Gluconeogenesis

Karoline Hanevik

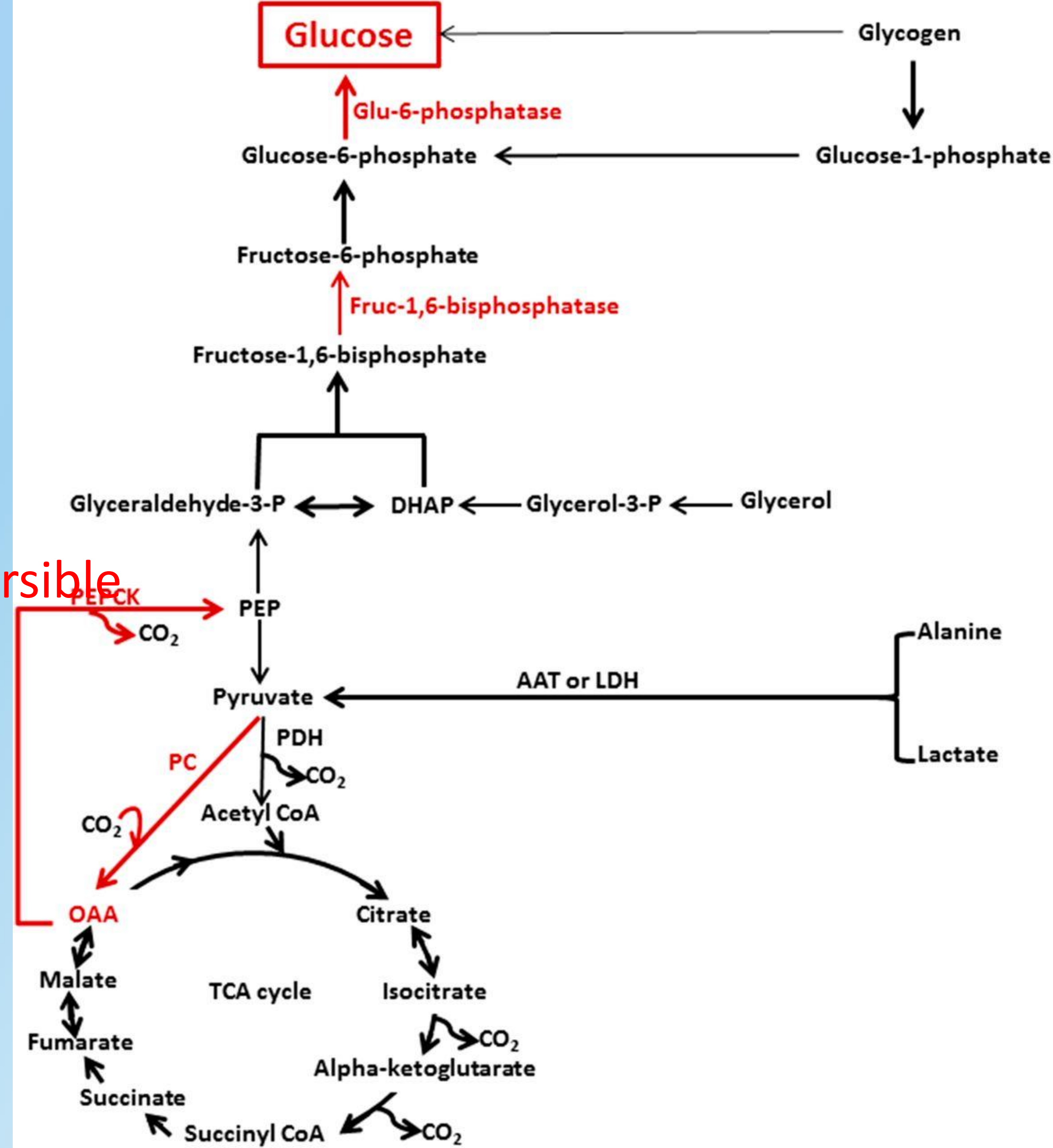

To Do List
1. Why gluconeogenesis?
2. Substrates/precursors
3. FOUR NEW ENZYMES
4. Regulation of gluconeogensis
5. QUIZ

The problem

- Glycogen reserves last max. 24 hours
- Brain, RBC, eye, kidney medulla +++ require **continuous glucose supply**
- In glycolysis, three enzymes are irreversible

Gluconeogenesis is the process that overcomes this!

BYPASS ENZYMES



Glucose

ATP → ADP

Hexose Kinase

Glucose-6-Phosphate

PhosphoGlucose Isomerase

Fructose-6-Phosphate

ATP → ADP

PhosphoFructose Kinase

Fructose-1,6-BisPhosphate

AldoLase

GLAP ← DHAP

Triose Phosphate Isomerase

$2NAD^+ + 2Pi$ → 2NADH

GLAP Hydrogenase

1,3-BisPhosphoGlycerate

2ADP → 2ATP

PhosphoGlycerate Kinase

3-PhosphoGlycerate

PhosphoGlycerate Mutase

2-PhosphoGlycerate

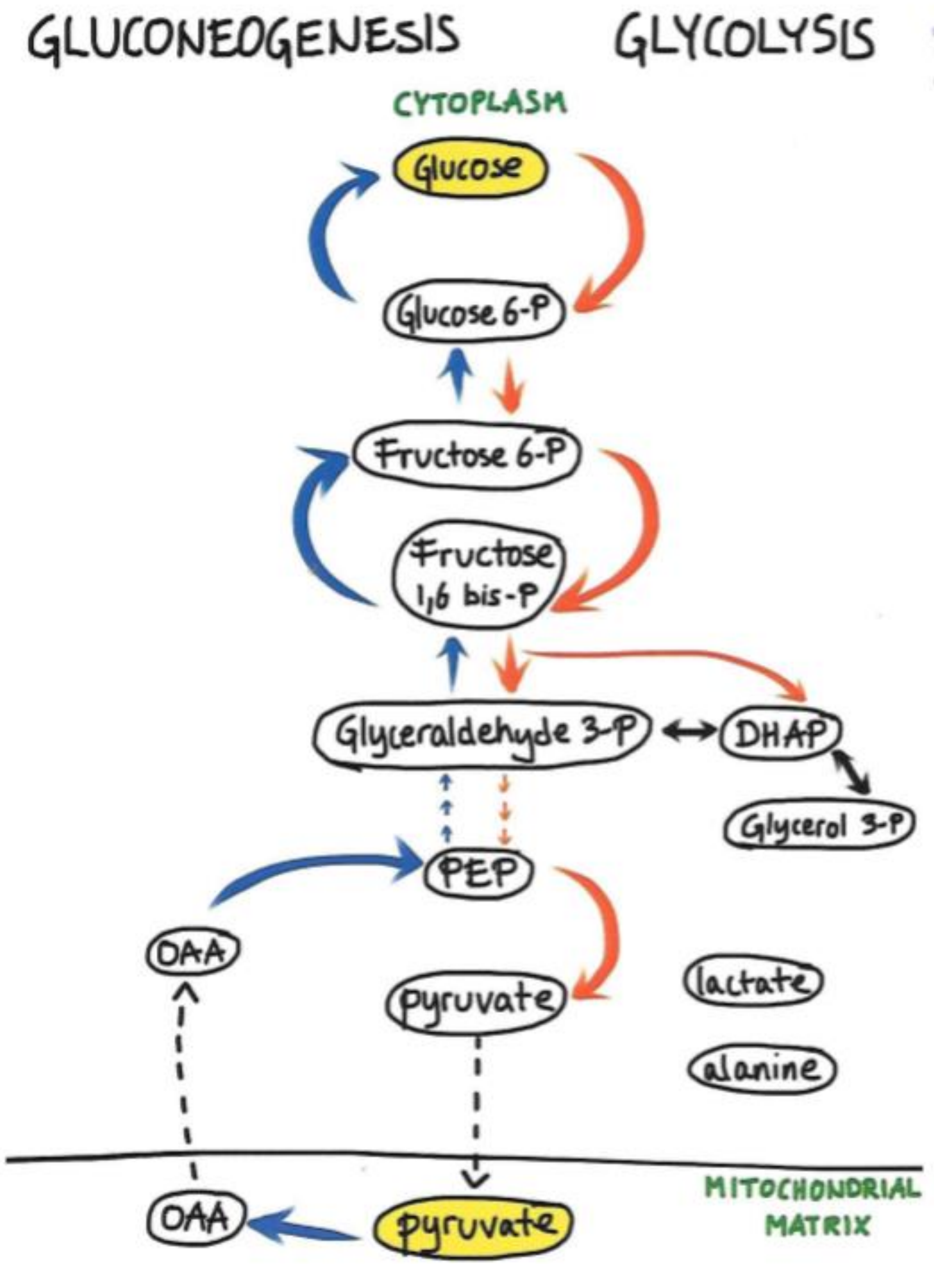
Enolase

PhosphoEnolPyruvate

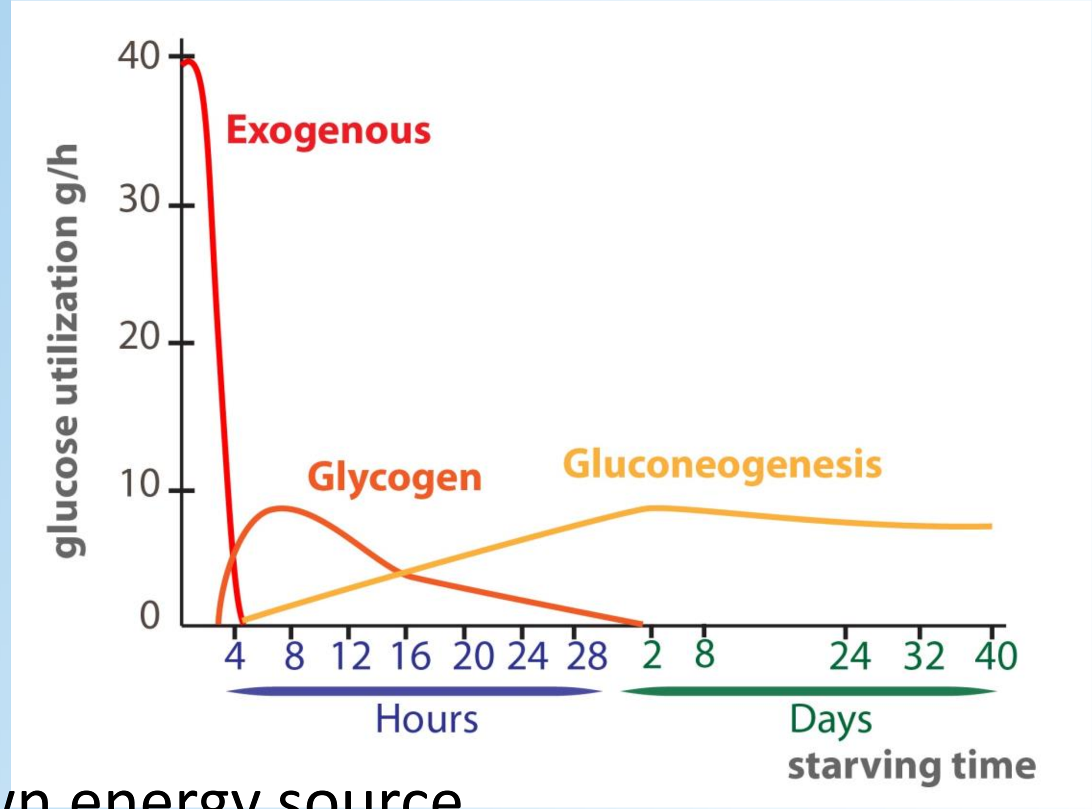
2ADP → 2ATP

Pyruvate Kinase

Pyruvate



Gluconeogenesis basics



- <u>Location:</u> liver (some kidney)
  - Starts in mitochondria with pyruvate
  - Finishes in cytoplasm as glucose

