CARBOHYDRATE METABOLISM – GLUCONEOGENESIS

Prof. Michele Di Foggia

Dipartimento di Scienze Biomediche e Neuromotorie – DIBINEM – via Irnerio 48, Bologna

GLUCONEOGENESIS

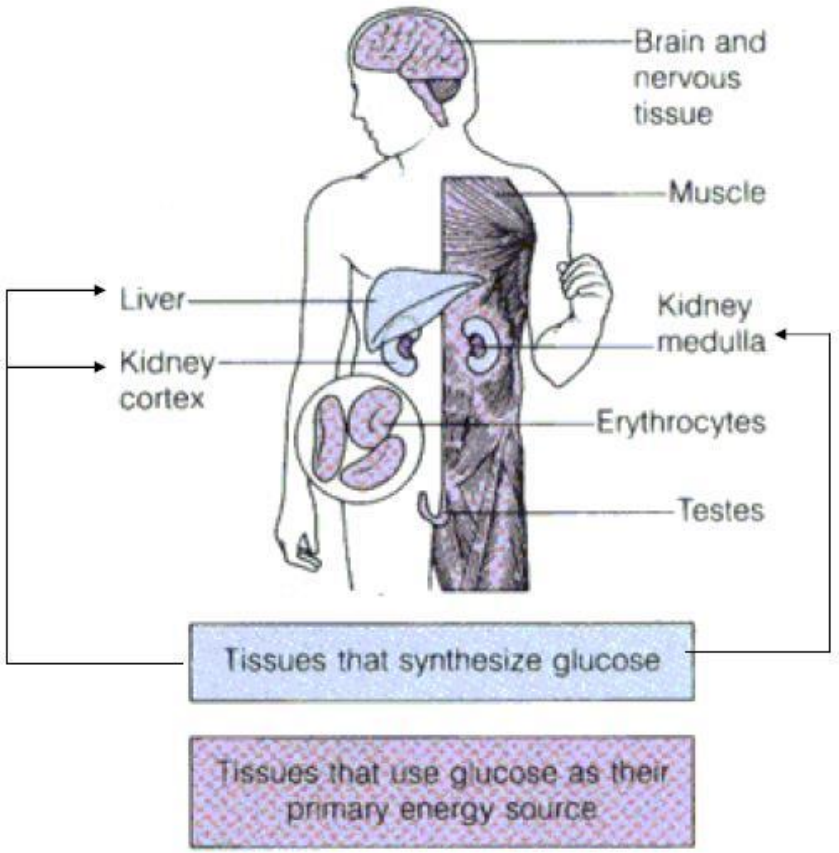
The **brain** depends on a continuous supply of **glucose**: brain cannot synthesize glucose or store much glycogen.

All the glucose found generally in the bloodstream can supply the brain for no more than one hour: low blood sugar (**hypoglycemia**) has rapid neural consequences.

When dietary sources are unavailable, and liver has exhausted glycogen, glucose must be synthesised from non-carbohydrate sources: **Gluconeogenesis** can provide a substantial part of blood glucose just a few hours after eating.

GLUCONEOGENESIS

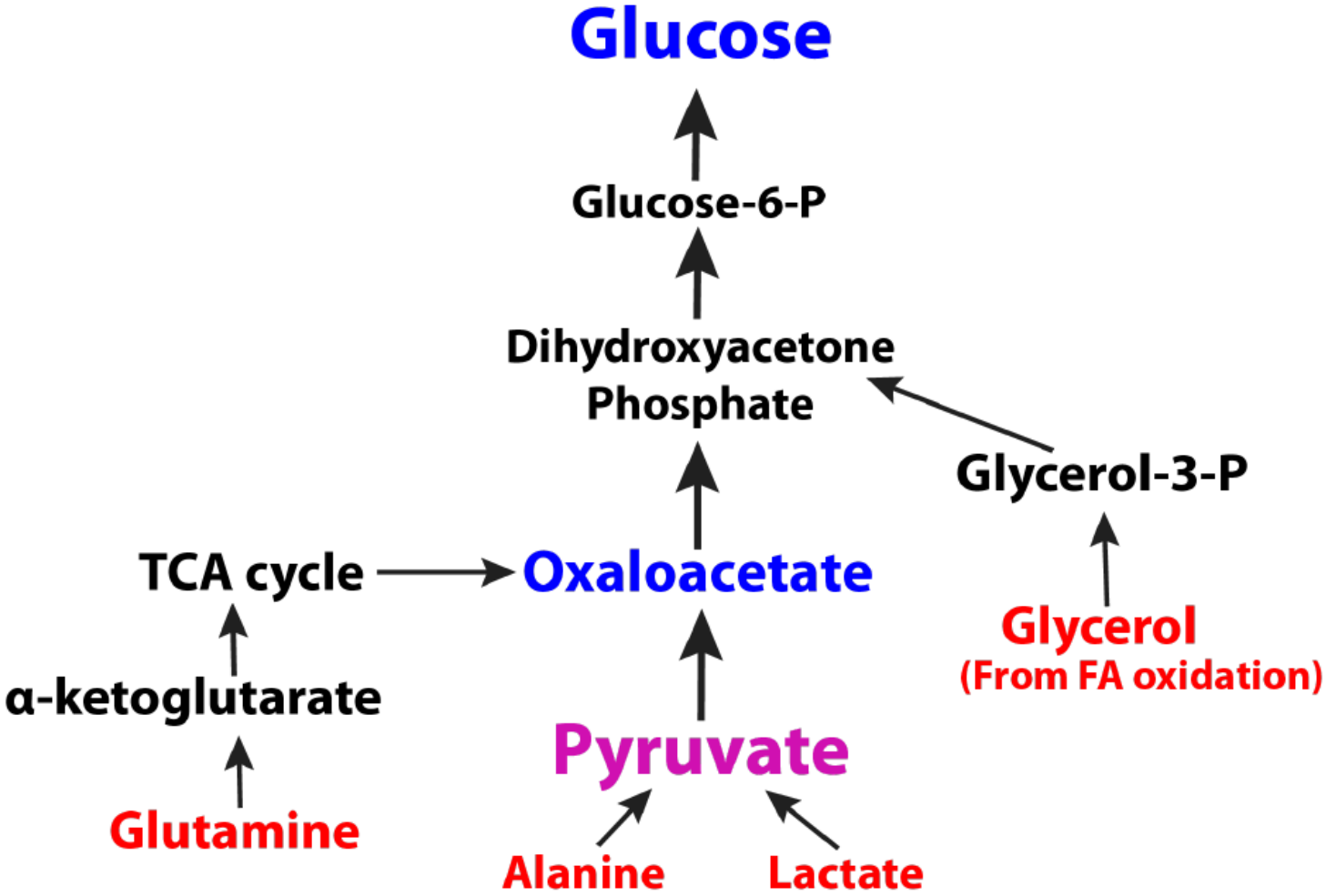
Liver, kidney cortex



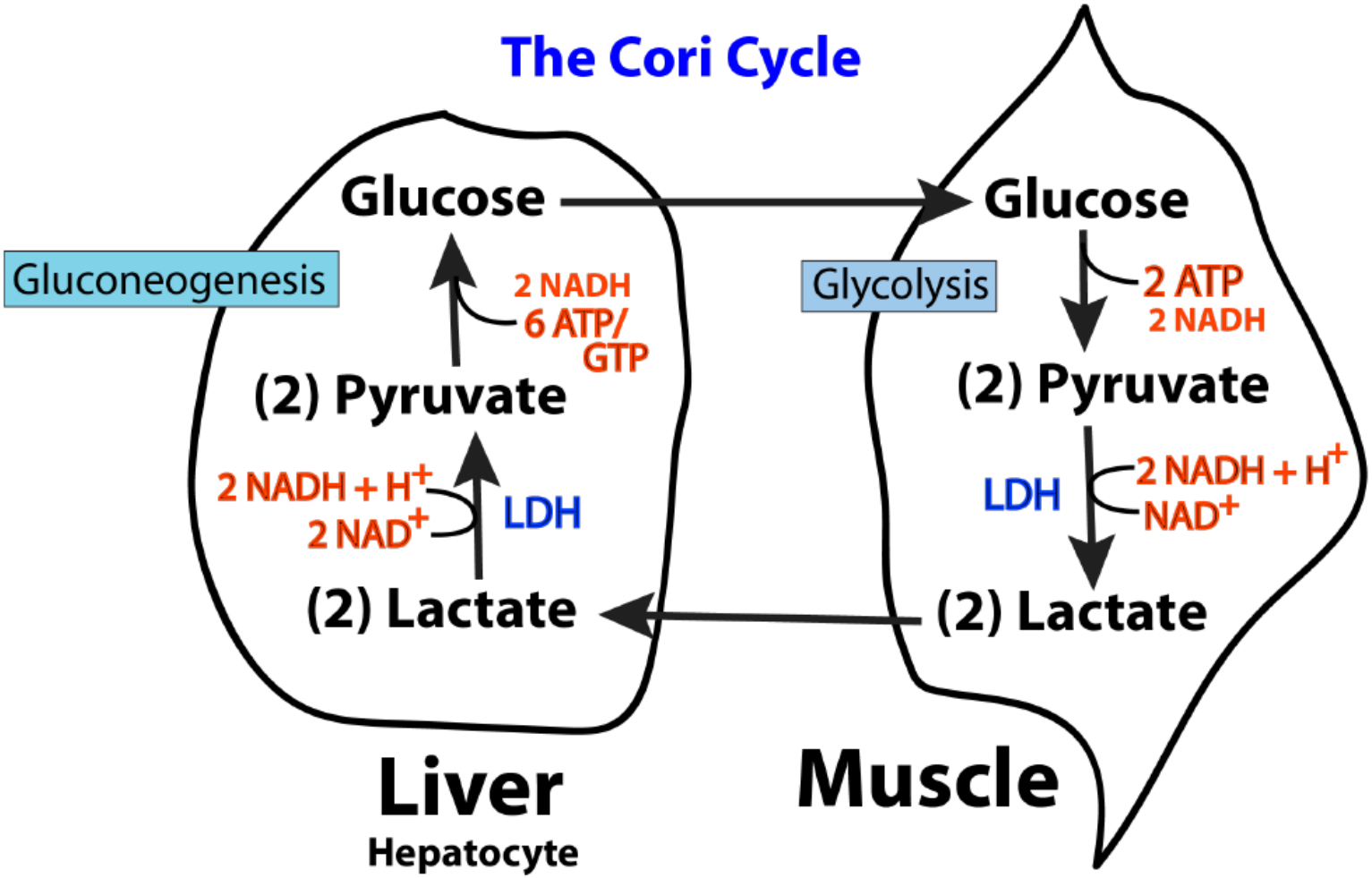
Gluconeogenic sources:

- *Lactate*
- *Pyruvate*
- *Glycerol*
- *Glucogenic Amino Acids* (Gly, **Ala**, Cys, Ser, Asp, Asn, Glu, **Gln**, Pro, Arg, His, Val, Met, Thr) or *partly glucogenic* (Trp, Ile, Phe, Tyr).

