or **GLUCOKINASE** . One of them can do the phosphorylation

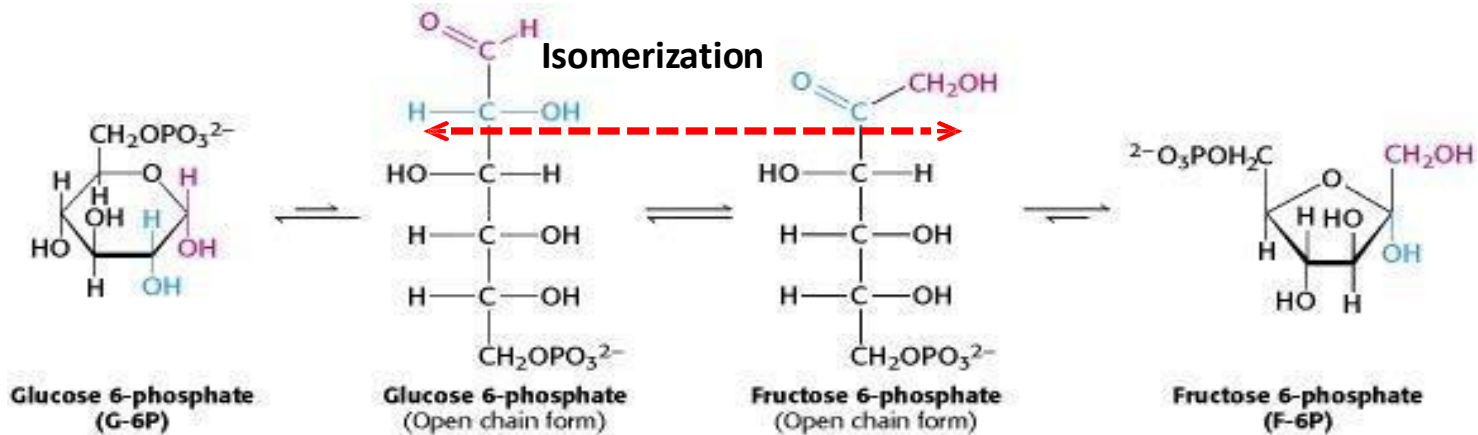
	Hexokinase	Glucokinase
Occurrence	In all tissues	In liver
Km	< 0.02 mM	10-20 mM
Specificity	Glc., Fruc, Man, Gal	Glc.
induction	Not induced	↑ insulin, Glc
Function	At any glucose level	Only > 100 mg/dl

- **HEXOKINASE** has a high expression in so many tissues in contrast to **GLUCOKINASE** , which is expressed in certain tissues -because of its specificity to glucose- like the liver which is very reactive and highly dynamic organ
- **HEXOKINASE** works whatever the concentration of the glucose is (constant function). As long as we have glucose , it is active. That's why it has a high affinity and low Km . On the other hand , we have to have a very high concentration of glucose for **GLUCOKINASE** to get activated because it has low affinity and high Km. So, it acts in spikes.
- **HEXOKINASE** as the name implies , can phosphorylate multiple hexoses like glucose , galactose , fructose , etc. It is not specific for glucose in contrast to **GLUCOKINASE** , which is specific to glucose

✓ This step (conversion of glucose to glucose-6-phosphate) is **NOT** a committed step , as glucose-6-phosphate may continue to the glycolytic pathway or it can into other pathways (**ALTHOUGH** it is considered as a glycolytic step , it doesn't necessarily continue to glycolysis

The SECOND Step

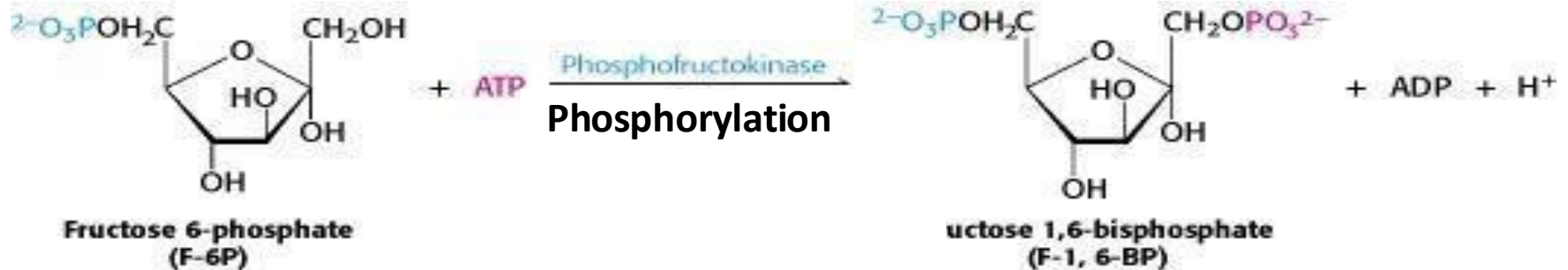
- ✓ This step includes the isomerization of glucose-6-phosphate to fructose-6-phosphate, so we have to open the ring structure into an open chain form