- <u>Purpose:</u>
  maintains blood glucose at 24hrs fasting

- Liver does NOT use gluconeogenesis as its own energy source

- **REQUIRES ACETYL-CoA FROM β-OXIDATION OF FATTY ACIDS IN LIVER**

1. Pyruvate carboxylase (ABC)
2. PEP carboxykinase
3. Fructose-1,6-bisphosphatase
4. Glucose-6-phosphatase

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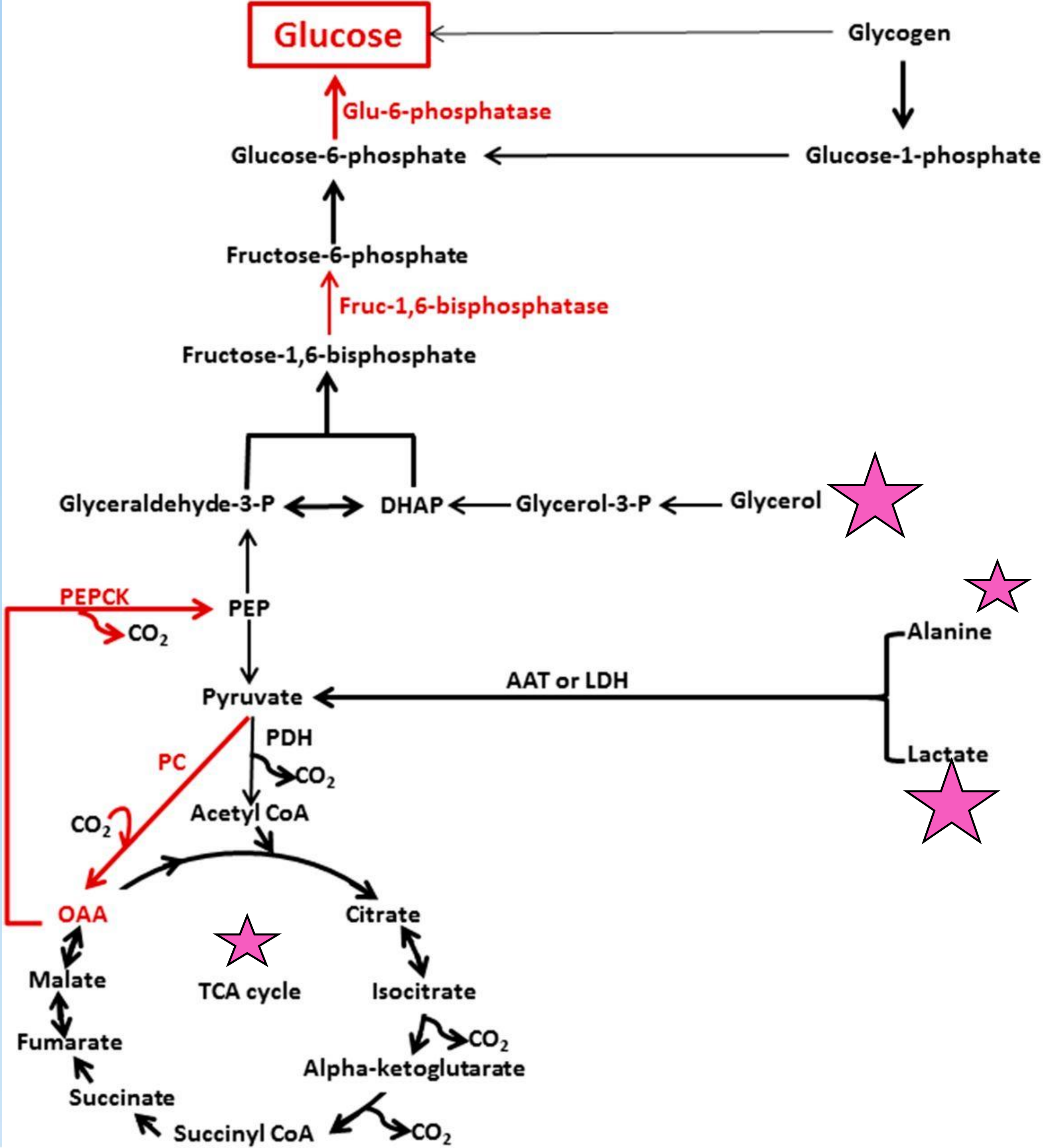
Substrates for gluconeogenesis

From the three different branches!

1. Glycerol-3-phosphate
(from triacylglycerol in fat)

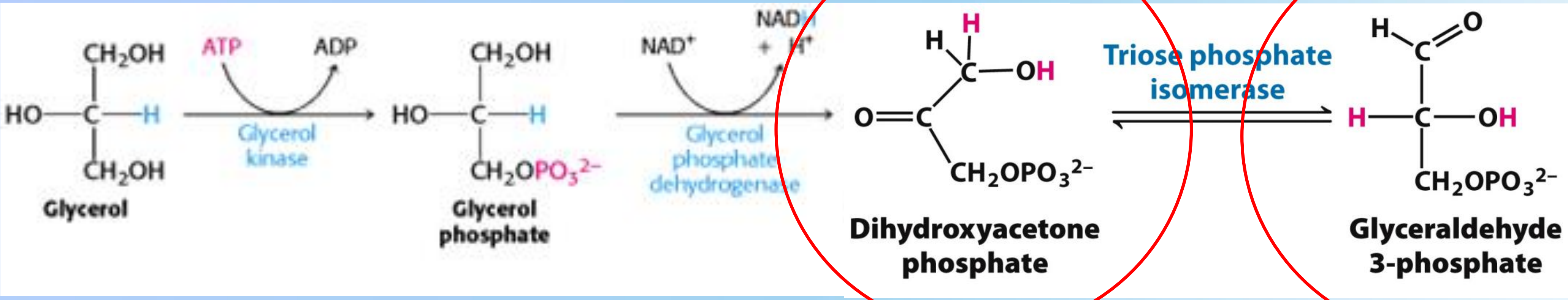
2. Lactate
(from anaerobic glycolysis of carbohydrates)

3. Gluconeogenic amino acids
(individual pathways, proteins)

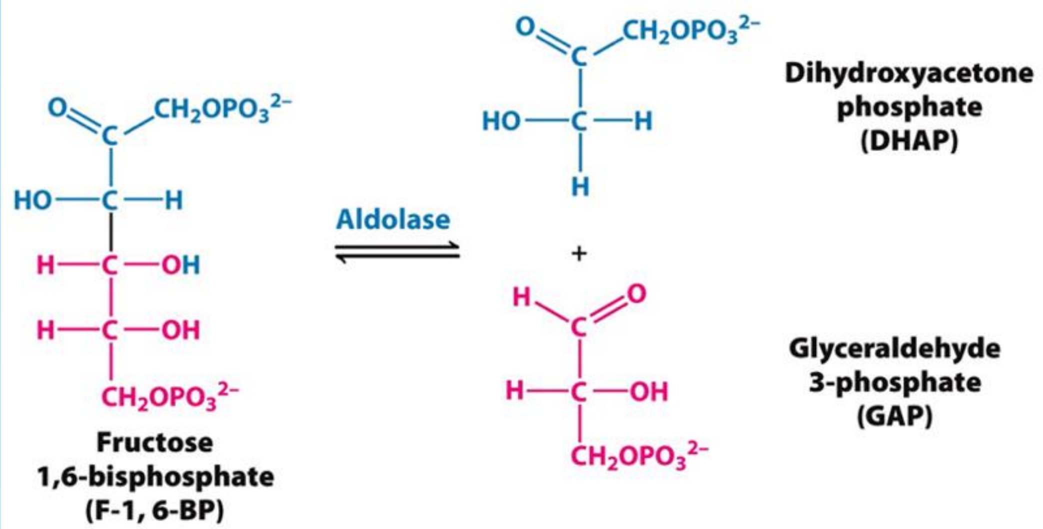


Substrates for gluconeogenesis

1. Glycerol-3-phosphate (fats)



- **Glycerol from** hydrolysis of **triacylglycerol in fat →** liver
- Glycerol kinase *(require ATP)* & glycerol phosphate dehydrogenase
- Converted to F-1,6-BP via aldolase (reversible enzyme)