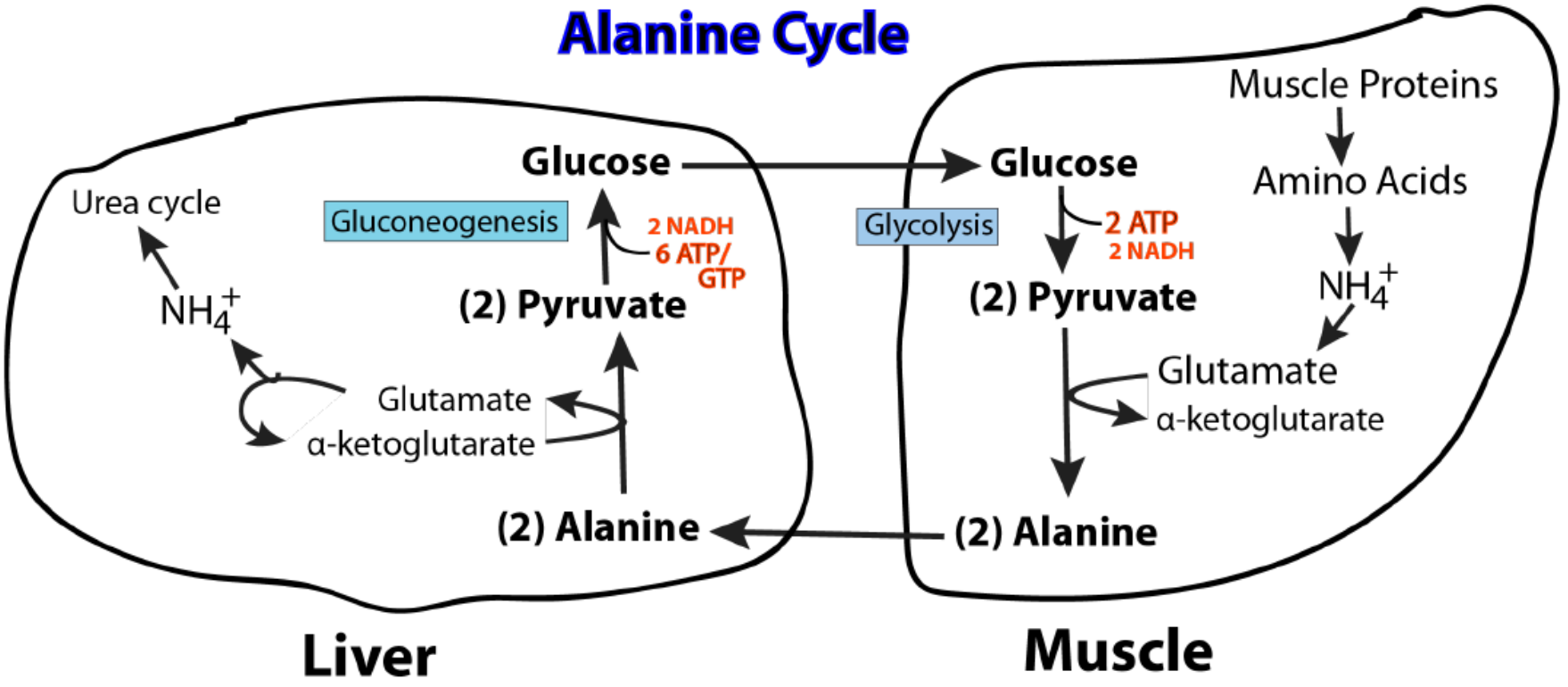
GLUCONEOGENESIS



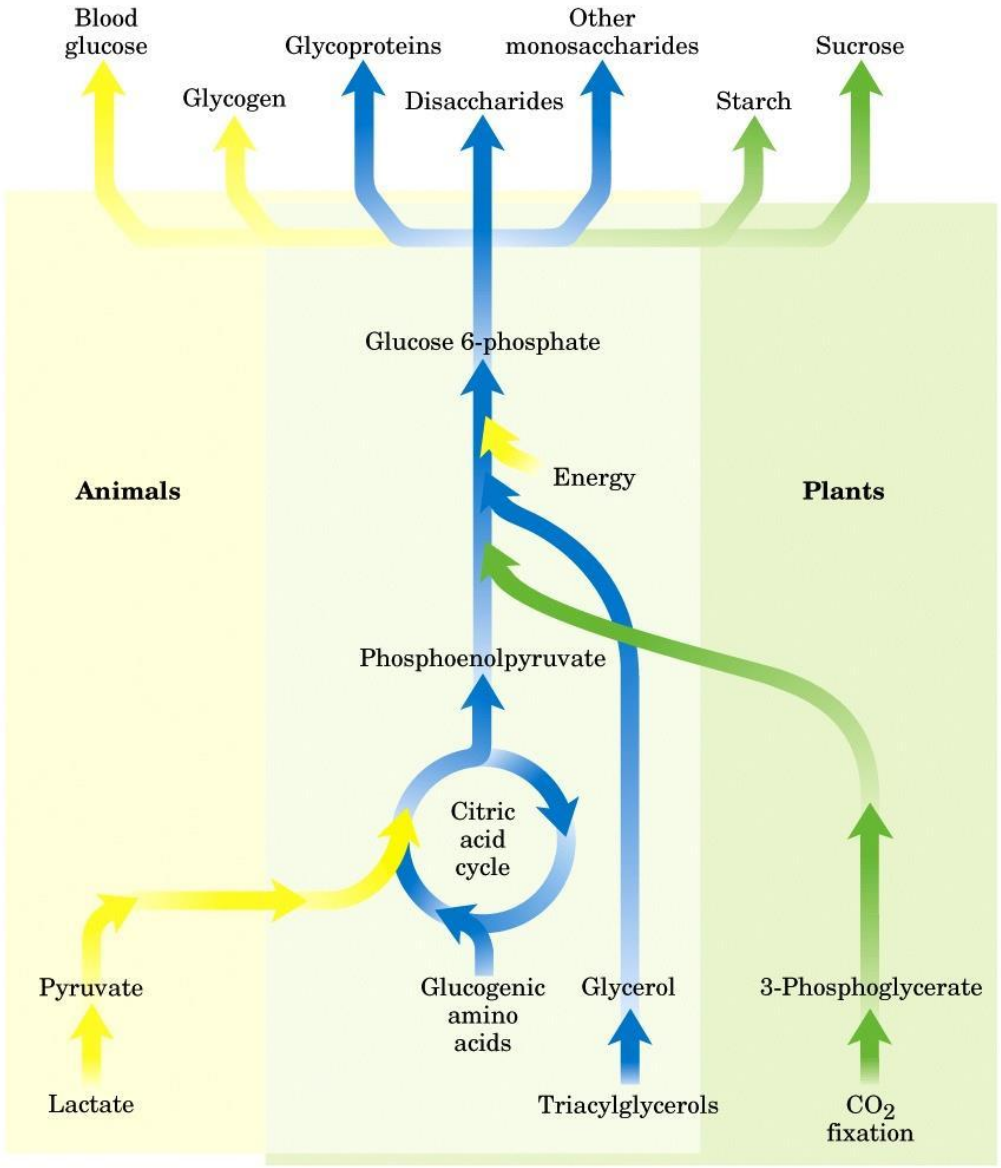
GLUCONEOGENESIS



GLUCONEOGENESIS



GLUCONEOGENESIS



GLUCONEOGENESIS

Gluconeogenesis follows the steps of glycolysis in reverse, except for the **three irreversible reactions**, which are bypassed by three distinct sets of reactions.

While glycolysis is totally cytosolic, the **first bypass** of gluconeogenesis follows some **mitochondrial** steps.

GLUCONEOGENESIS: first bypass

Pyruvate kinase

P-enolpyruvate + ADP → pyruvate + ATP

Bypass:

- *Pyruvate carboxylase*

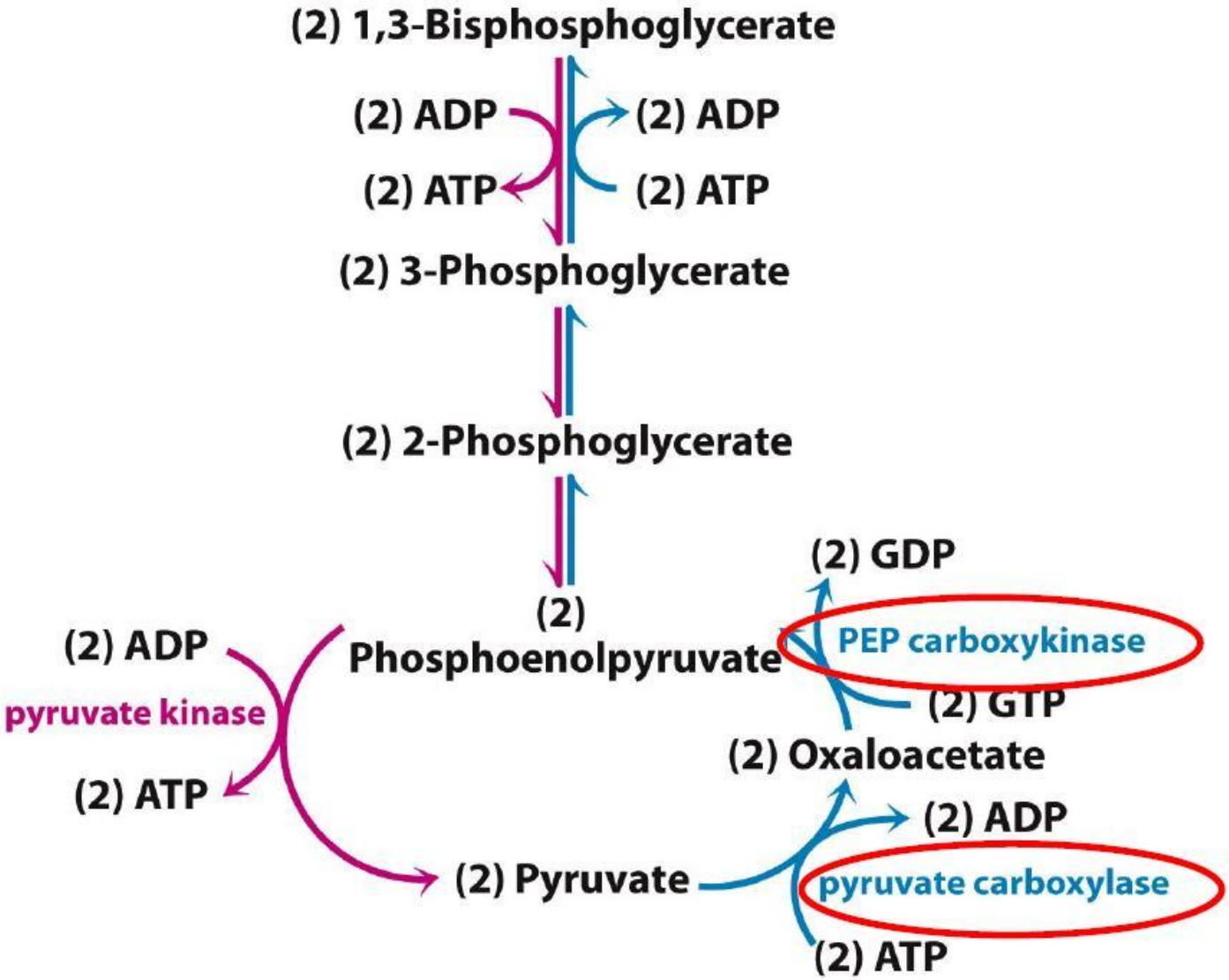
  Pyruvate + HCO_3^- + ATP → Oxaloacetate + ADP + P_i

- *P-enolpyruvate carboxykinase (PEPCK)*

  Oxaloacetate + GTP → P-enolpyruvate + CO_2 + GDP

The actual free energy is strongly negative (−25 kJ/mol) making the bypass effectively irreversible.

GLUCONEOGENESIS: first bypass



GLUCONEOGENESIS: second bypass

Phosphofructokinase (PFK-1)

Fructose-6-P + ATP → fructose-1,6-bisP + ADP

Bypass:

- *Fructose-1,6-bisphosphatase (FBPase)*

Fructose-1,6 bisP + H_2O → fructose-6-P + P_i

GLUCONEOGENESIS: third bypass

Glucokinase (Hexokinase)

Glucose + ATP → glucose-6-P + ADP

Bypass:

- *Glucose-6-phosphatase*

Glucose-6-P + H_2O → glucose + P_i

GLUCONEOGENESIS: second & third bypass

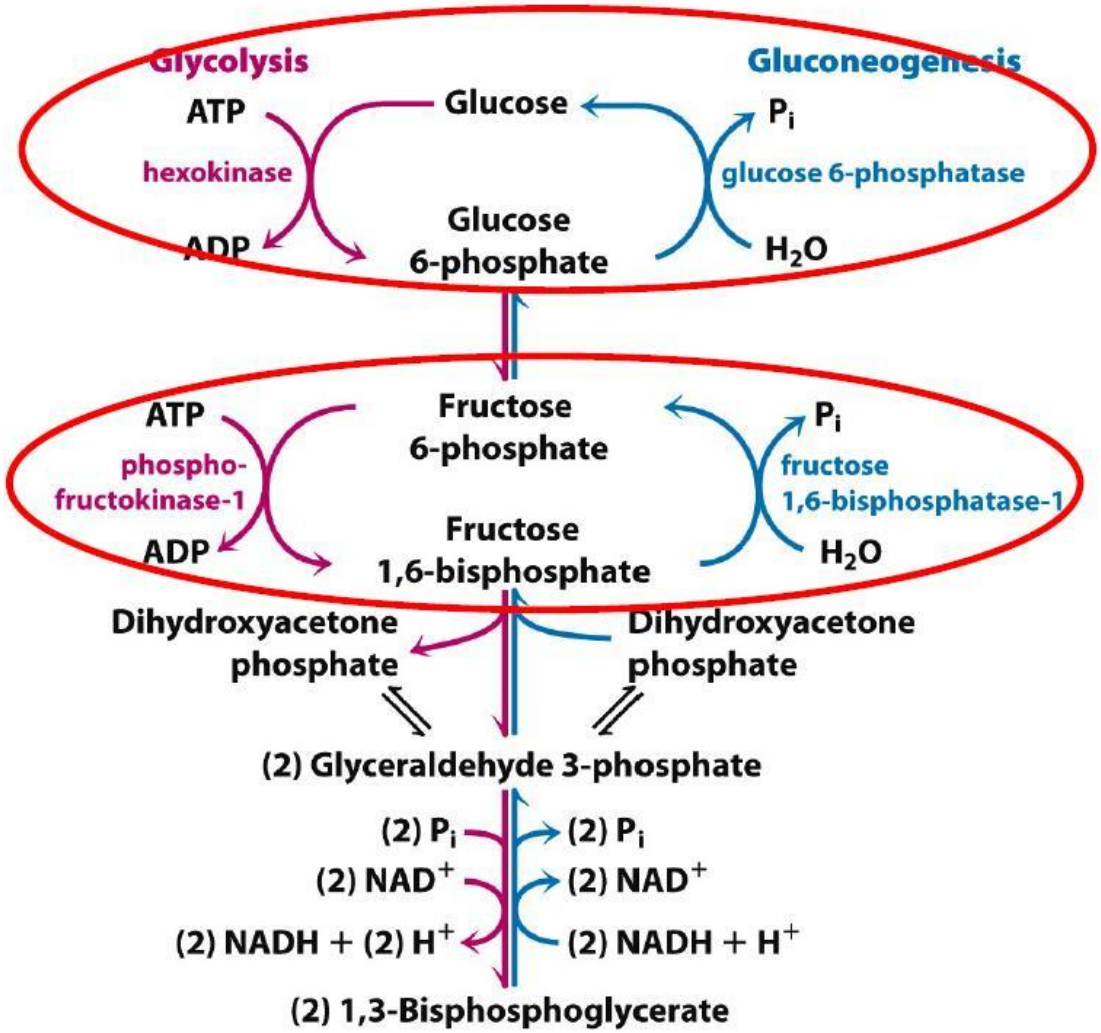


Figure 14-16 part 1
Lehninger Principles of Biochemistry, Fifth Edition

PYRUVATE CARBOXYLASE

Exclusively **mitochondrial**

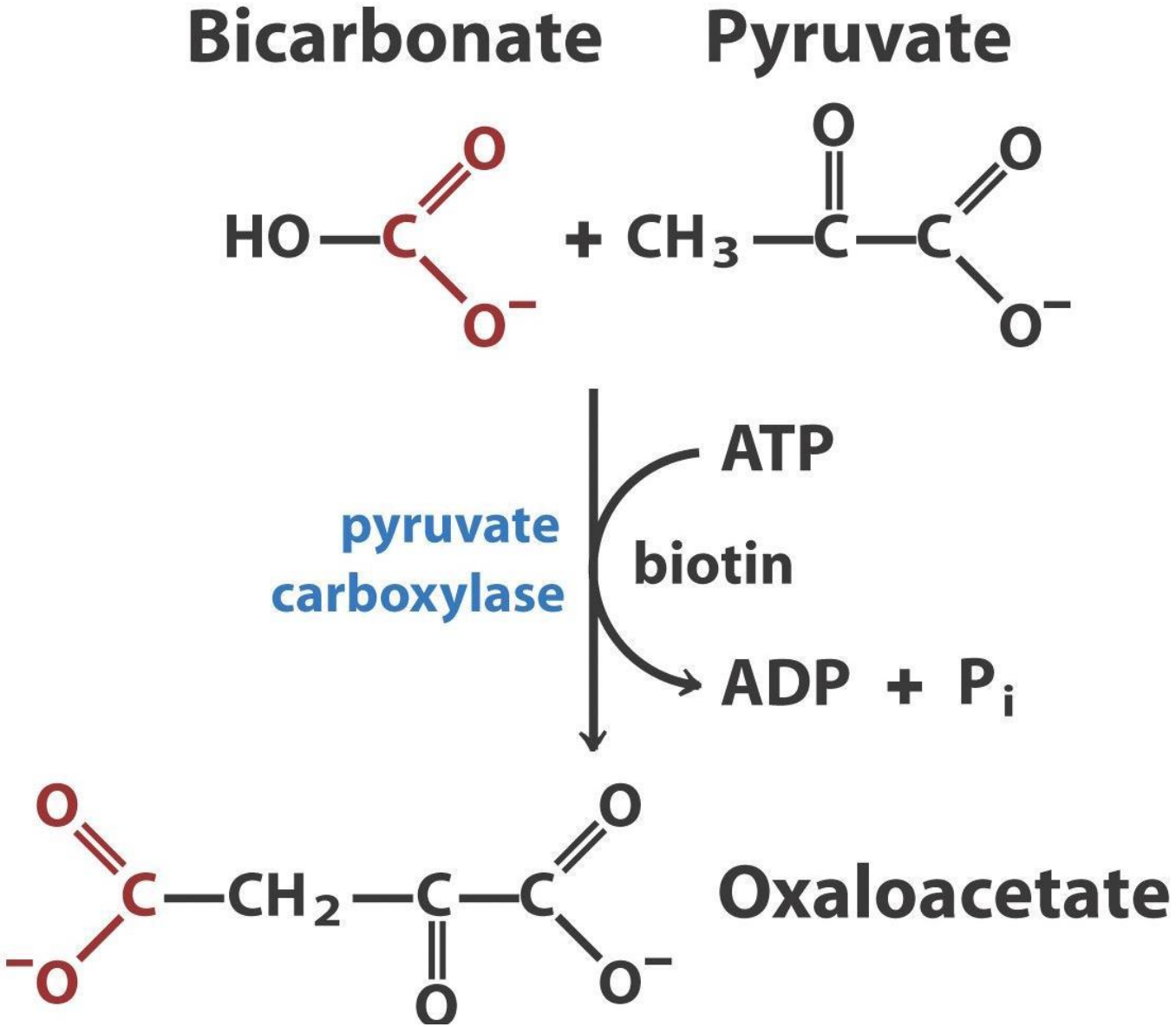
Four subunits:

- ATP + HCO_3^- binding site
- Biotin-binding site (mobile arm)
- Pyruvate-binding site
- Allosteric site for acetyl-CoA

Positively regulated by glucagon.

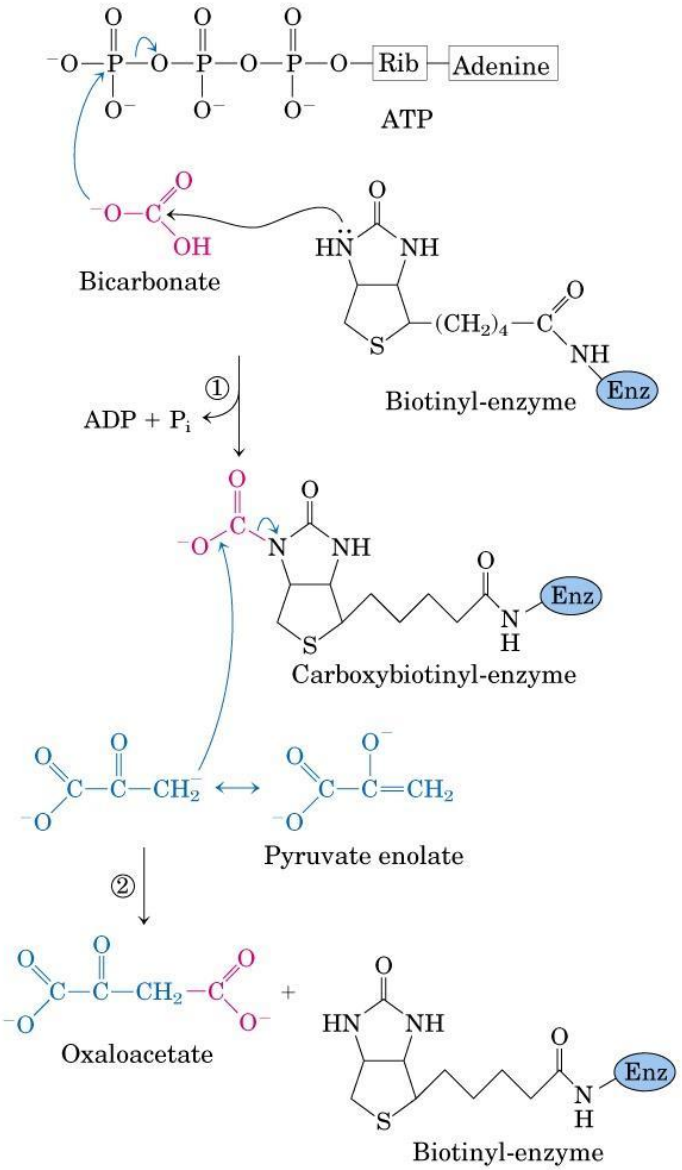
Negatively regulated by insulin.

PYRUVATE CARBOXYLASE

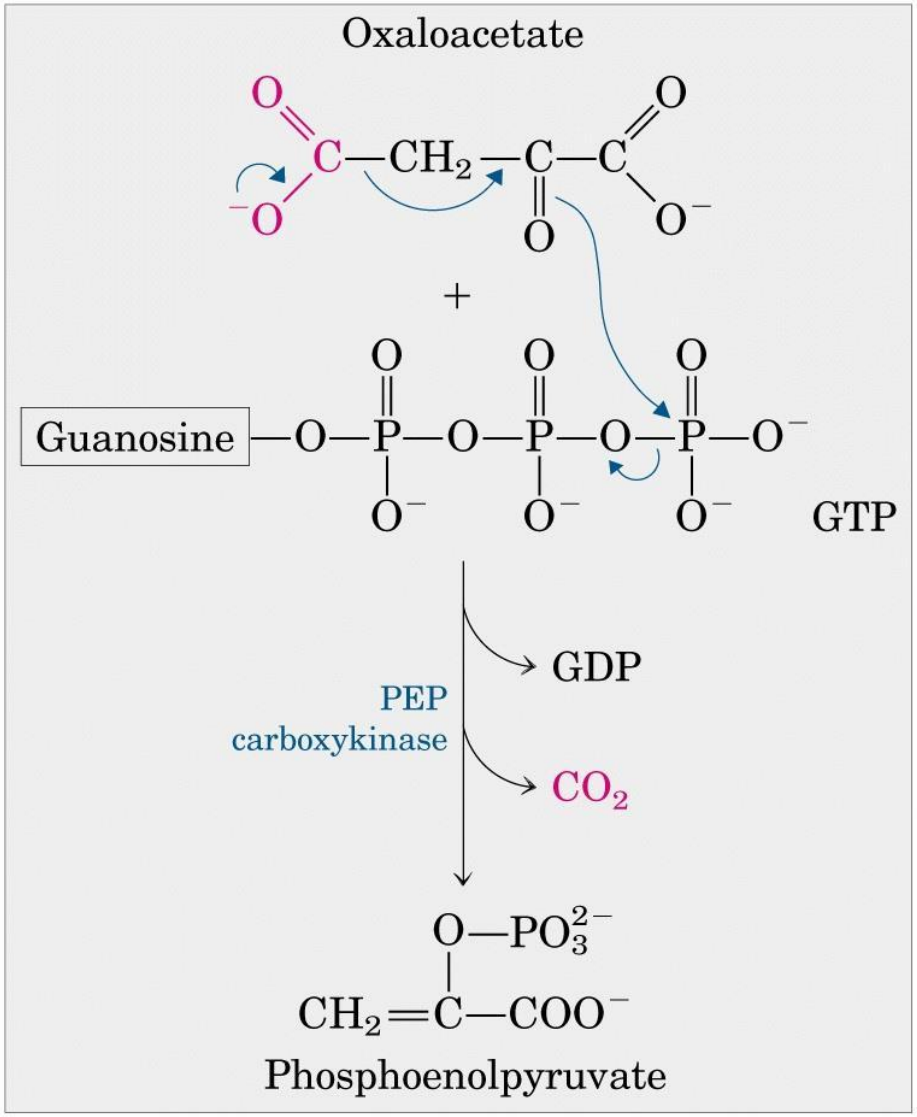


PYRUVATE CARBOXYLASE

Biotin is a water-soluble Vitamin (B7 or H).



PEP CARBOXYKINASE



PEP CARBOXYKINASE

Both **mitochondrial** and **cytosolic.**

Phosphorylation from GTP and decarboxylation:

- The C removed was the same previously added by pyruvate carboxylase

REDUCING POWER FOR GLUCONEOGENESIS

Gluconeogenesis requires **cytosolic NADH** for the oxidation of 1,3-BPG to glyceraldehyde-P.