- This step is a kind of transmission of the carbonyl group by electron rearrangement from C1 to C2 to make fructose-6-phosphate, then the ring forms again to have the ring structure of fructose-6-phosphate
- The enzyme which catalyses this reaction is isomerase (isomerases usually catalyses reversible reactions-in both ways)
- The enzyme is called PHOSPHOGLUCOSE isomerase → Reversible Reaction

The THIRD Step

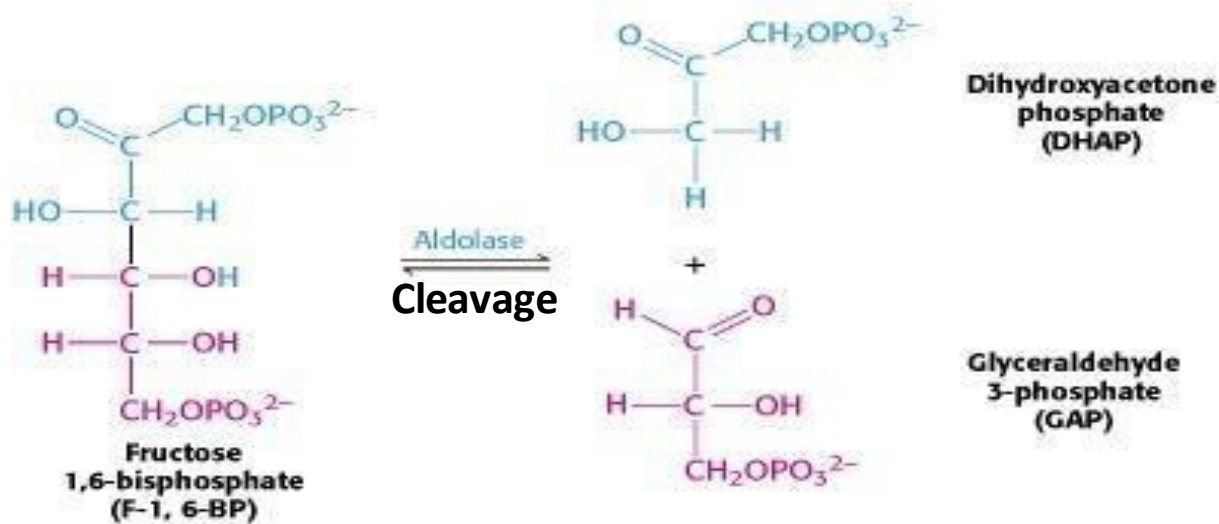
- ✓ This step includes the phosphorylation of Fructose-6-phosphate to fructose 1,6-bisphosphate (addition of phosphate to C1) in order to end the preparative phase



- This reaction is very slow, and it has positive delta G value, so I have to couple it to ATP hydrolysis reaction to provide phosphate and energy for this reaction
- Since it is the slowest step, it is the rate limiting step of the whole pathway. It is also the committed step as it is the first irreversible step after the first one which wasn't committed
- Enzymes that utilize this step is called PHOSPHOFRUCTOKINASE 1 (there is PHOSPHOFRUCTOKINASE 2, but it does different things that are not directly related to glycolysis)
- Until this step, we have used two ATP molecules so far in phosphorylation steps
- By this step, we end the first phase of glycolysis (preparative phase)