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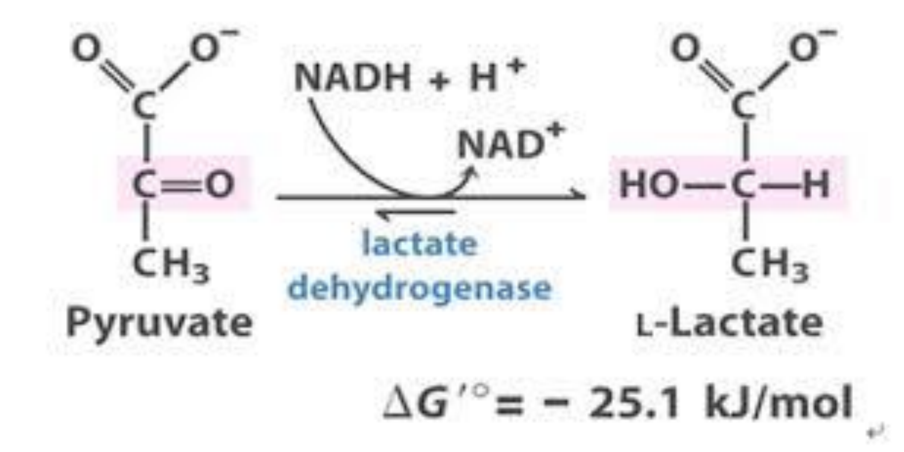
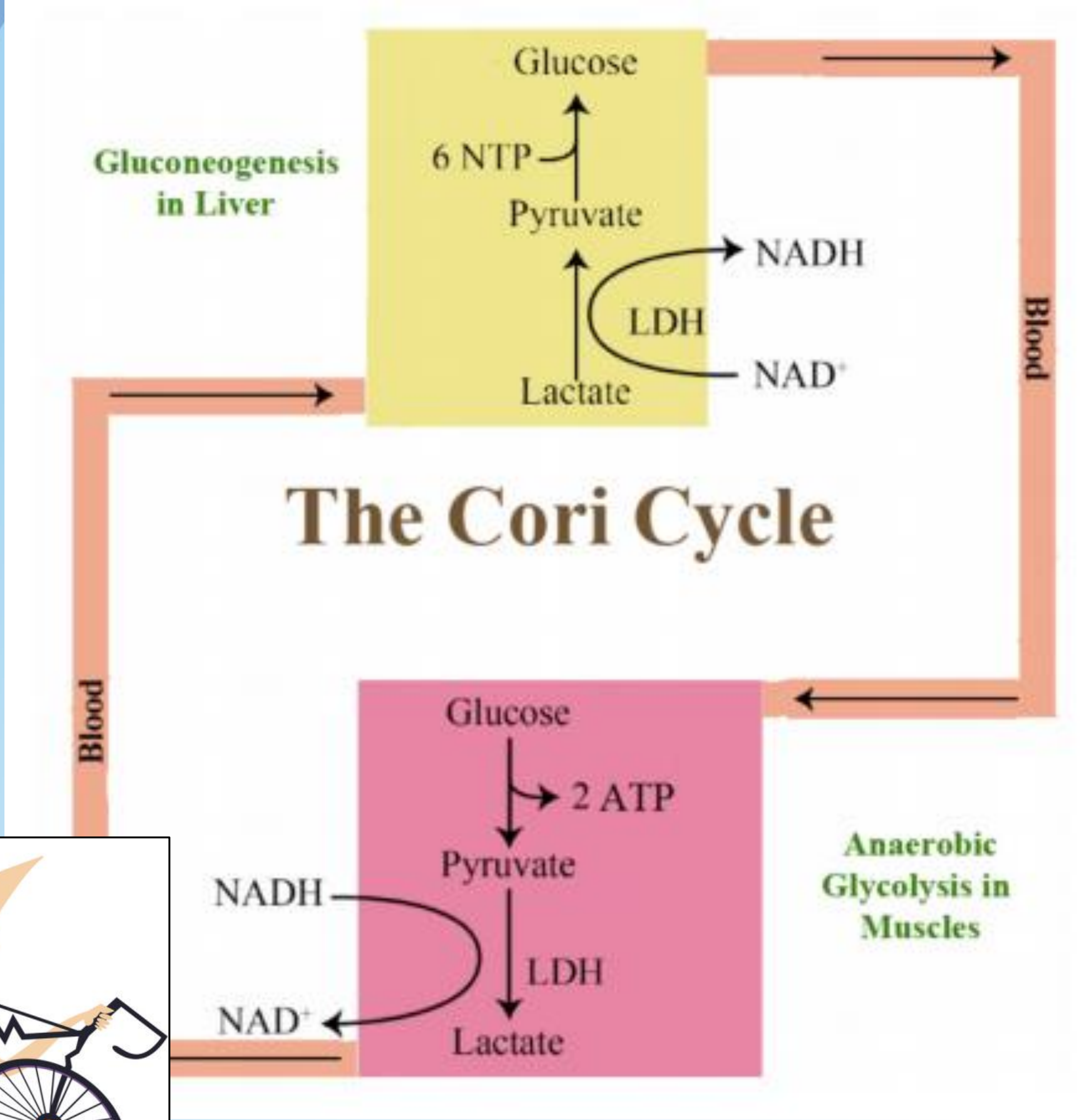
Substrates for gluconeogenesis

2. Lactate (carbohydrate)

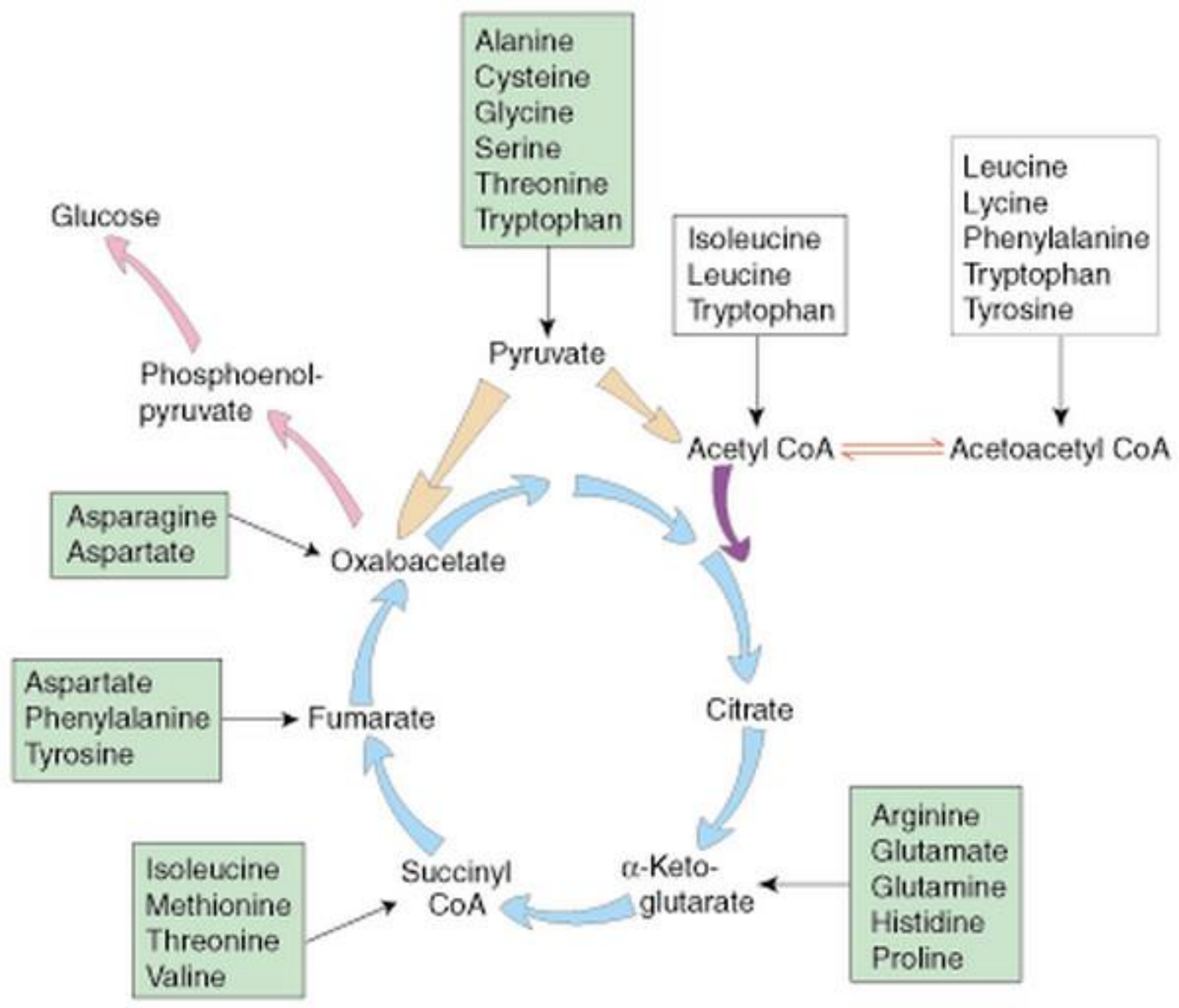
- From anaerobic glycolysis in exercising skeletal muscle and RBCs
- *THE CORI CYCLE*
- Pyruvate to lactate resupplies NAD+ for glyceraldehyde-3-P dehydrogenase





Substrates for gluconeogenesis

3. Gluconeogenic amino acids



All except leucine and lysine!

Major source of glucose during a fast!

Individual pathway conversion to citric acid cycle → malate → *malate shuttle* → OAA → PEP