- If the carbon source is **lactate**, NADH derives from oxidation of lactate to pyruvate in the cytosol:

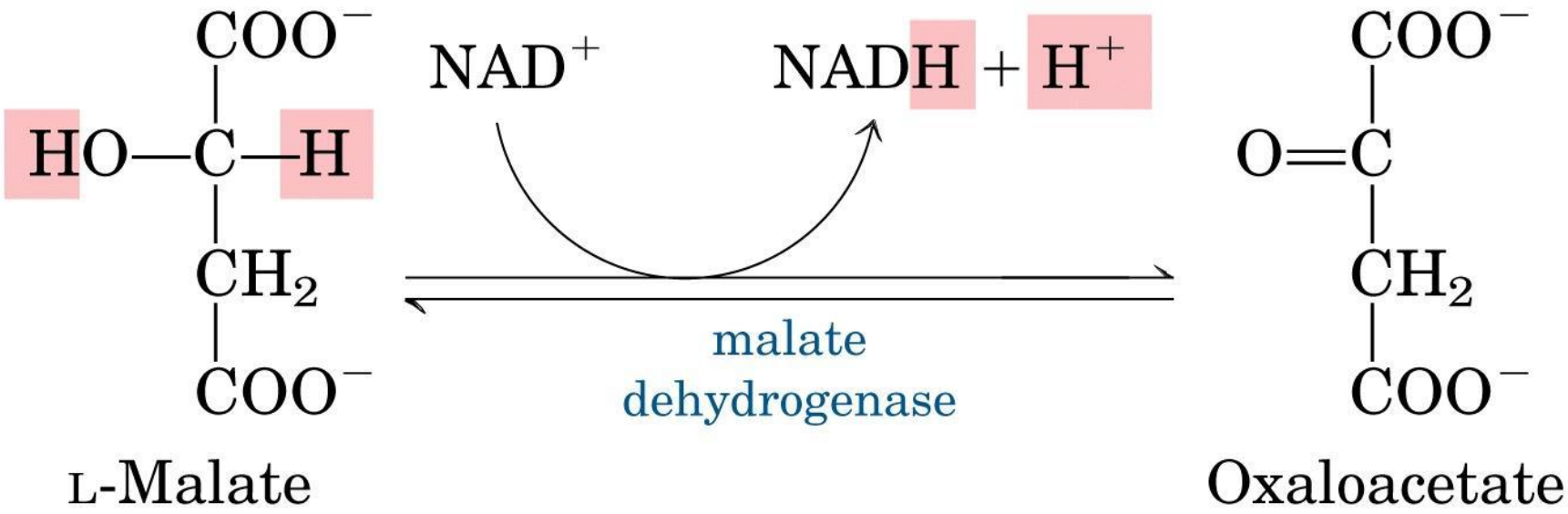
$$\text{lactate} + NAD^+ \rightarrow \text{pyruvate} + NADH + H^+$$

- If the carbon source are **amino acids**, NADH derives from malate export from the mitochondrion followed by its oxidation to oxaloacetate in the cytosol

$$\text{malate} + NAD^+ \rightarrow \text{oxaloacetate} + NADH + H^+$$

Mitochondrial malate may derive from pyruvate via oxaloacetate, but also from other sources in TCA cycle (from several amino acids)

REDUCING POWER FOR GLUCONEOGENESIS



L-Malate

Oxaloacetate

$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$

GLUCONEOGENESIS FROM AMINO ACIDS

- **Alanine**, others → Pyruvate → Oxaloacetate
- **Aspartate** → Oxaloacetate

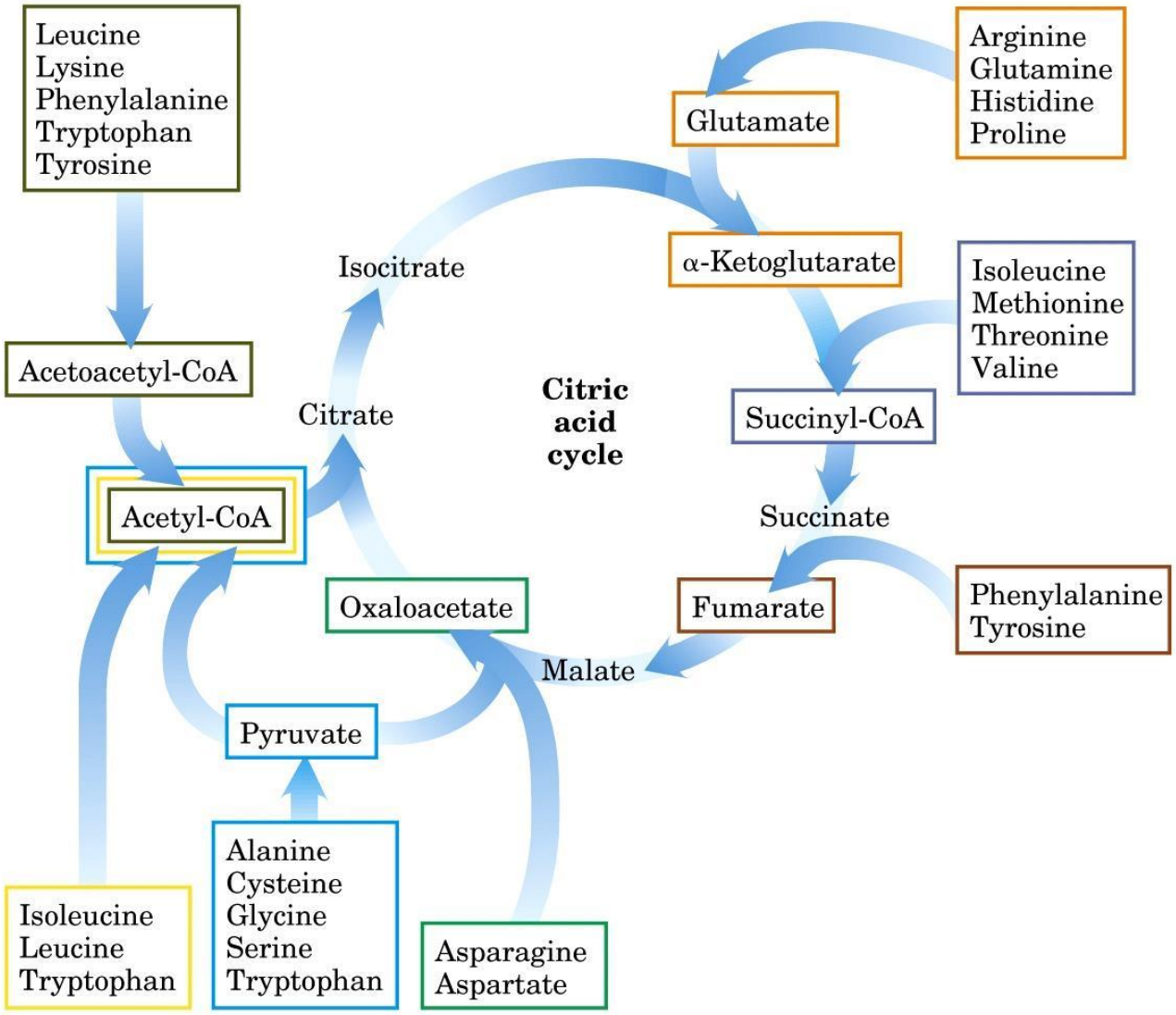
$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{Malate} + \text{NAD}^+$$

- Other aminoacids → TCA cycle intermediates → Malate → to cytosol

$$\text{Malate} + \text{NAD}^+ \rightarrow \text{Oxaloacetate} + \text{NADH} + \text{H}^+$$

$$\text{Oxaloacetate} \rightarrow \text{Phosphoenolpyruvate}$$

GLUCONEOGENESIS FROM AMINO ACIDS



GLUCONEOGENESIS FROM AMINO ACIDS

table 20–3

Glucogenic Amino Acids, Grouped by Site of Entry*

Pyruvate	**Succinyl-CoA**
Alanine	Isoleucine†
Cysteine	Methionine
Glycine	Threonine
Serine	Valine
Tryptophan†	
α-Ketoglutarate	**Fumarate**
Arginine	Phenylalanine†
Glutamate	Tyrosine†
Glutamine	
Histidine	**Oxaloacetate**
Proline	Asparagine
	Aspartate

*These amino acids are precursors of blood glucose or liver glycogen because they can be converted to pyruvate or citric acid cycle intermediates. Only leucine and lysine are unable to furnish carbon for net glucose synthesis.

†These amino acids are also ketogenic (see Fig. 18–19).

GLUCONEOGENESIS FROM AMINO ACIDS

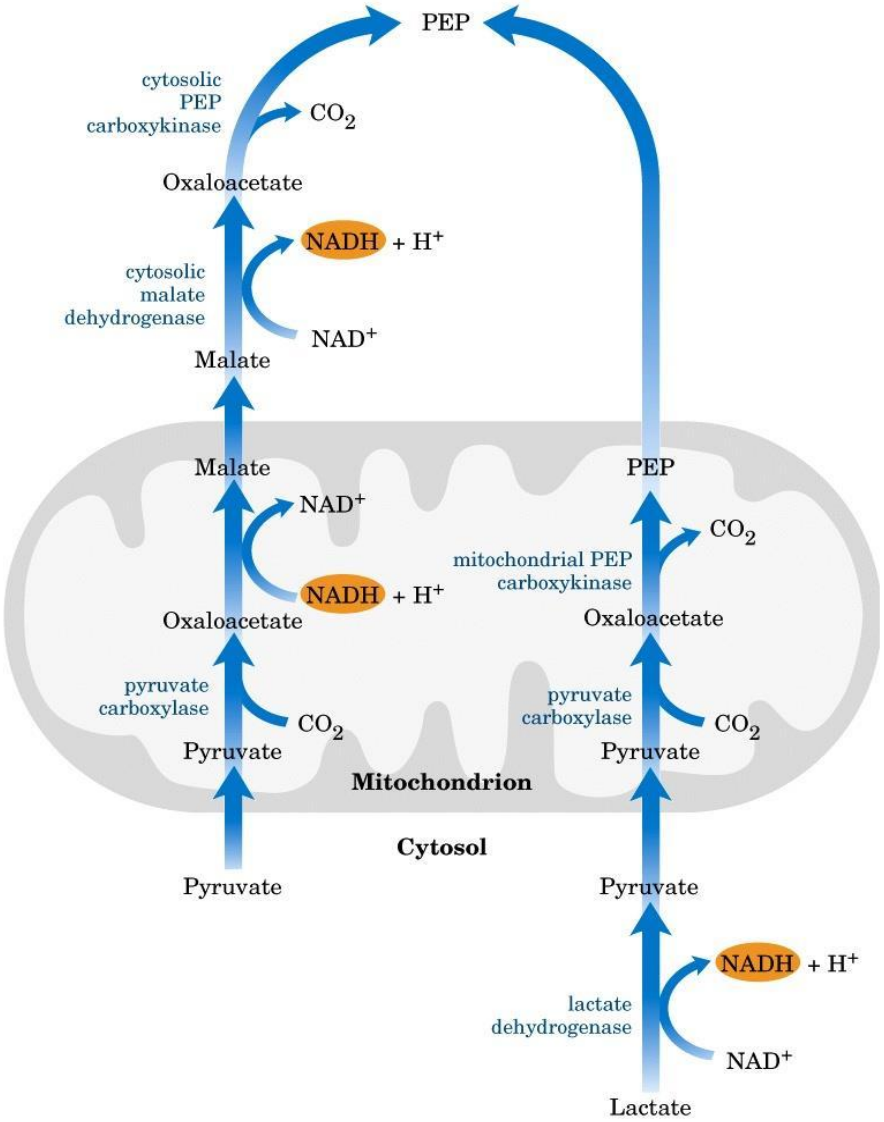
Alanine → pyruvate → oxaloacetate → malate → to cytosol

Aspartate → oxaloacetate → malate → to cytosol

Glutamate → α-ketoglutarate → succinyl CoA → → malate → to cytosol

Valine → succinyl CoA → → malate → to cytosol

GLUCONEOGENESIS



When lactate is the glucogenic precursor, a second bypass prevails.