The FOURTH Step

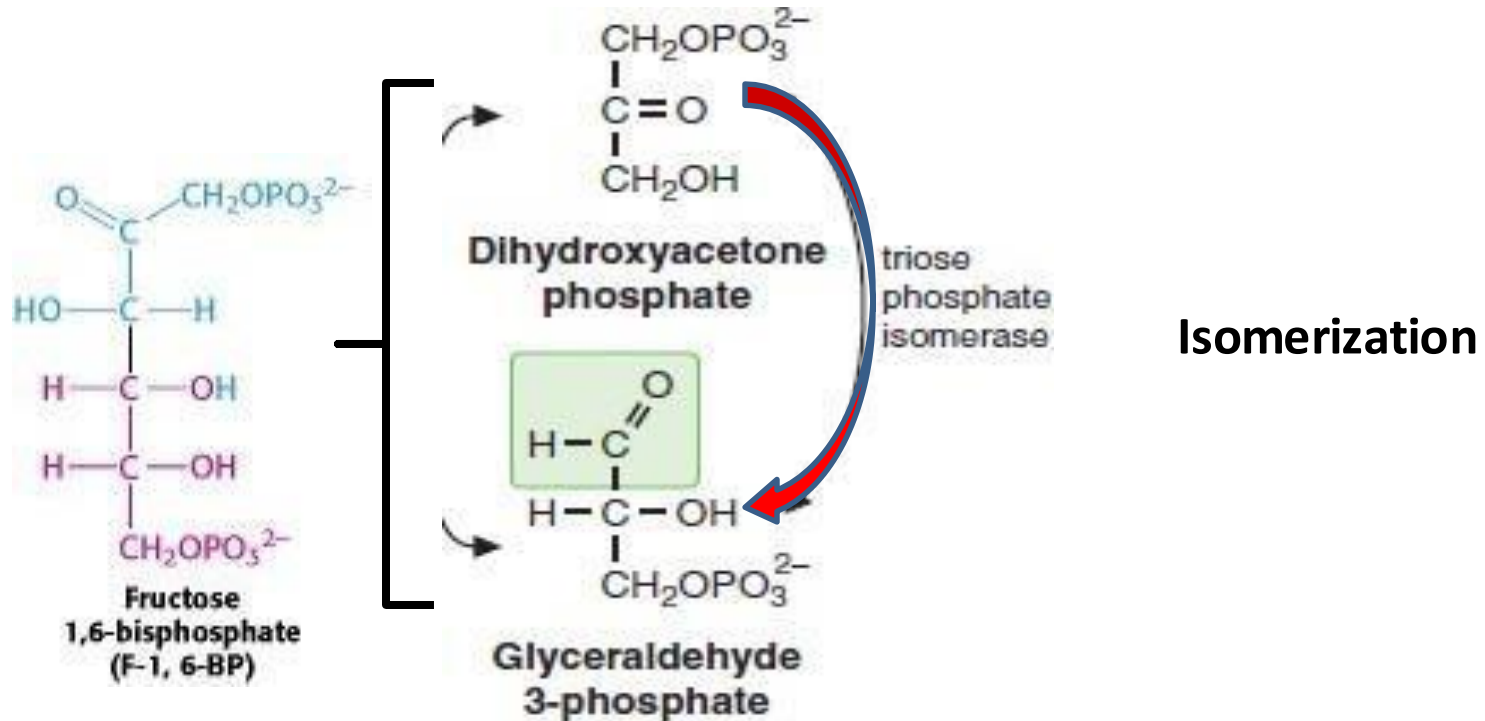
- ✓ This step includes the **CLEAVAGE**/opening the structure of fructose 1,6-bisphosphate



- If I cleaved the first three carbons together (the blue part) , dihydroxyacetone phosphate (DHAP) would be produced
- The second part (the pink part) will produce Glyceraldehyde 3-phosphate (GAP)
- After cleavage , C4 loses a bond , so we take a H-atom from the adjacent O-atom and bind it to the third carbon (and double bond forms)
- The enzyme that catalyzes the cleavage is called **ALDOLASE** , which can catalyze the reverse reaction -> **REVERSIBLE REACTION**
- Now I have two trioses with phosphate groups . GAP can continue to into the subsequent steps . However, DHAP cannot because it has to be isomerized to GAP to be able to continue . See step 5