MAIN AMINO ACID = **ALANINE**

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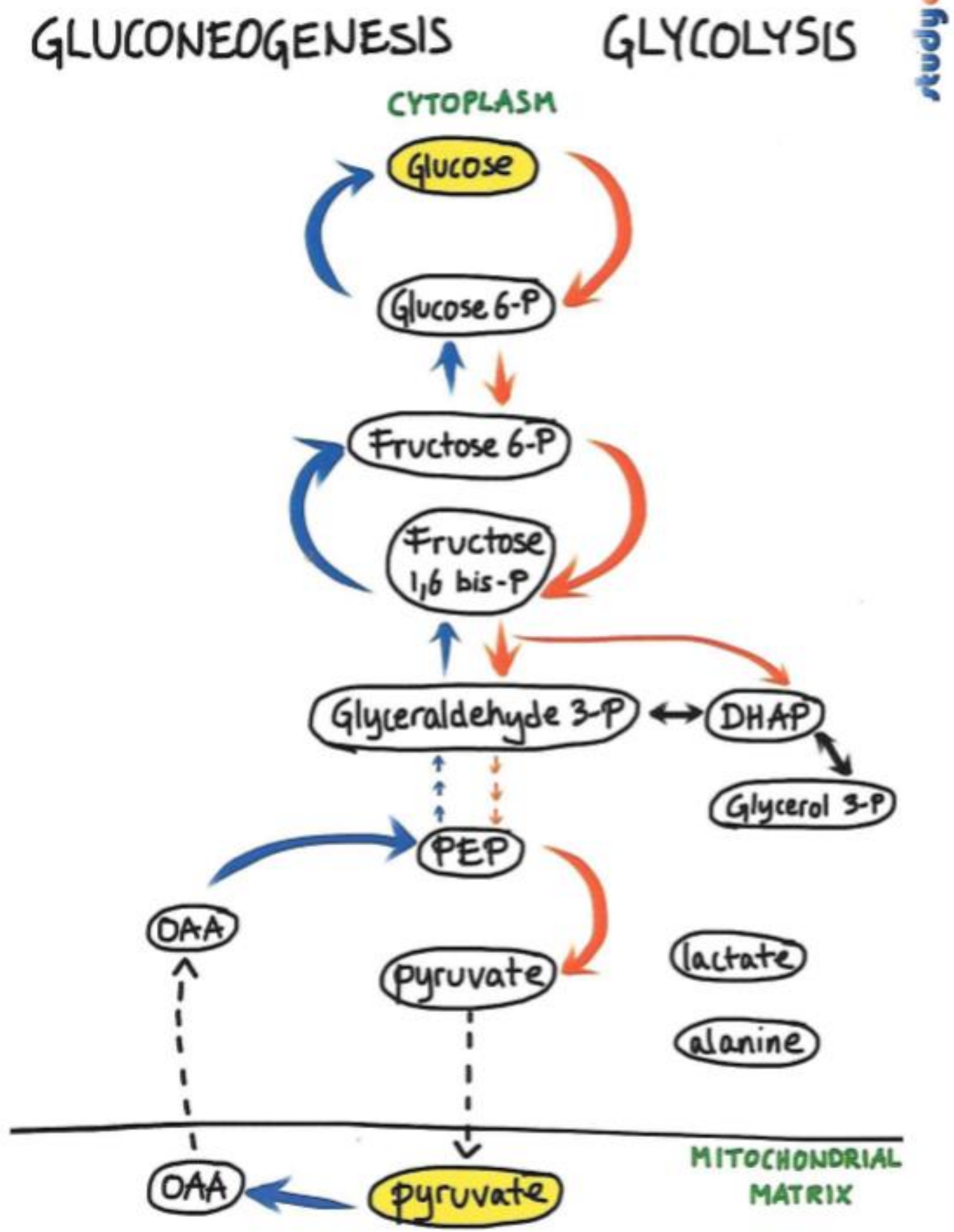
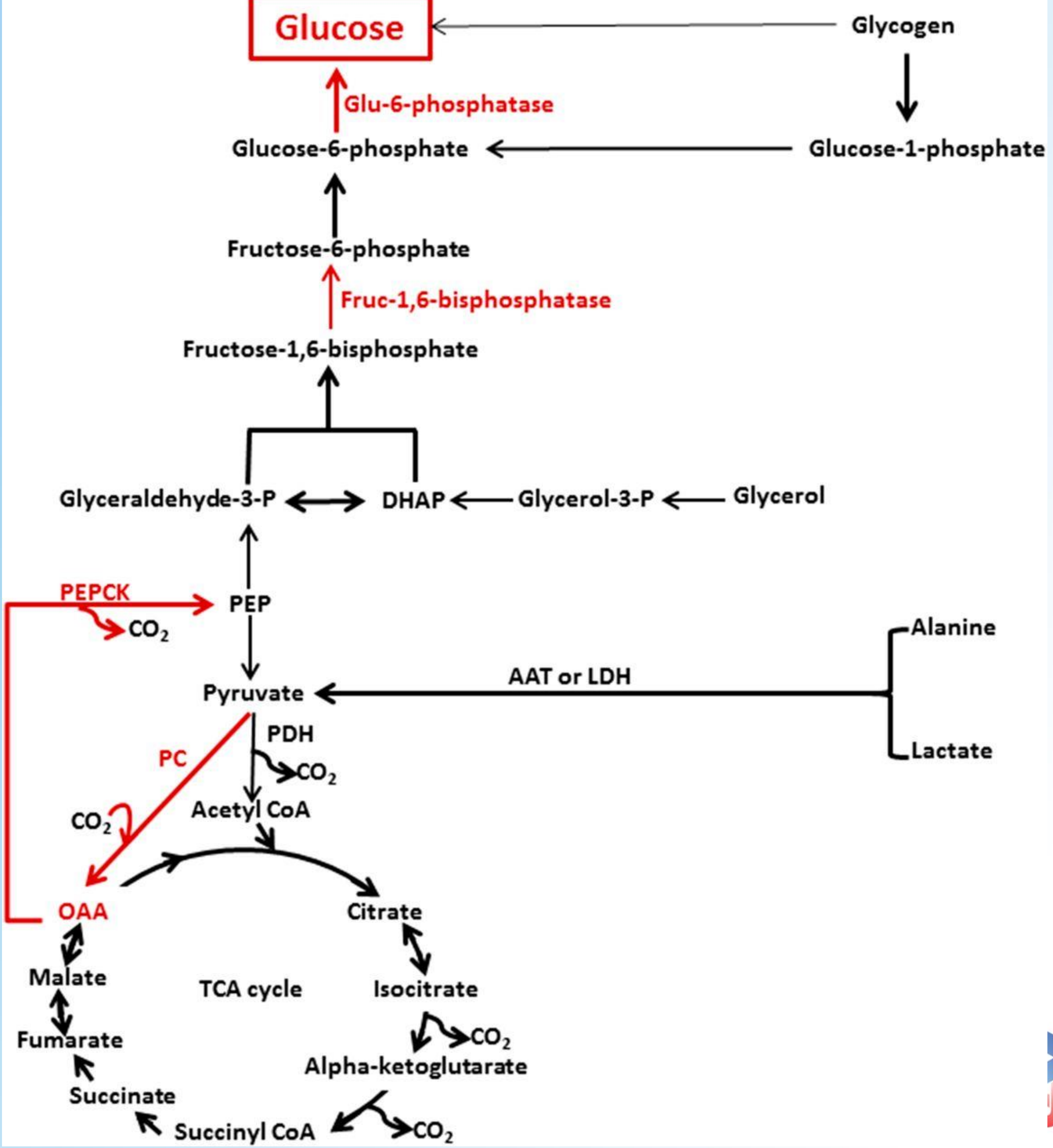

GLUCONEOGENESIS
GLYCOLYSIS
CYTOPLASM
Glucose
Glucose 6-P
Fructose 6-P
Fructose 1,6 bis-P
Glyceraldehyde 3-P
DHAP
Glycerol 3-P
PEP
OAA
pyruvate
lactate
alanine
OAA
pyruvate
MITOCHONDRIAL MATRIX


Glucose
Glycogen
Glu-6-phosphatase
Glucose-6-phosphate
Glucose-1-phosphate
Fructose-6-phosphate
Fruc-1,6-bisphosphatase
Fructose-1,6-bisphosphate
Glyceraldehyde-3-P
DHAP
Glycerol-3-P
Glycerol
PEPCK
PEP
CO_2
Alanine
AAT or LDH
Pyruvate
Lactate
PDH
PC
CO_2
CO_2
Acetyl CoA
OAA
Citrate
Malate
TCA cycle
Isocitrate
Fumarate
CO_2
Alpha-ketoglutarate
Succinate
Succinyl CoA
CO_2

<table>
<tr><th>BYPASS ENZYME</th><th>What is bypassed?</th><th>Note!</th></tr>
<tr><td>Pyruvate carboxylase</td><td rowspan="2">Pyruvate kinase
and
Link reaction</td><td><u>Location:</u> Mitochondrial enzyme
<u>Reaction</u>: pyruvate → oxaloacetate → malate
<u>Activated by:</u> ACETYL-CoA from beta-oxidation!

ABC enzyme! (Uses CO2 from PEPCK)

Malate leaves mitochondria via malate shuttle</td></tr>
<tr><td>Phosphoenol-pyruvate carboxykinase</td><td><u>Location</u>: Cytoplasmic enzyme
<u>Reaction</u>: oxaloacetate → malate → oxaloacetate → PEP + CO2
<u>Activated by:</u> glucagon, cortisol

Reaction requires 2 GTP, releases 2 GDP.</td></tr>
<tr><td>Fructose-1,6-bisphosphatase</td><td>PFK-1</td><td><u>Location</u>: Cytoplasmic enzyme
<u>Reaction</u>: F-1,6-BP → Fructose-6-phosphate
<u>Activated by</u>: ATP
<u>Inhibited by</u>: AMP, fructose-2,6-BP</td></tr>
<tr><td>Glucose-6-phoshatase</td><td>Hexokinase/
glucokinase</td><td><u>Location</u>: In the lumen of the endoplasmic reticulum, ONLY PRESENT IN THE LIVER
<u>Reaction</u>: G-6-P → glucose</td></tr>
</table>

Pyruvate carboxylase

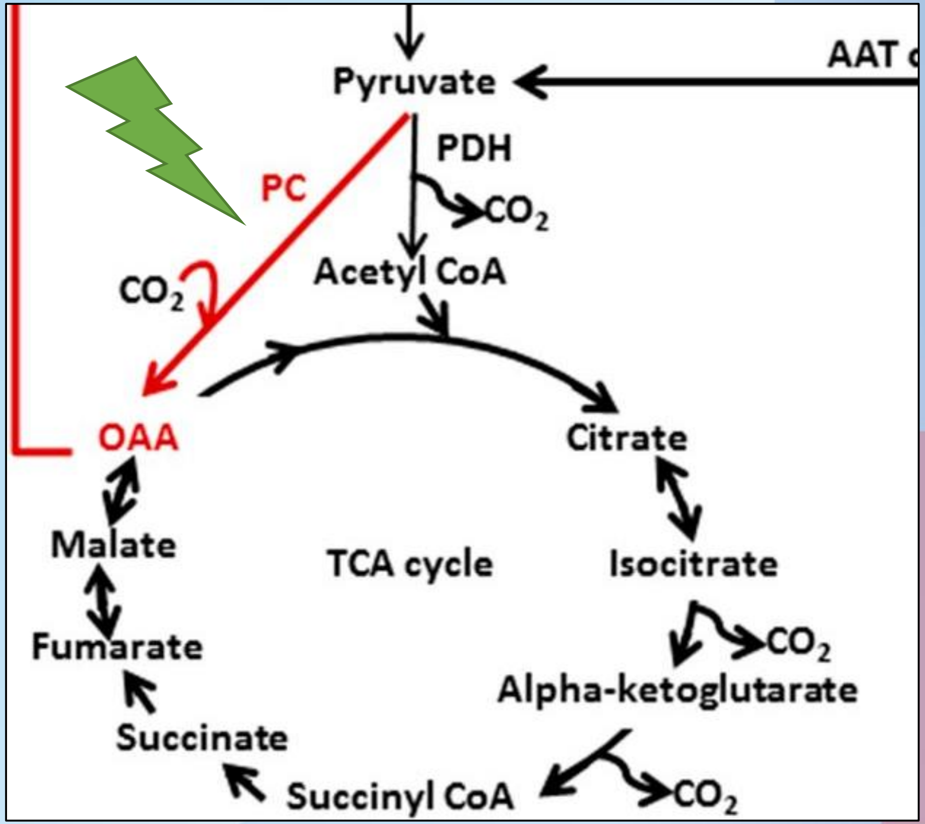
Bypasses: pyruvate kinase and link reaction