GLUCONEOGENESIS

table 20–2

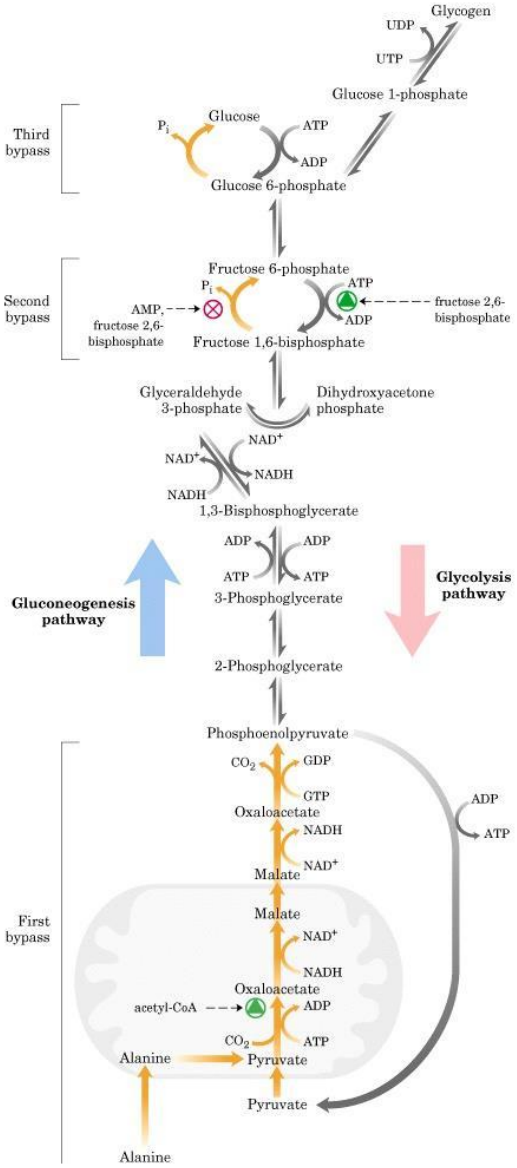
Sequential Reactions in Gluconeogenesis Starting from Pyruvate*

Pyruvate + HCO_3^- + ATP ⟶ oxaloacetate + ADP + P_i + H^+	×2
Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO_2 + GDP	×2
Phosphoenolpyruvate + H_2O ⇌ 2-phosphoglycerate	×2
2-Phosphoglycerate ⇌ 3-phosphoglycerate	×2
3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP + H^+	×2
1,3-Bisphosphoglycerate + NADH + H^+ ⇌ glyceraldehyde 3-phosphate + NAD^+ + P_i	×2
Glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate	
Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ fructose 1,6-bisphosphate	
Fructose 1,6-bisphosphate + H_2O ⟶ fructose 6-phosphate + P_i	
Fructose 6-phosphate ⇌ glucose 6-phosphate	
Glucose 6-phosphate + H_2O ⟶ glucose + P_i	

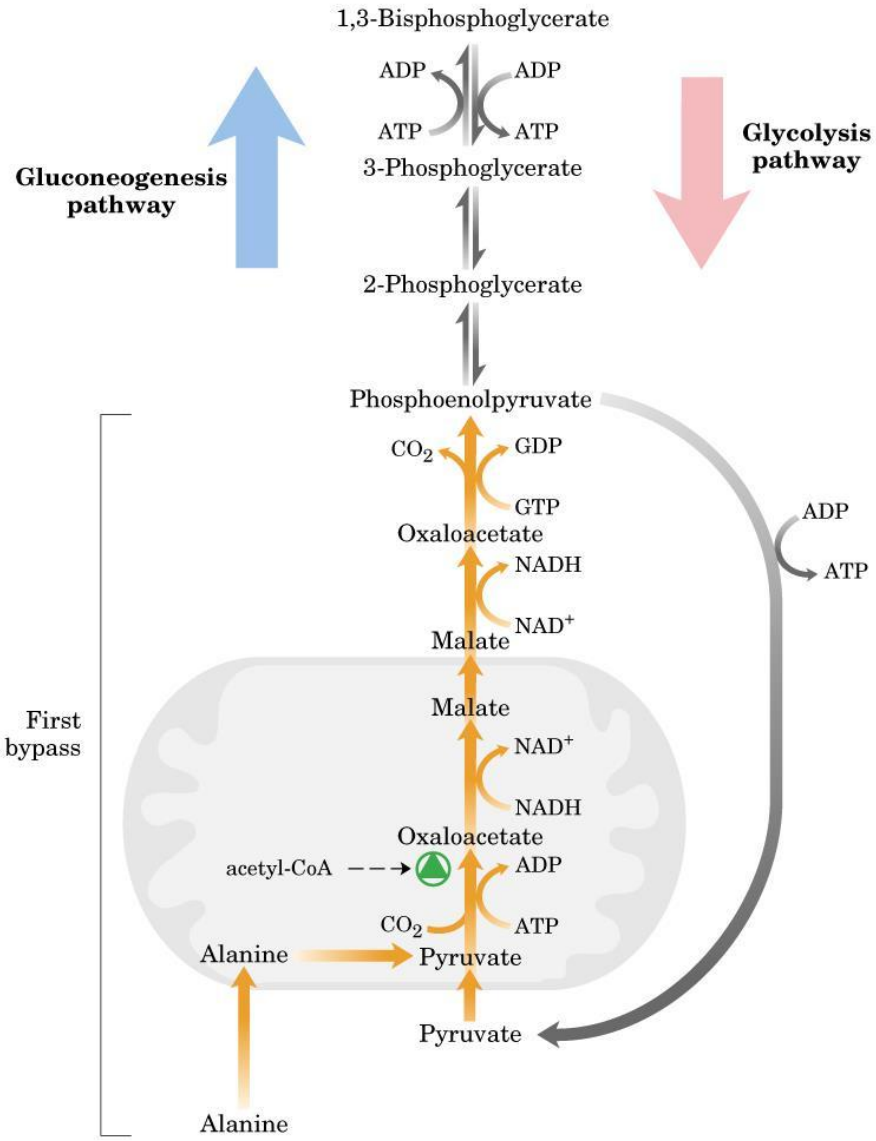
Sum: 2 Pyruvate + 4ATP + 2GTP + 2NADH + $4H_2O$ ⟶ glucose + 4ADP + 2GDP + $6P_i$ + $2NAD^+$ + $2H^+$

*The bypass reactions are in red; all other reactions are reversible steps of glycolysis. The figures at the right indicate that the reaction is to be counted twice, because two three-carbon precursors are required to make a molecule of glucose. Note that the reactions required to replace the cytosolic NADH consumed in the glyceraldehyde 3-phosphate dehydrogenase reaction (the conversion of lactate to pyruvate in the cytosol or the transport of reducing equivalents from mitochondria to the cytosol in the form of malate) are not considered in this summary.

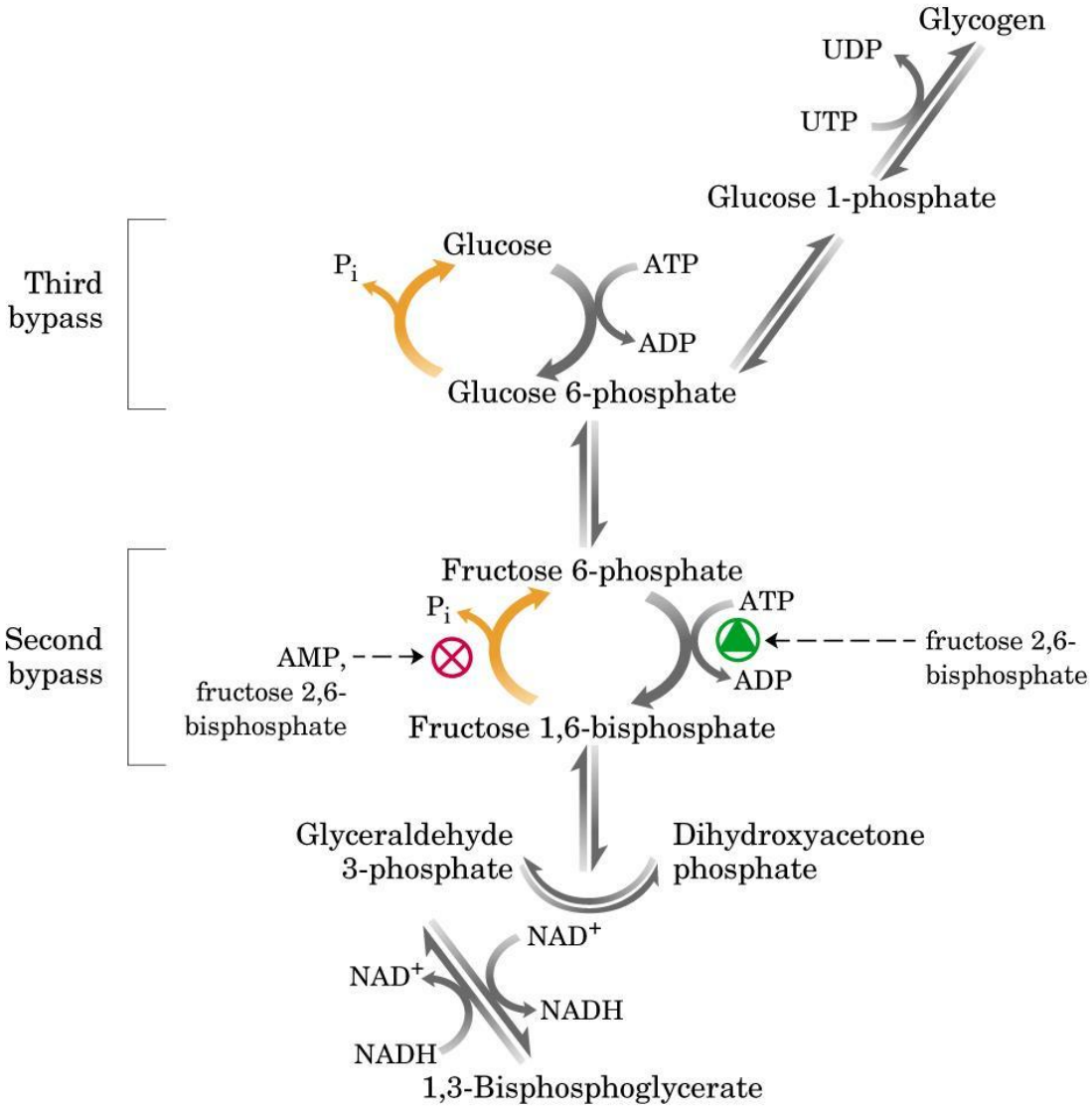
GLUCONEOGENESIS



GLUCONEOGENESIS



GLUCONEOGENESIS



GLUCONEOGENESIS

2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 4 H_2O → Glucose + 4 ADP + 2 GDP + 6 P_i + 2 NAD^+

Costs **4 ATP**, **2 GTP**, and **2 NADH**

Physiologically necessary: brain, nervous system, and red blood cells generate ATP mainly from glucose.

Allows generation of glucose when glycogen stores are depleted:

- during starvation
- during vigorous exercise
- can generate glucose from many amino acids, but not fatty acids

GLUCONEOGENESIS

Gluconeogenesis fuel is initially amino acids.

Gradually, body shifts to protect critical proteins and enzymes.

Brain metabolism shifts from all glucose to some glucose plus ketone bodies (acetone, acetoacetate, β-hydroxybutyrate) derived from FA oxidation.