The FIFTH Step

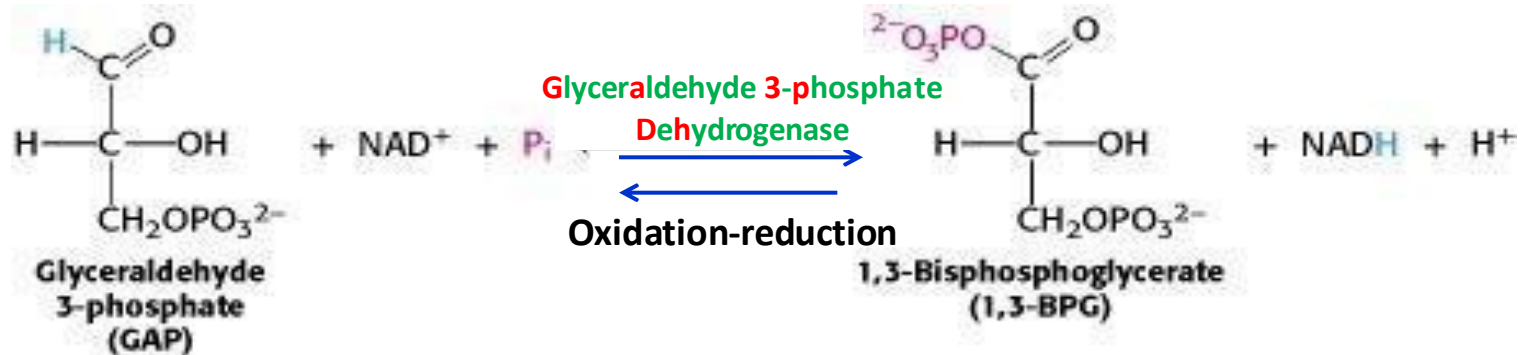
- ✓ This step includes isomerization of DHAP to GAP by **TRIOSE PHOSPHATE isomerase** in a **REVERSIBLE** reaction (can also convert GAP to DHAP)



- Because GAP is being consumed in this case, the equilibrium is shifted towards production of GAP rather than DHAP
- Now I have two molecules of GAP, so I need to repeat the reactions of the second phase twice (specifically from step 6-10)

The SIXTH Step

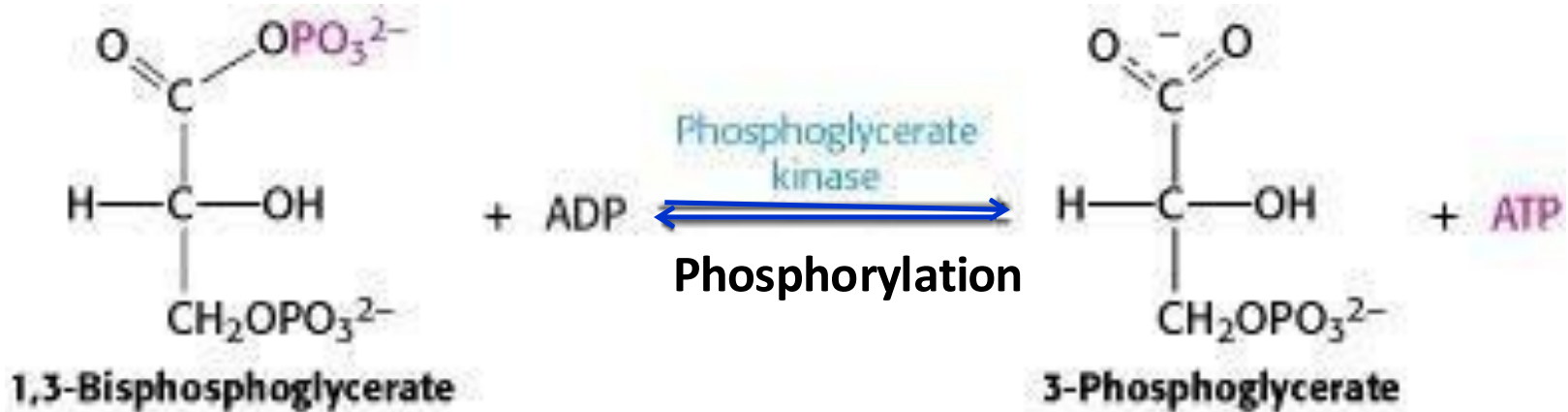
- ✓ In this step, GAP will enter oxidation-reduction reaction to be oxidized and to reduce NAD^+ to NADH