Location: Mitochondria

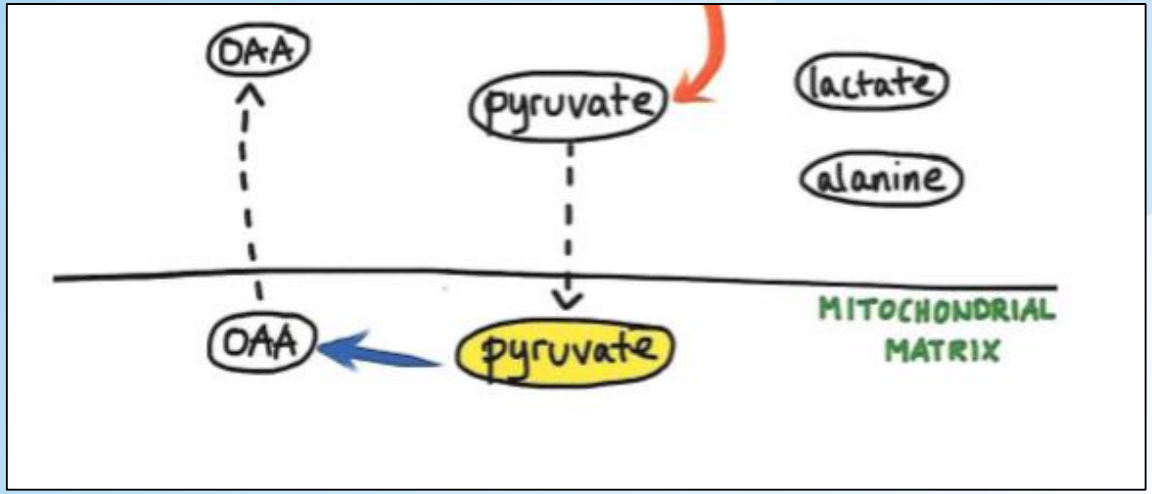
Reaction: **pyruvate → oxaloacetate →** malate

Activated by: ACETYL-CoA from beta-oxidation!

ABC enzyme! (Uses CO2 from PEPCK)



Malate leaves mitochondria via malate shuttle



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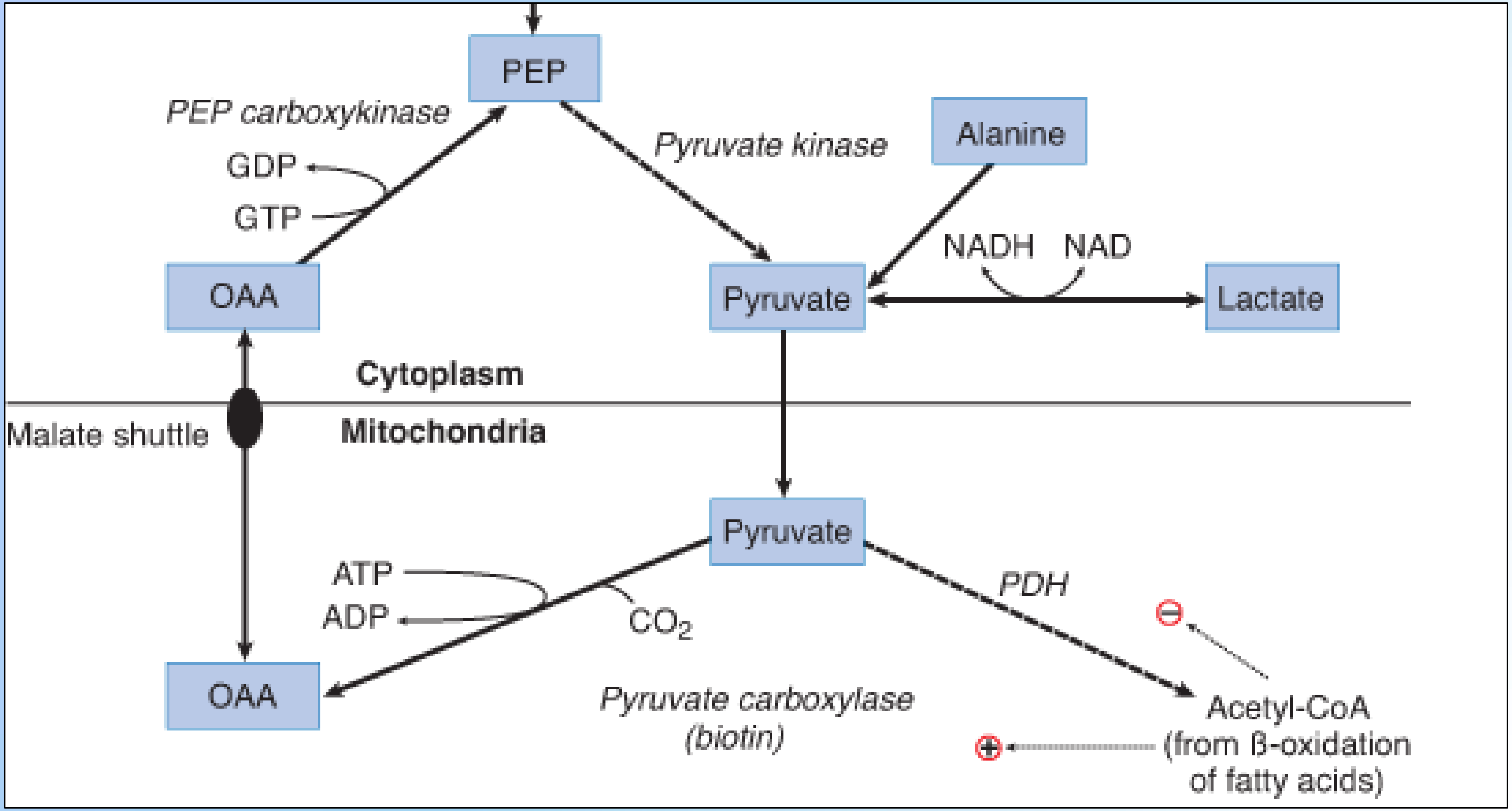
PEP
PEP carboxykinase
GDP
GTP
Pyruvate kinase
Alanine
OAA
NADH
NAD
Pyruvate
Lactate
Cytoplasm
Malate shuttle
Mitochondria
Pyruvate
ATP
ADP
CO_2
PDH
OAA
Pyruvate carboxylase
(biotin)
Acetyl-CoA
(from ß-oxidation
of fatty acids)

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Phosphoenolpyruvate carboxykinase