GLUCONEOGENESIS: disorders

Glucose-6-phosphatase deficiency

- 1/100,000
- Glycogen storage disease

Pyruvate carboxylase deficiency

- <1/250,000
- No survival beyond infancy

PEP carboxykinase deficiency

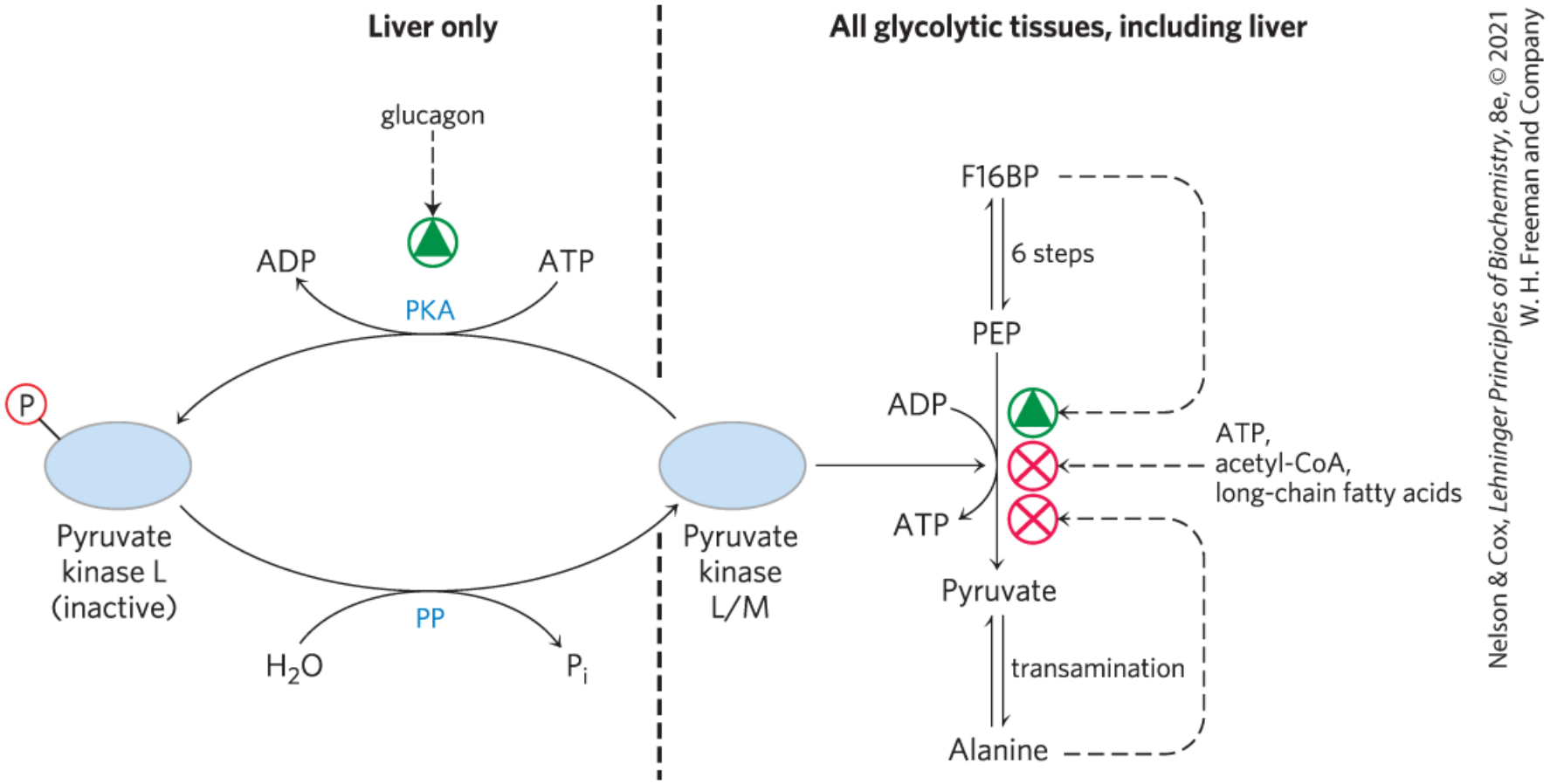
- Very rare (5-6 patients seen)
- No survival beyond infancy

GLUCONEOGENESIS: disorders

Fructose-1,6-bisphosphatase deficiency

- 1/500,000
- Clinical management via diet: avoid fructose and sucrose, infusion of glucose
- Rapid onset of hypoglycemia in fasting
- Lactic acidosis (NADH buildup; nausea, vomiting, weakness)

GLUCONEOGENESIS

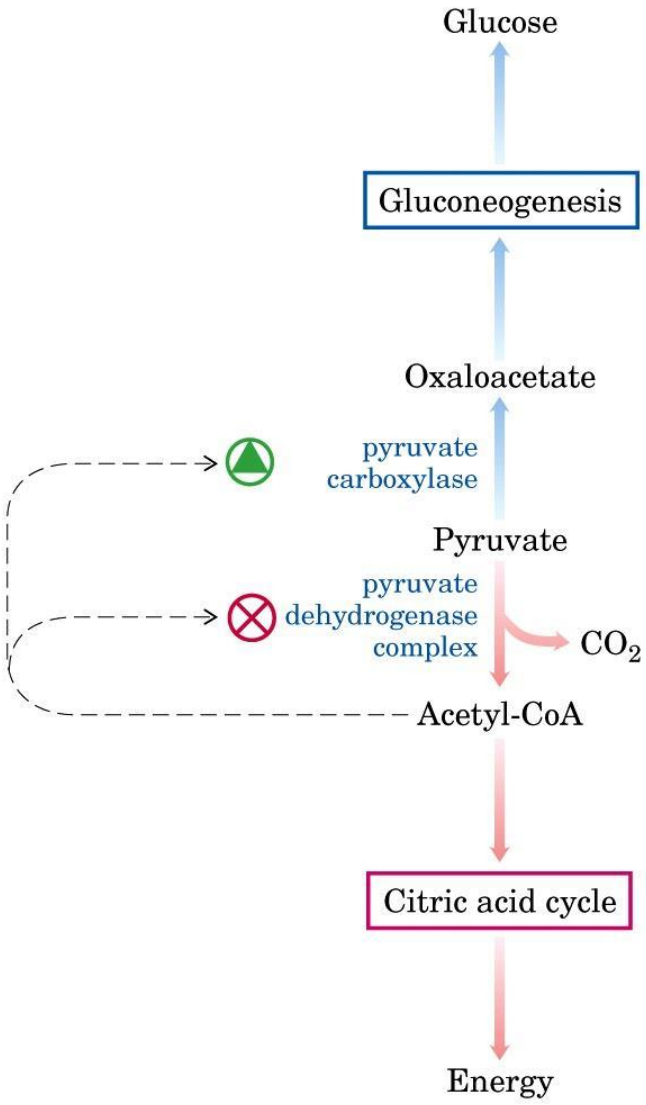


Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

GLUCONEOGENESIS

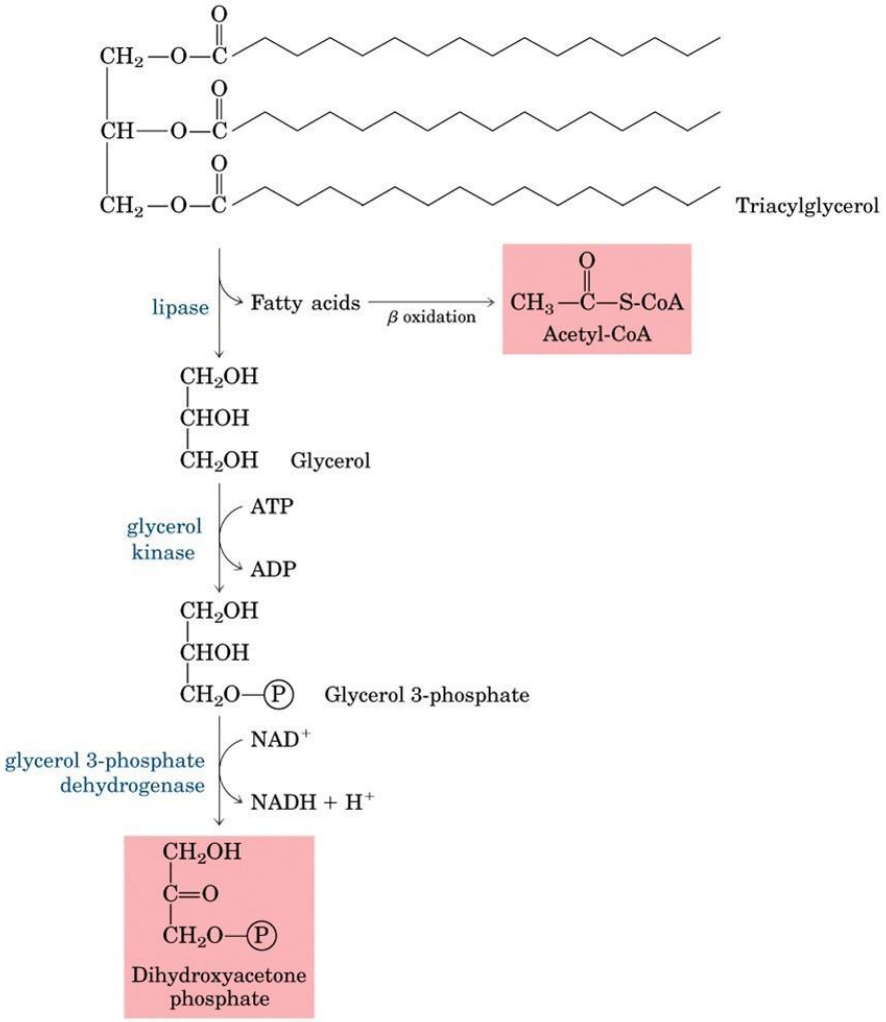
Acetyl-CoA signals that further glucose oxidation is not needed

- allosterically stimulates pyruvate carboxylase
- allosterically inhibits pyruvate dehydrogenase



GLUCONEOGENESIS FROM GLYCEROL

Glycerol is the only glucogenic part of lipids.



GLUCONEOGENESIS

NOT Fuel for Gluconeogenesis

- Acetyl-CoA
- Fatty acids
- Leucine and Lysine (broken down only to acetyl-CoA)

There is no path for the NET synthesis of Oxaloacetate from acetyl-CoA.

However, fatty acid oxidation provides much of the ATP for gluconeogenesis.