- At the same time, I am going to invest the negative delta G value of this reaction to add an inorganic phosphate (without hydrolysis of ATP) because we don't need energy for this reaction
- This phosphate will be added to the carbonyl carbon, resulting in the formation of 1,3-bisphosphoglycerate
- The enzyme that catalyzes this reaction is called Glyceraldehyde 3-phosphate Dehydrogenase (redox reaction)
- It is a REVERSIBLE step
- The NADH produced is cytosolic (not mitochondrial), so it could be a source of energy (and could not). It could also produce 2 or 3 molecules of ATP depending on how it enters the mitochondria

The SEVENTH Step

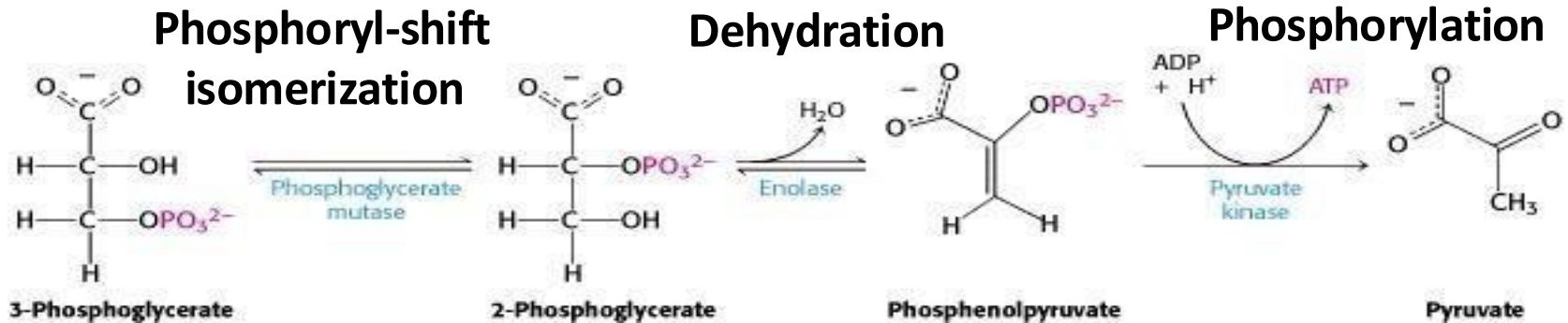
- ✓ This step includes phosphorylation of ADP (I want to get out the same phosphate group which enters in the previous step as an energy molecule)



- The O-PO₃ is a high energy bond (even if it enters the molecular without ATP hydrolysis), so it is going to release high energy when hydrolyzed. So, I want to invest this high energy released to phosphorylate ADP and produce ATP
- The enzyme that catalyzes this reaction and forms the first ATP is called Glycerate kinase
- The 1,3-Bisphosphoglycerate loses one phosphate group and becomes 3-phosphoglycerate
- It is a REVERSIBLE step

Steps 8-10

- ✓ In step 8 , I want to isomerize the 3-phosphoglycerate by transferring the phosphate group from C3 to C2 , to produce 2-phosphoglycerate



- ✓ Since this isomerization occurs by transferring a phosphate group , we call the enzyme (mutase) not isomerase → phosphoglycerate mutase
- ✓ It is a REVERSIBLE step

- ✓ In step 9 , dehydration reaction occurs. H₂O molecule is removed from C₂(H) & C₃(OH) and double bond forms .
- ✓ This step is catalyzed by Enolase enzyme in a REVERSIBLE reaction and produces phosphoenolpyruvate

- Pyruvate doesn't have phosphate group, so it should be removed in step 10
- The structure of pyruvate is carboxyl-carbonyl-methyl groups sequence
- The O-PO₃ is a high energy bond, so the bond is broken, ADP is phosphorylated to ATP by pyruvate kinase enzyme
- After the O-PO₃ bond is broken, new double bond forms, and the old one breaks. Also, one H-atom binds to the last carbon to form the methyl group.

✓ If the phosphate group on 3-phosphoglycerate remained on C2 (step 8 didn't happen) → The new formed double bond in pyruvate will not be able to form

- ✓ The last step produces energy (ATP) and it is the third IRREVERSIBLE step in glycolysis
- ✓ Step 1 & 3 & 10 are IRREVERSIBLE STEPS

- The steps from 6-10 are repeated twice (because of the production of two GAP molecules)
- Two steps consume ATP molecules
- Two steps produce ATP → 4 ATP molecules are produced
- One step produces NADH → 2 NADH molecules are produced
- The NET is production of 2 ATP molecules and 2 NADH molecules

✓ We always say that Glycolysis is the main source of energy, so we predict to produce high energy (knowing that Krebs Cycle produces more energy). Despite this, for cells like the RBCs (which has a very low energy demand), this pathway is enough, but in other cells it needs to continue the process (Acetyl Co-A and Krebs Cycle).