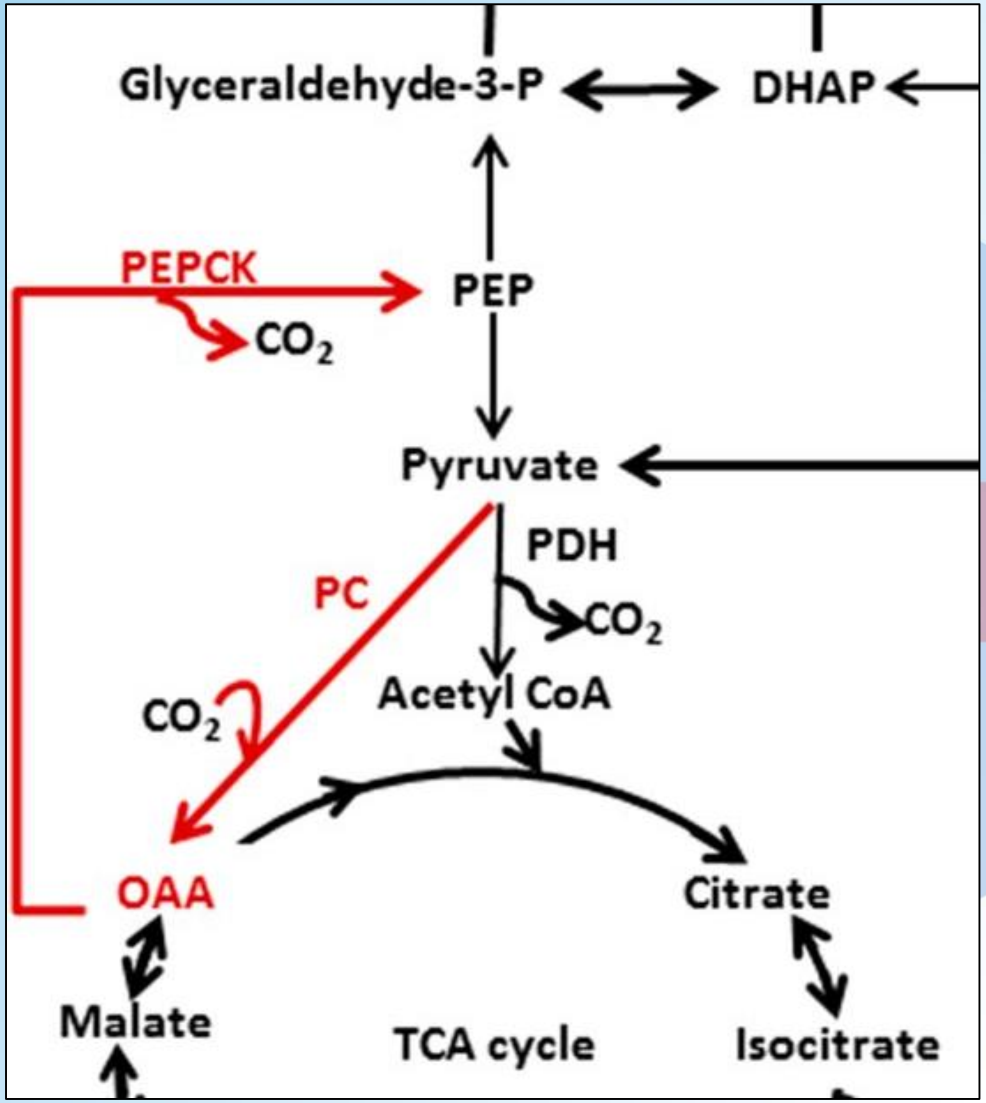
Location: Cytoplasm

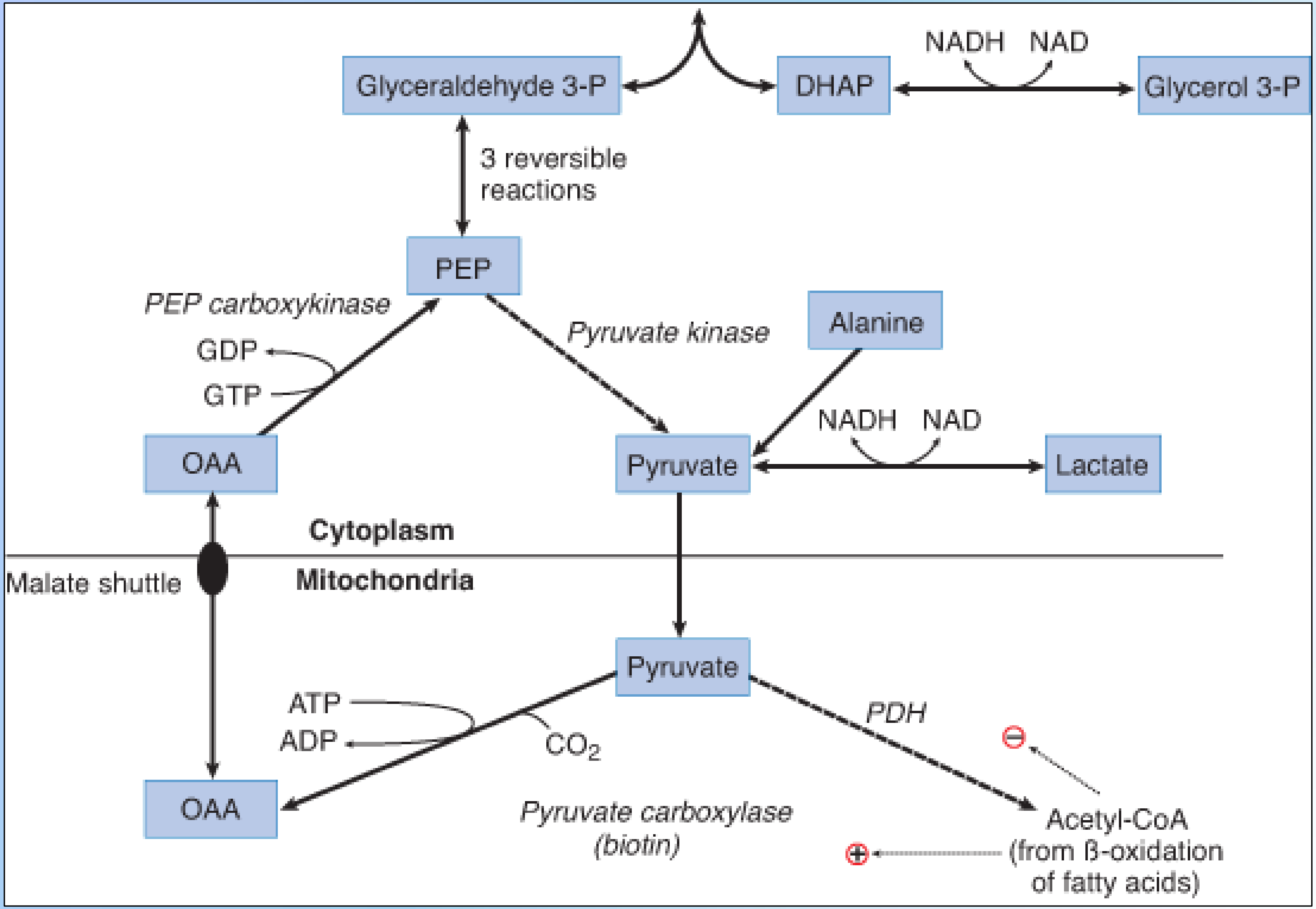
Reaction: **oxaloacetate →** *malate shuttle* **→ oxaloacetate → PEP + CO2**

Activated by:

glucagon, cortisol

Reaction requires GTP, releases GDP *(two molecules required to make glucose)*





Glyceraldehyde 3-P
DHAP
NADH
NAD
Glycerol 3-P
3 reversible reactions
PEP
PEP carboxykinase
GDP
GTP
Pyruvate kinase
Alanine
NADH
NAD
OAA
Pyruvate
Lactate
Cytoplasm
Malate shuttle
Mitochondria
Pyruvate
ATP
ADP
CO_2
PDH
OAA
Pyruvate carboxylase (biotin)
Acetyl-CoA (from β-oxidation of fatty acids)

Fructose-1,6-bisphosphatase

Rate-determining enzyme!!

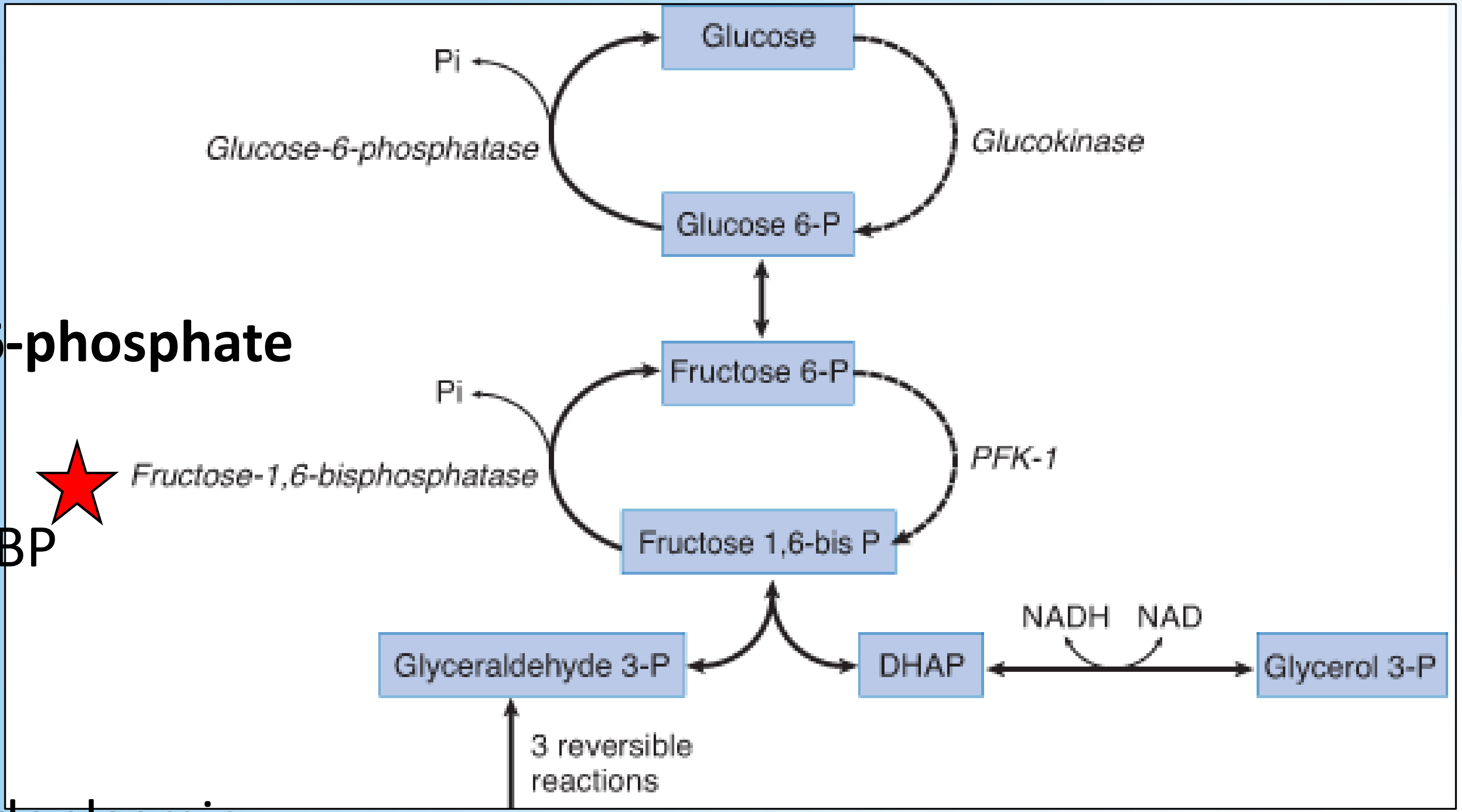
<u>Location</u>: Cytoplasm

<u>Bypassing:</u> PFK-1

<u>Reaction</u>: **F-1,6-BP → Fructose-6-phosphate**

<u>Activated by</u>: ATP

<u>Inhibited by</u>: AMP, fructose-2,6-BP



Glucose-6-phosphatase

<u>Location</u>: In the lumen of the endoplasmic reticulum, *ONLY PRESENT IN THE LIVER*