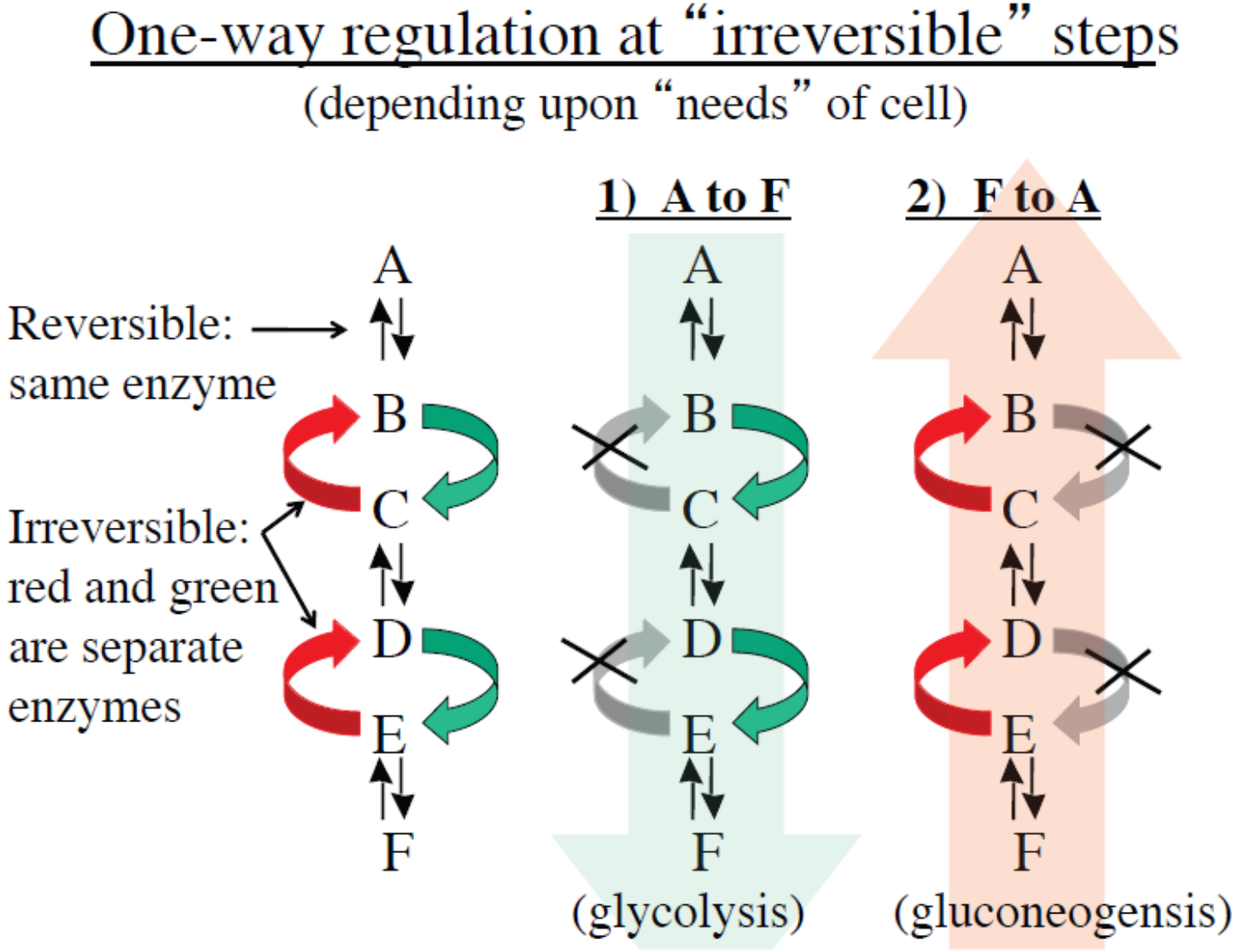
REGULATION OF GLUCONEOGENESIS IN LIVER

Glucagon: stimulates gluconeogenesis, inhibits glycolysis

Insulin: stimulates glycolysis, inhibits gluconeogenesis

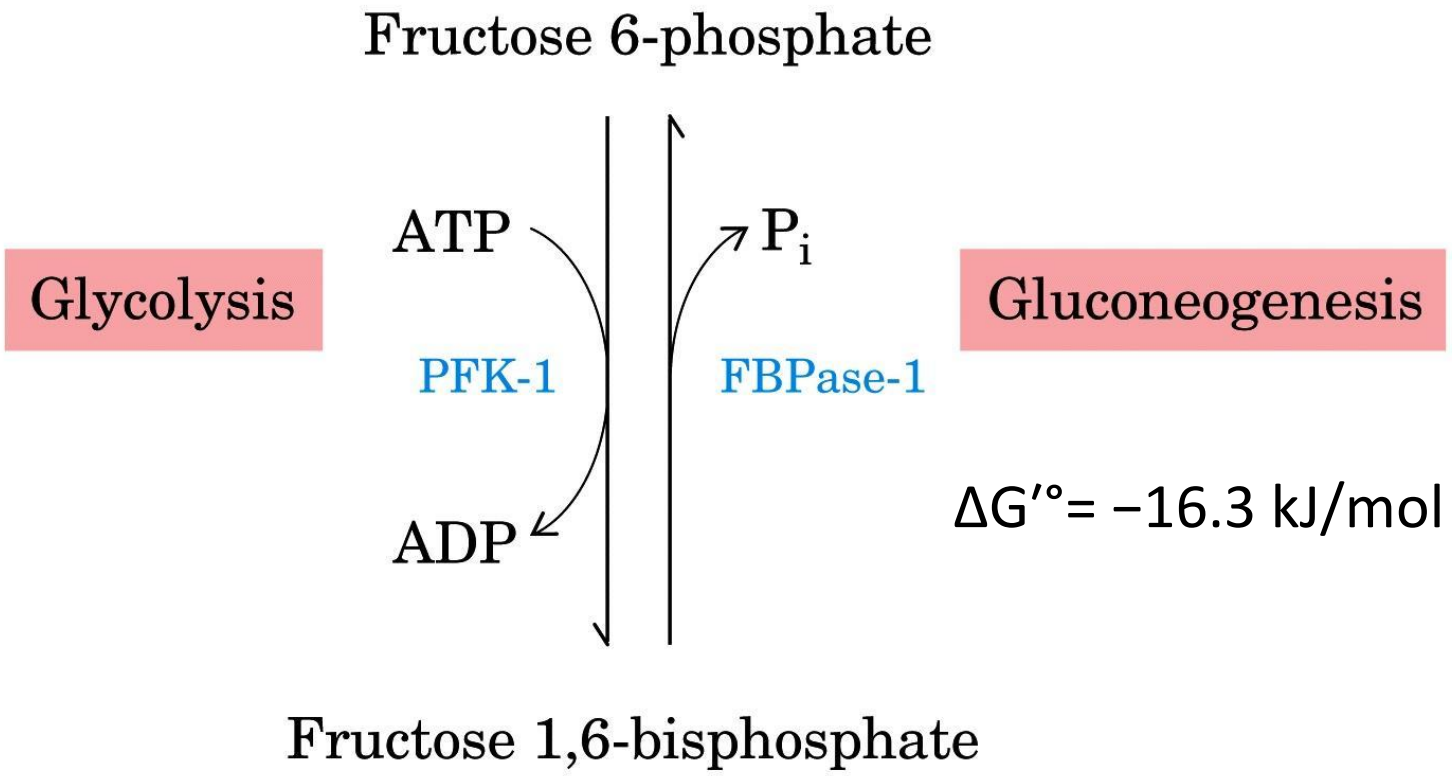
Cortisol: stimulates gluconeogenesis

REGULATION OF GLUCONEOGENESIS IN LIVER



REGULATION OF GLUCONEOGENESIS IN LIVER

The major regulation step is at the level of PFK and FBPase.



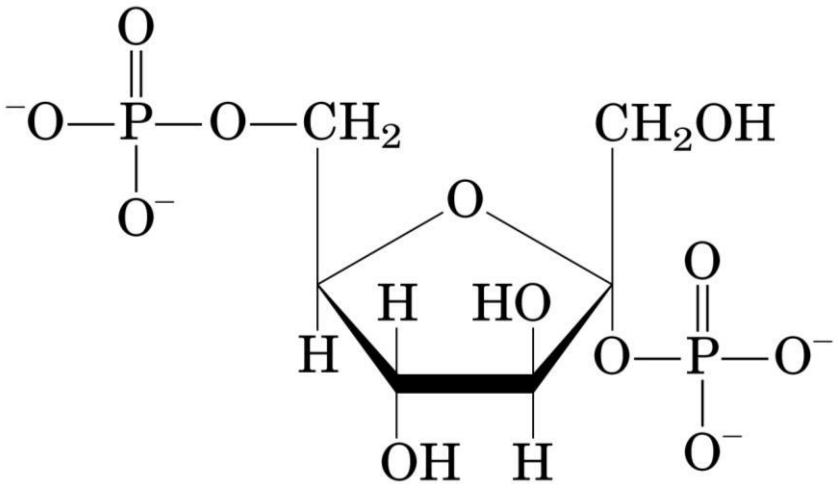
$\Delta G'^{\circ} = -16.3$ kJ/mol

REGULATION OF GLUCONEOGENESIS IN LIVER

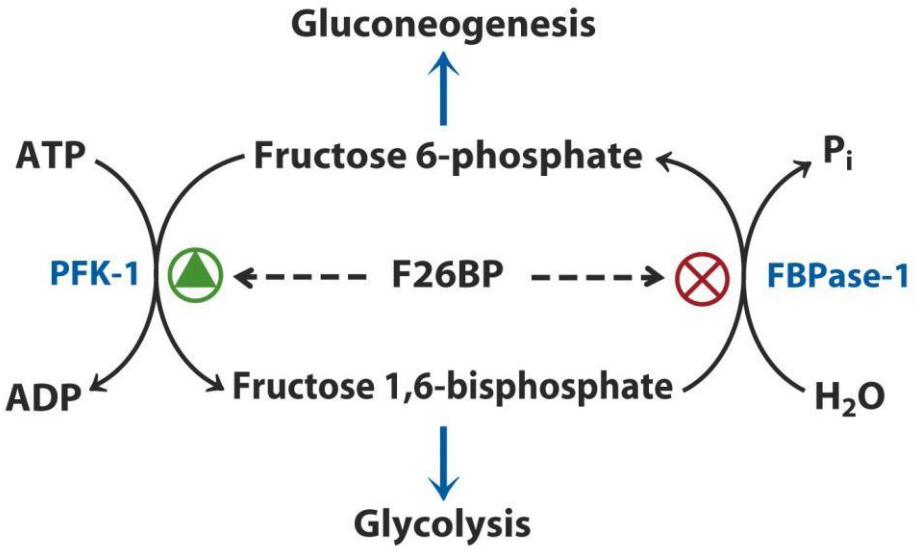
Fructose-2,6-bisphosphate is not an intermediate, it's a regulator only.

F-2,6-bP allosterically ACTIVATES PFK-1 (Glycolysis)

INACTIVATES FBPase1 (Gluconeogenesis)



Fructose 2,6-bisphosphate



REGULATION OF GLUCONEOGENESIS IN LIVER

Concentration of F-2,6-bP is regulated by the action of a tandem enzyme:

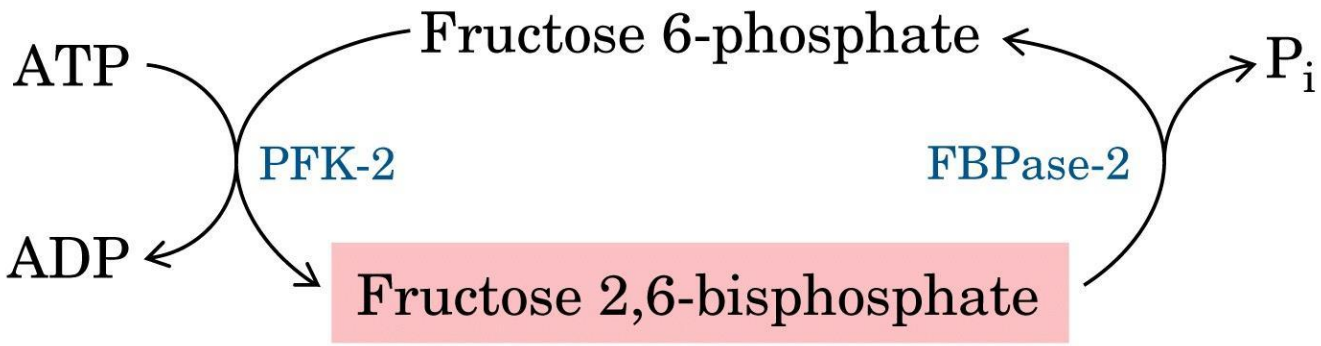
PFK-2 makes F-2,6-bP

FBPase-2 breaks down F-2,6-bP

Hormone stimulated-phosphorylation also regulates this step:

Glucagon will ↑ blood glucose: ↓ PFK-1 & ↑ FBPase-2 in liver

Insulin will ↓ blood glucose: ↑ PFK-1 in liver and muscle

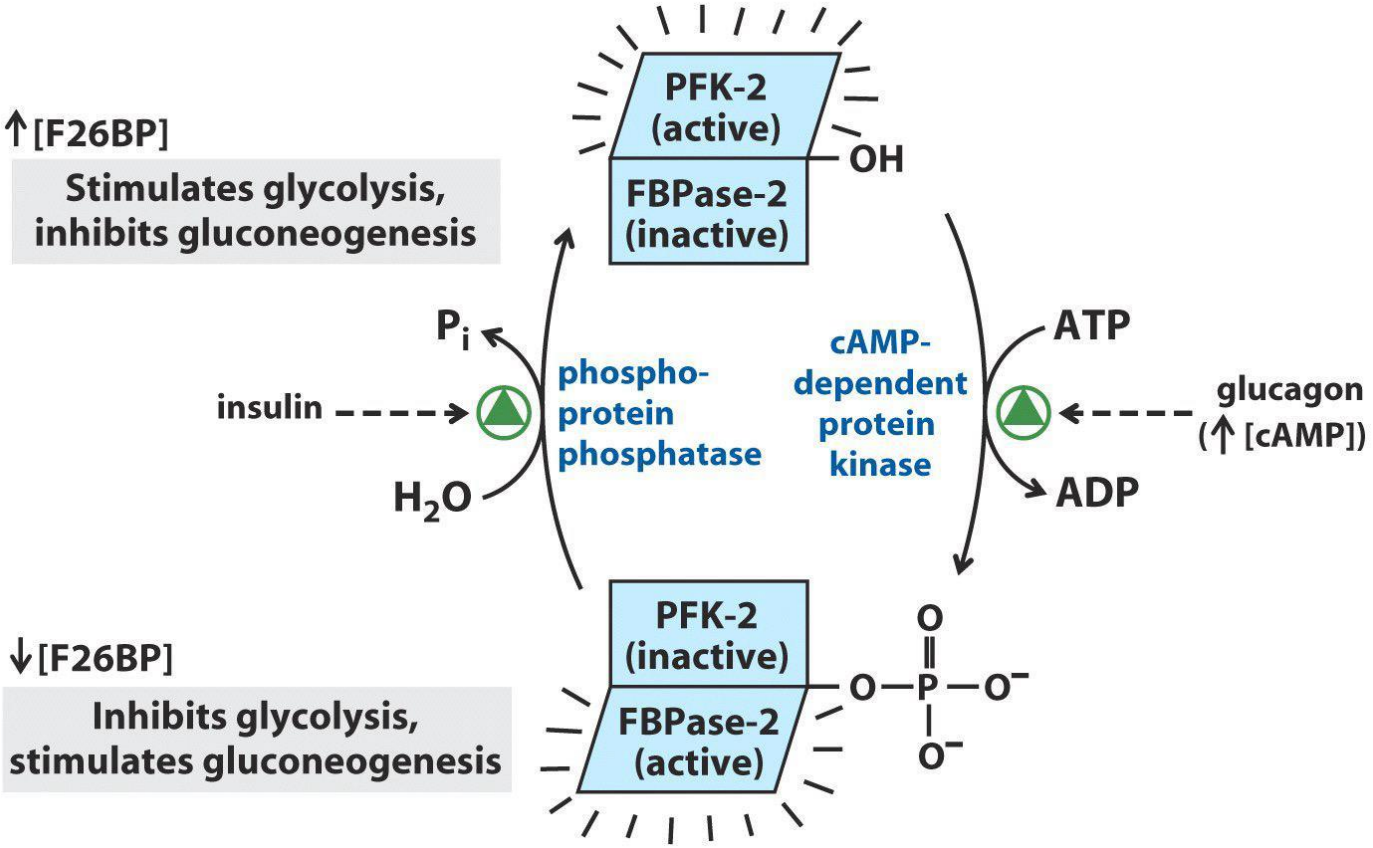


REGULATION OF GLUCONEOGENESIS IN LIVER

PFK-2 and FBPase-2 are contained in the same protein.