Energy Need and Production

Glucose 6-P ↔ Glucose

↓↑
Fructose 6-P

-ATP

↔
Fructose 1,6-bis-P

-ATP

↔
Glyceraldehyde 3-P ↔ Dihydroxy acetone-P

2 NADH

↓↑
1,3-bis-Phosphoglycerate

2 ATP

↓↑
3-Phosphoglycerate

↓↑
2-Phosphoglycerate

Is Oxygen needed?

➤ Glycolysis is independent of oxygen

↓↑
Phosphoenolpyruvate

↓
Pyruvate

2ATP

SUMMERY TABLE

# of step	Type of reaction	Reactants	Products	Enzyme	Reversible/Irreversible	Produces/Consumes ATP	Produces NADH
Step 1	Phosphorylation	Glucose	Glucose-6-phosphate	Hexokinase OR Glucokinase	Irreversible	Consumption	-
Step 2	Isomerization	Glucose-6-phosphate	Fructose-6-phosphate	Phosphoglucose isomerase	Reversible	-	-
Step 3	Phosphorylation	Fructose-6-phosphate	Fructose-1,6-phosphate	Phosphofructokinase	Irreversible	Consumption	-
Step 4	Cleavage	Fructose-1,6-phosphate	DHAP and GAP	Aldolase	Reversible	-	-
Step 5	Isomerization	DHAP	GAP	Triose phosphate isomerase	Reversible		
Step 6	Oxidation-Reduction	GAP	1,3-bisphosphoglycerate	GAP DH	Reversible	-	Yes
Step 7	Phosphorylation	1,3-bisphosphoglycerate	3-phosphoglycerate	Phosphoglycerase kinase	Reversible	Production	-
Step 8	Isomerization	3-phosphoglycerate	2-phosphoglycerate	Phosphoglycerate mutase	Reversible	-	-
Step 9	Dehydration	2-phosphoglycerate	Phosphoenolpyruvate	Enolase	Reversible	-	-
Step 10	Phosphorylation	Phosphoenolpyruvate	Pyruvate	Pyruvate kinase	Irreversible	Production	-

For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
v0 → v1	3	Tricycle	Triacylglycerol
	25	Fourth carbon	Third carbon
v1 → v2			

REFERENCES :

Lippincott Illustrated Reviews:
Biochemistry, Seventh Edition.

أَقِفْ اليوم على مفترقِ مركزيِّ في حياتي..

تجاه ديني وجسدي وعقلي وقلبي وخطوتي ومشاريعي وكُلِّ
محاولاتي! أمام أمةٍ جريحة! وبلاءاتٍ شديدة، تحتاج إلى
صدقٍ وجهدٍ وتعبٍ وغرسٍ أكبر بكثير مما نُحاول فعله!
أكبر من مزاجيةٍ لحظية، ونفسيةٍ مُتعبة، وجسدٍ مهترئ،
ومتابعةٍ ساكنة! أكبرُ من شعورٍ ينطفئ، وخطوةٍ تتعثّر،
ومنشورٍ يُكتب!

وهذا عهد تغيير مع الله، عهدٌ أحاسب عليه؛ ألا يقبضني إلا
وأفديتُ أنفاسي صادقاً له، وأوصلتُ أيامي تعباً مثمرًا لدينه،
وأتعبتُ شيطاني خلف ظلي، وتركتُ لله كُلَّ ما يُبعدني عنه،
وراغمتُ العدوَّ إعدادًا ليوم أراه، وزاحمتُ الدنيا بالآخرة مع
كُلِّ نفسٍ، وأسأله صدقًا وثباتًا وألا أخاف، ويدًا تلقى العصا
دون ارتجاف!

لأجل أولئك الثابتين، أعني على إتمام المسار، وغرس
البذرة، ونبت الفكرة، دون انقطاع ولا ذبولٍ ولا تشنُّت،
غرسًا دائمًا يا رب، حتى لو لم أرَ الثمر!