<u>Bypassing:</u> Glucokinase

<u>Reaction</u>: **G-6-P → glucose**

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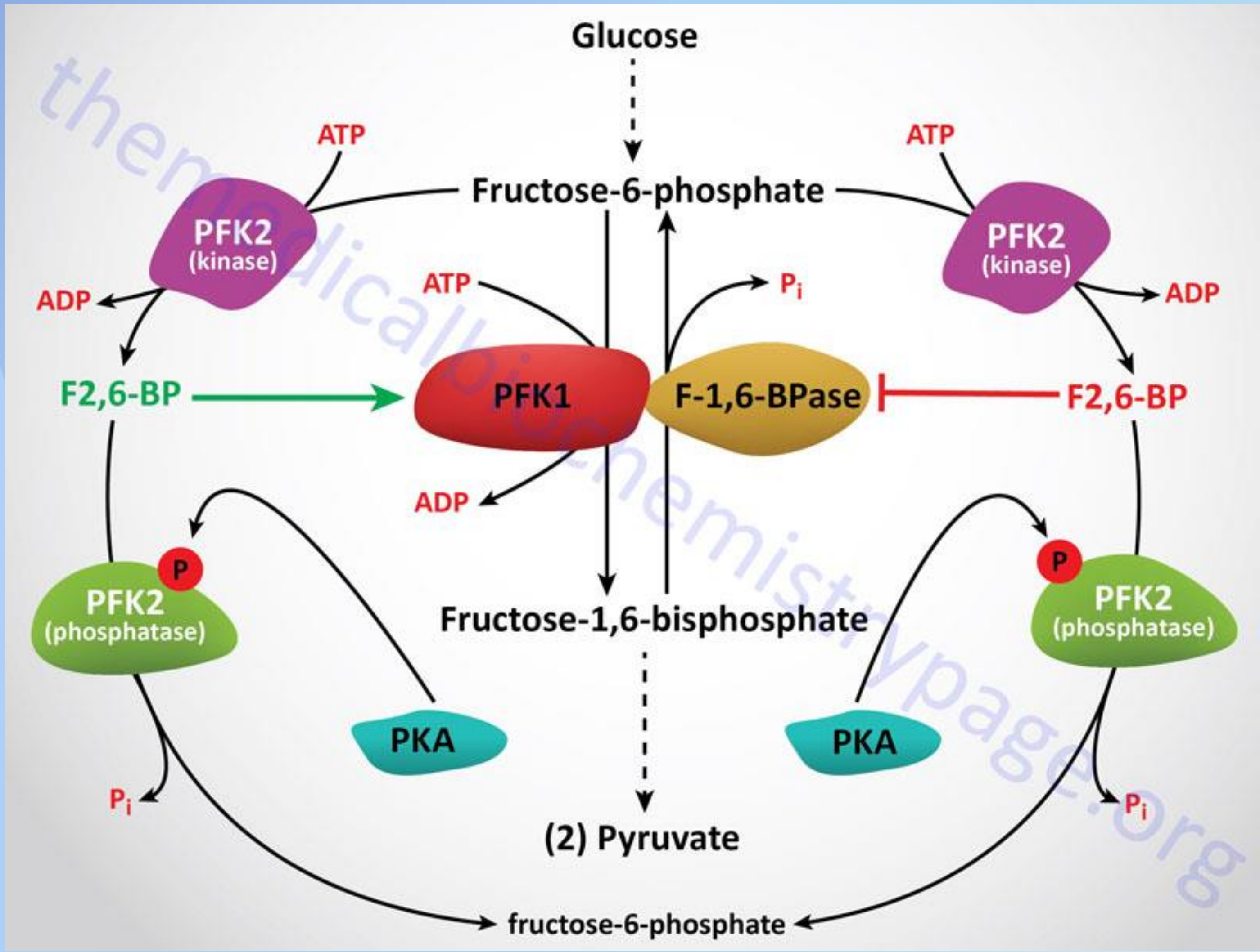
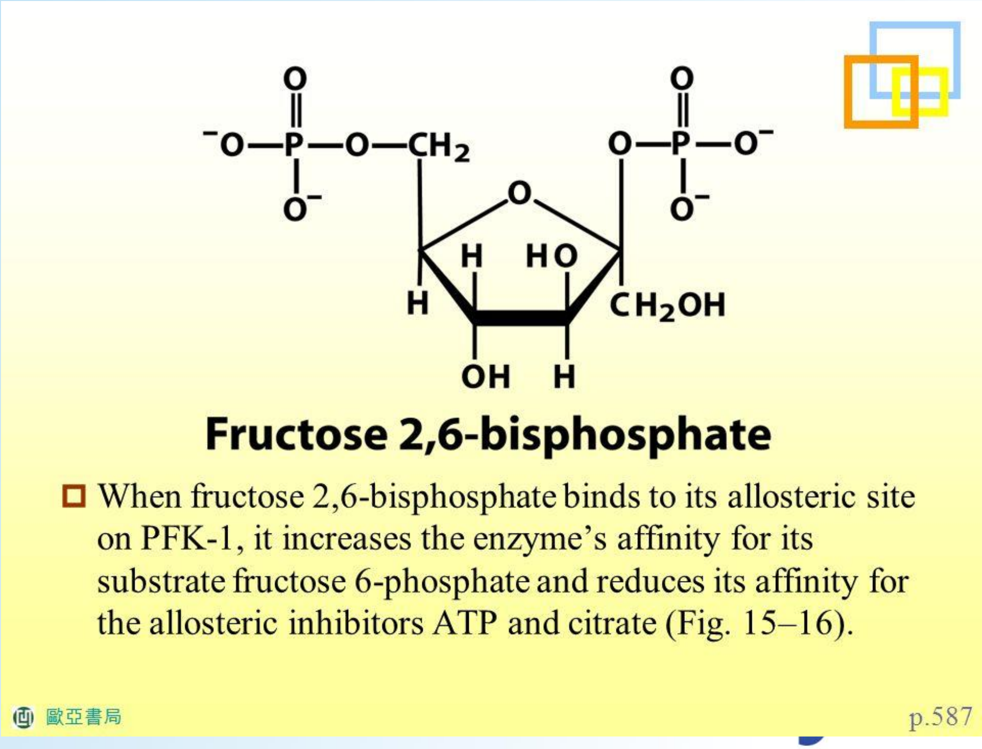
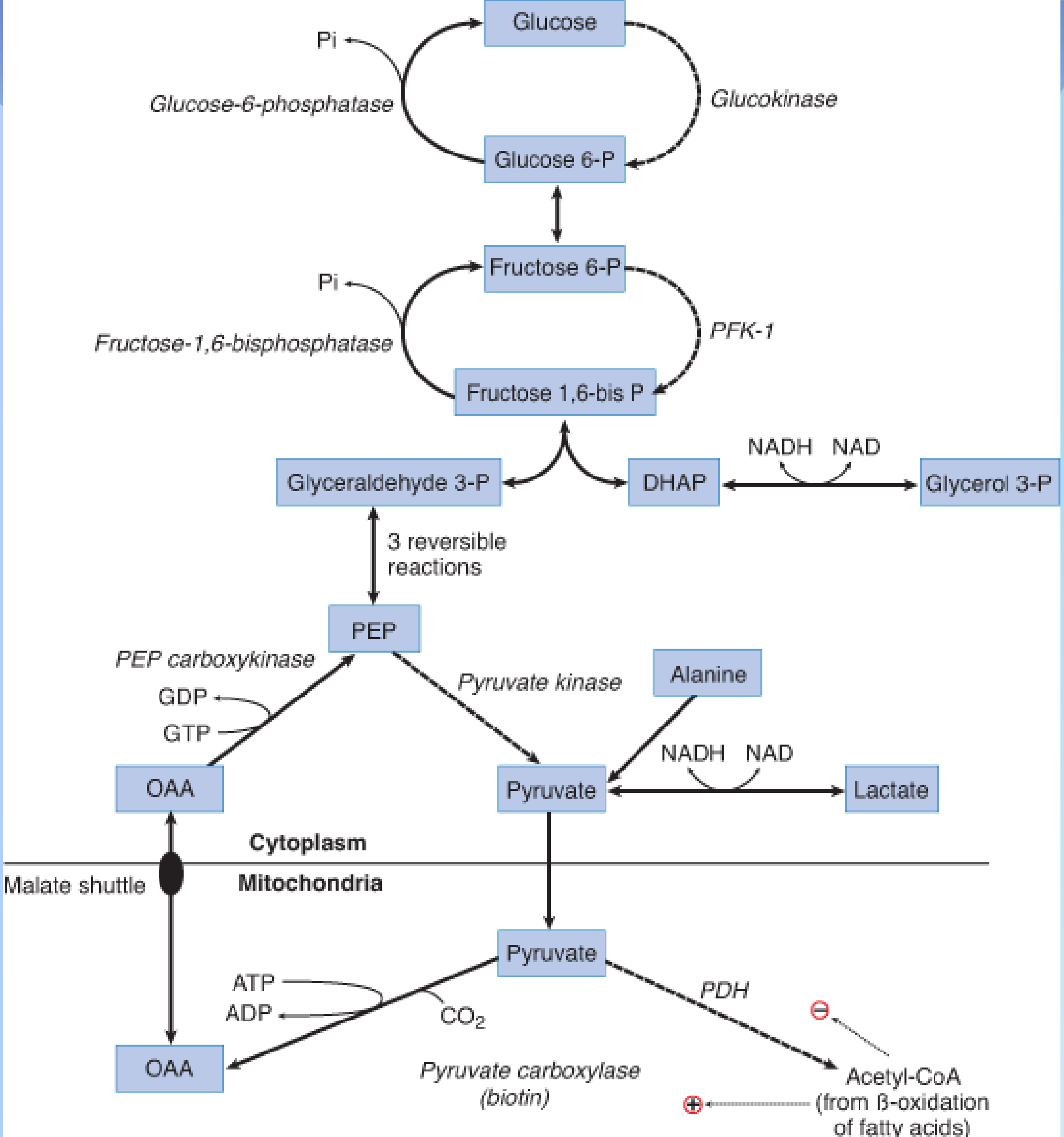
The importance of fructose-2,6-bisphosphate



- F2,6-BP simulates glycolysis, and inhibits gluconeogenesis
  - A sister and a Big Brother
- PFK2 = bifunctional enzyme




Glucose
Pi
Glucose-6-phosphatase
Glucokinase
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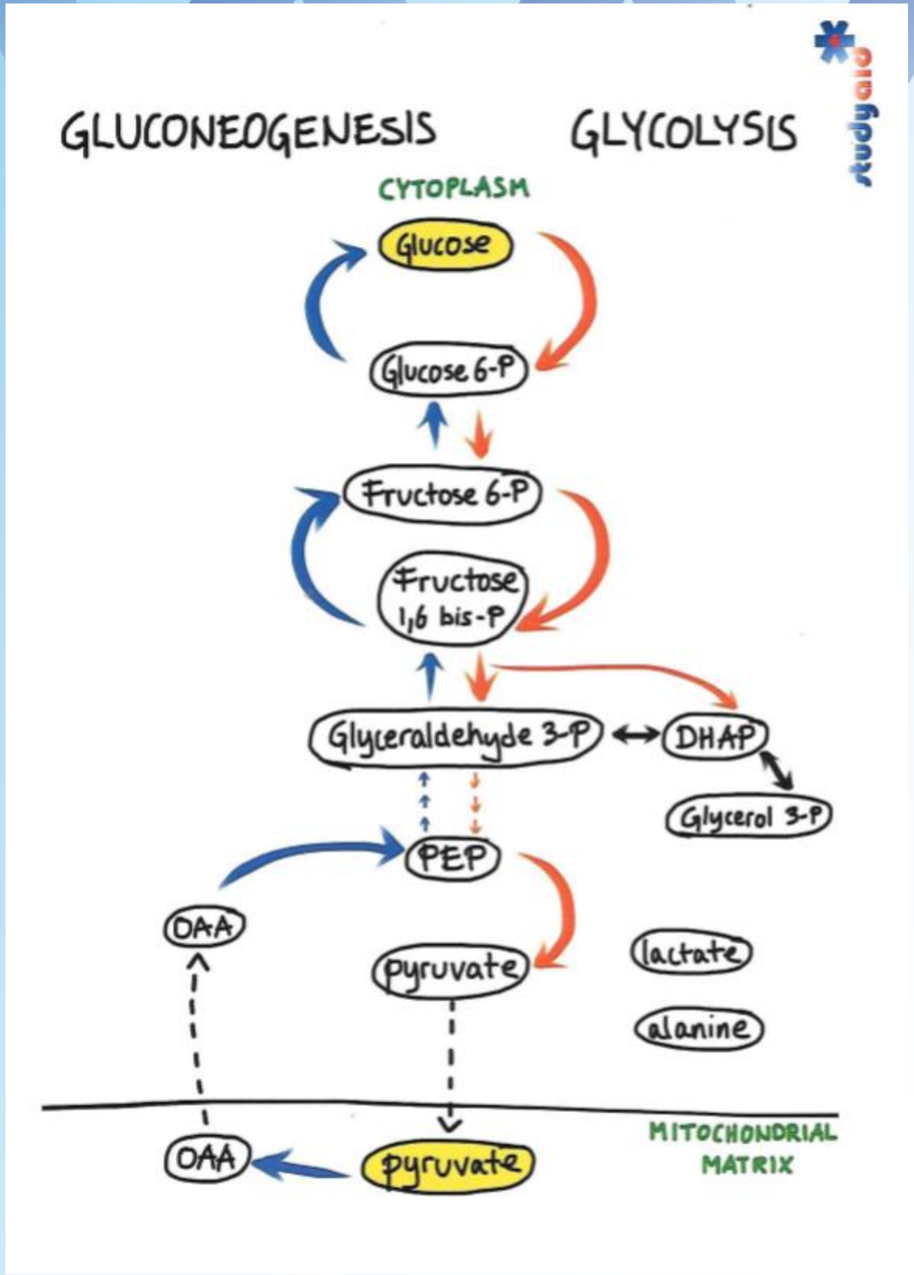
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MITOCHONDRIAL MATRIX

BYPASS ENZYME	What is bypassed?	Note!
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Phosphoenol-pyruvate carboxykinase		Location: Cytoplasmic enzyme Reaction: **oxaloacetate →** malate **→ oxaloacetate → PEP + CO_2** Activated by: glucagon, cortisol Reaction requires GTP, releases GDP.
Fructose-1,6-bisphosphatase	PFK-1	Location: Cytoplasmic enzyme Reaction: **F-1,6-BP → Fructose-6-phosphate** Activated by: ATP Inhibited by: AMP, fructose-2,6-BP
Glucose-6-phoshatase	Glucokinase	Location: In the lumen of the endoplasmic reticulum, ONLY PRESENT IN THE LIVER Reaction: **G-6-P → glucose**


To Do List
1. ~~Why gluconeogenesis?~~
2. ~~Substrates, location, precursors~~
3. ~~FOUR NEW ENZYMES~~
4. Regulation of gluconeogensis
5. QUIZ
2019

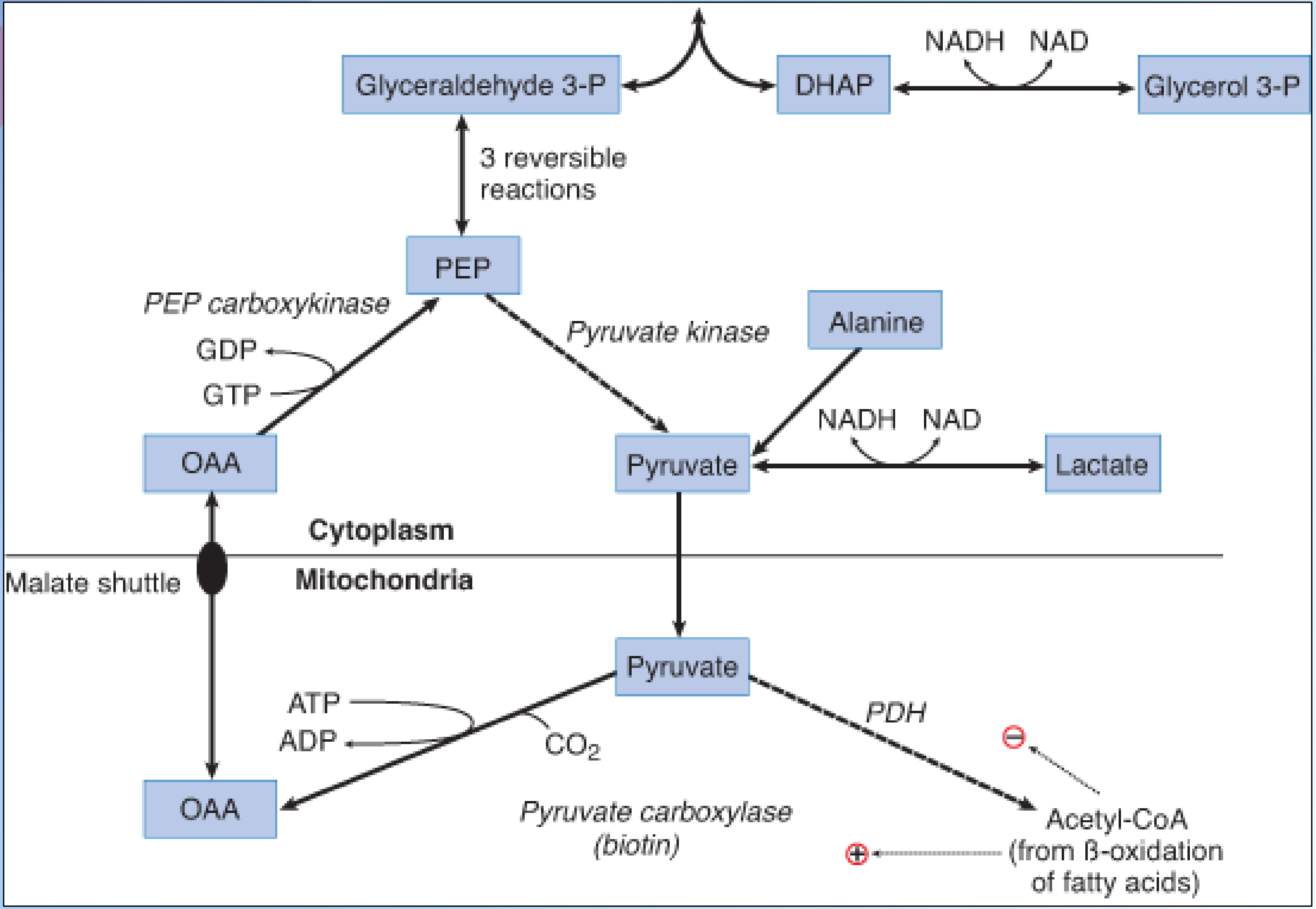
Regulation of gluconeogenesis x4

1. **GLUCAGON**
   - Gs coupling = ↑ cAMP = ↑ PKA activity = makes PFK-2 into a phosphatase = ↓ levels of frucose-2,6-bisphosphate = ↓ inhibition of F-1,6-bisphosphatase
   - ↑ PKA also inactivates hepatic pyruvate kinase
   - Increases transcription of PEP carboxykinase

2. Substrate availability

3. **Allosteric activation by acetyl coenzyme A**

4. Allosteric inhibition by AMP



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Cytoplasm
Malate shuttle
Mitochondria
Pyruvate
ATP
ADP
CO_2
PDH
OAA
Pyruvate carboxylase
(biotin)
Acetyl-CoA
(from ß-oxidation
of fatty acids)

QUIZ

Which of the following compounds cannot serve as a substrate for gluconeogenesis?

a) Glycerol-3-phosphate
b) Oxaloacetate
c) α-ketoglutarate
d) Lactate
e) Acetoacetate

Answer: e

Glycerol, lactate and ketoacids (gluco/ketogenic amino acids) are the three main pathways to create new glucose during a prolonged fast. Acetoacetate is an ketone body and is converted via the TCA directly to ATP, never to glucose.

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Which of the following is an allosteric activator of glycolysis and inhibitor of gluconeogenesis?

a) AMP
b) ATP
c) Citrate
d) F-1,6-BP
e) Acetyl-CoA

Answer: a

AMP (and F-2,6-BP) activate glycolysis and also inhibit gluconeogenesis. F-2,6-BP does so through its positive action on PFK1 (enhancing glycolysis as the rate-limiting step), while it also negatively affects F-2,6-Bpase (at the rate-limiting step).

Many enzymes are involved in reactions concerning pyruvate. Recall which enzyme acts in which pathway.

a) Pyruvate carboxylase
b) Lactate dehydrogenase
c) Pyruvate kinase
d) Pyruvate dehydrogenase

a) Pyruvate carboxylase = gluconeogenesis. This mitochondrial enzyme requires ATP, biotin and CO2. The obligate activator is acetyl CoA. The product, oxaloacetate, is acted upon by PEPCK to create PEP in the cytosol.

b) Lactate dehydrogenase = Cori cycle. The reaction requires vitamin B3 (NAD+/NADH). Lactate is the end-product of anaerobic metabolism, which is the major energy pathway in RBCs, WBCs, kidney medulla, lens, testes and cornea.

c) Pyruvate kinase = glycolysis. It converts PEP to pyruvate during glycolysis. It is stimulated by F-1,6-BP, and inhibited by ATP and alanine.

d) Pyruvate dehydrogenase = Link reaction, converting pyruvate to acetyl CoA. It requires vitamin B1, B2, B3, B5 and lipoic acid. The reaction is inhibited by acetyl CoA.

Which effect is NOT seen by glucagon in gluconeogenesis?

a) Induces degradation of F-2,6-BP

b) Inhibition of PFK-1

c) Gi-coupling inactivates hepatic PFK through phosphorylation

d) Gs-coupling inactivates hepatic PFK through phosphorylation

e) Increases Vmax of oxaloacetate to phosphoenolpyruvate

Answer: C

Glucagon stimulates Gs receptors and indirectly causes phosphorylation of liver PFK2, turning PFK2 into a phosphatase enzyme. This leads to hydrolysis/degradation of F-2,6-BP. Glucagon also inactivates PFK-1 through phosphorylation. In addition, glucagon increases transcription of PEPCK, increasing the Vmax of the reaction.

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Which of the following is true of the Cori cycle?

a. Synthesizes glucose in muscle during strenuous activity
b. Primarily occurs during sleep
c. Produces glucose in the liver from alkaline metabolites in skeletal muscle and red blood cells
d. Never used by red blood cells
e. Hepatocytes recycle lactate from exercising muscle to glucose

Answer: e

The Cori cycle recycles lactate in exercising skeletal muscle and red blood cells by transporting lactate to the liver and converting it to glucose via gluconeogenesis. Glucose production in skeletal muscle during strenous activity is via glycogenolysis. Lactate is an acidic molecule, causing an anion gap metabolic acidosis with excessive amounts. Because red blood cells lack a mitochondrion for TCA/ETC, lactate is always the end-product of glycolysis and RBCs heavily rely on the Cori Cycle to prevent hemolysis.

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Which amino acid is purely ketogenic?

a. Glycine

b. Serine

c. Leucine

d. Isoleucine

e. Alanine

Answer: C

Leucine and lysine are not able to be converted into glucose. Instead, they are converted into ketone bodies