Phosphorylation activates **FBPase-2** (No F-2,6-bP).

Dephosphorylation activates **PFK-2** (High F-2,6-bP).



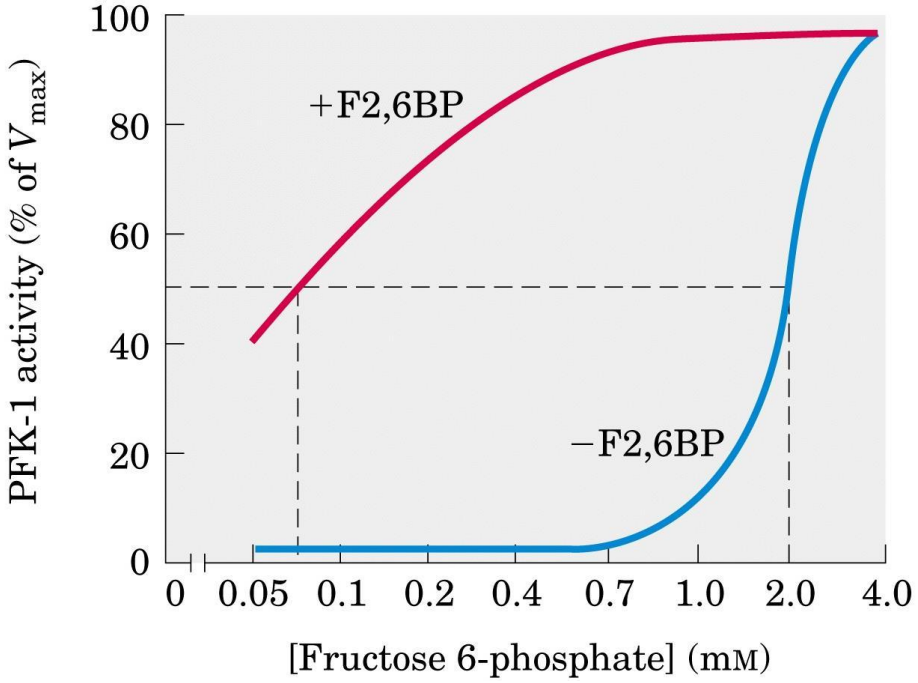
REGULATION OF GLUCONEOGENESIS IN LIVER

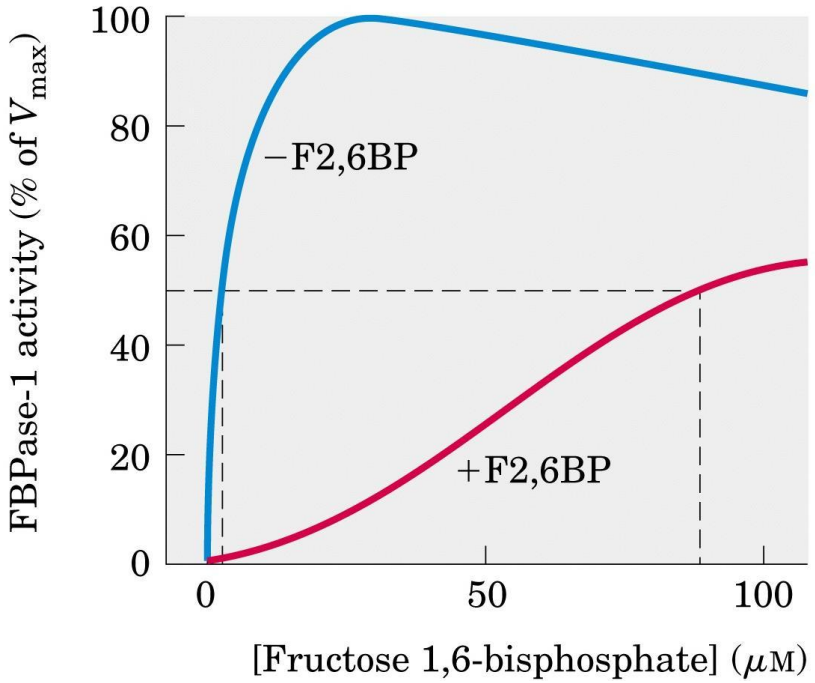
Insulin → PPPase-2a* → **Tandem enzyme dephosphorylated** → kinase → Fructose-2,6-bisP → PFK + → **Glycolysis +**

Glucagon → cAMP → PKA → **Tandem enzyme phosphorylated** → phosphatase → no Fructose-2,6-bisP → FBPase + → **Gluconeogenesis +**

PPPase-2a is also activated by xylulose-5-P.

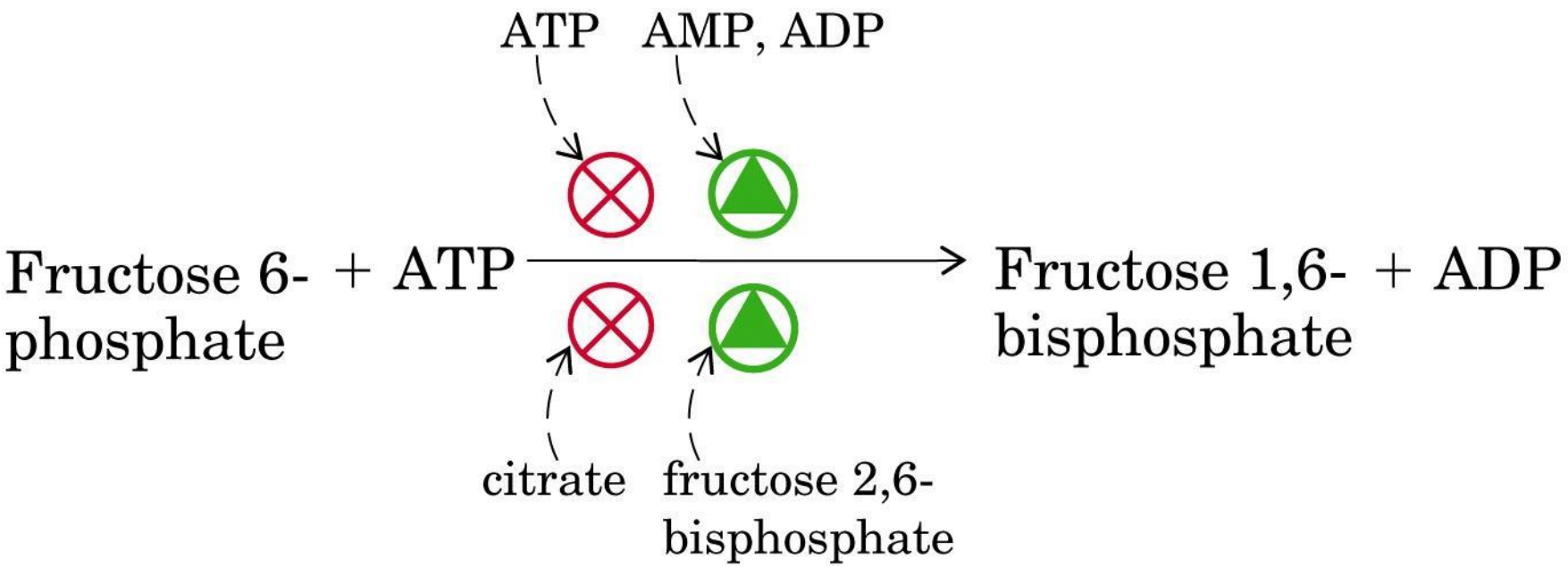
REGULATION OF GLUCONEOGENESIS IN LIVER





REGULATION OF GLUCONEOGENESIS IN LIVER

ATP is a substrate AND a negative effector



(c)

REGULATION OF GLUCONEOGENESIS IN LIVER

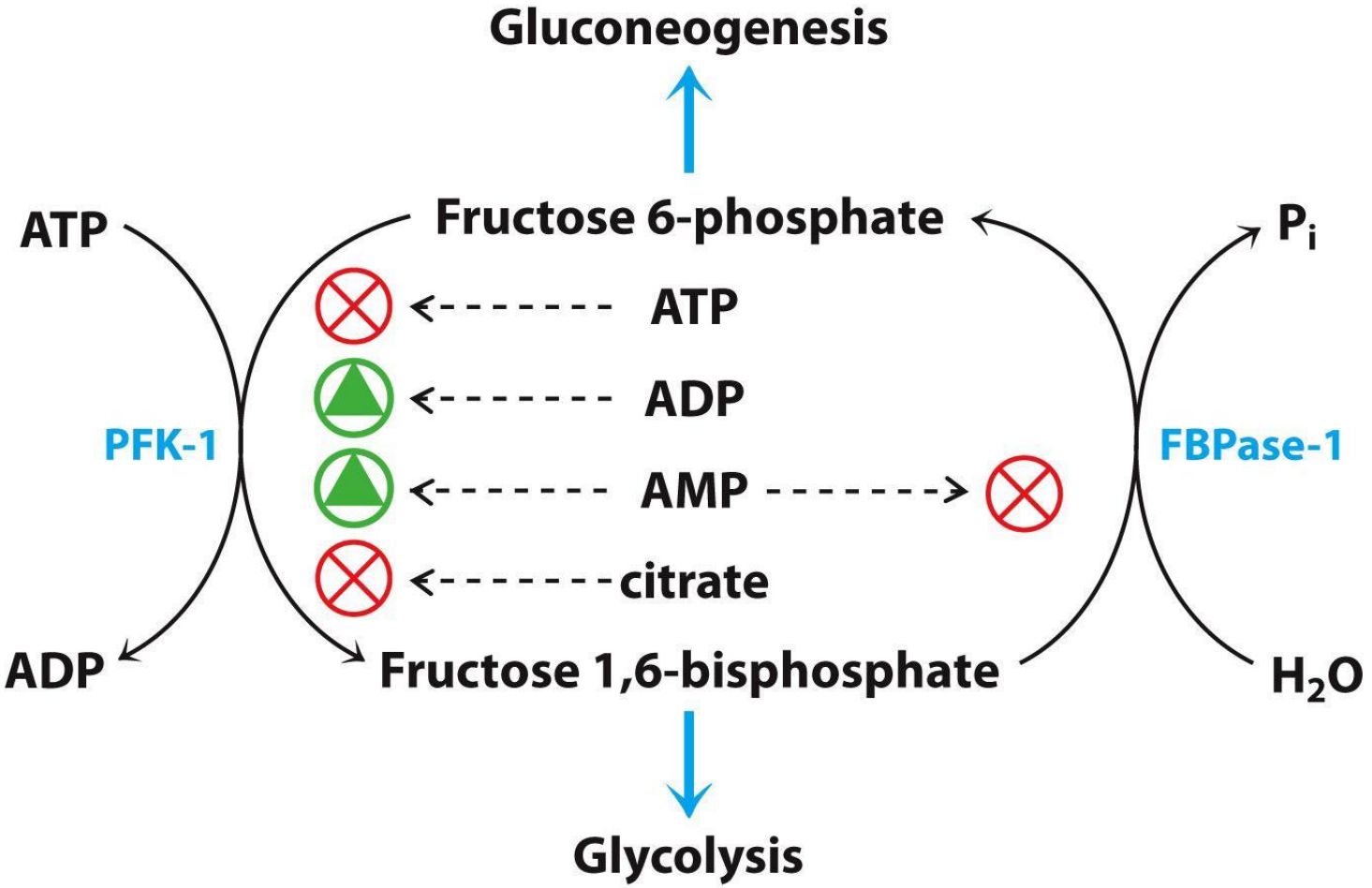


Figure 15-17
Lehninger Principles of Biochemistry, Seventh Edition

TRANSCRIPTIONAL REGULATION

- **CREB** (cyclic AMP responsive element binding protein):

  glucagon → cAMP → gluconeogenetic enzymes

- **ChREBP** (Carbohydrate responsive element binding protein):

  Insulin → PPPase2a → glycolytic and other insulin-dependent enzymes

- **SREBP** (Sterol responsive element binding protein) activated by insulin to express glycolytic and other enzymes and to repress glucogenic enzymes
- **FOXO1** stimulates gluconeogenetic enzymes. Insulin phosphorylates FOXO via PKB, leading it to ubiquitin-mediated degradation.

NUTRITION SENSORS OF METABOLISM

Sensors of low energy state, low nutritional intake (activation of catabolic pathways, gluconeogenesis, mitochondrial function):

- AMP-dependent protein kinase
- Sirtuins (protein deacetylases)
- PGC1α (PPAR γ coactivator 1α) activates mitochondrial biogenesis
- FOXO (transcription factor)

Sensors of high nutritional state (activation of anabolic pathways, protein synthesis, growth)

- insulin
- insulin-like Growth Factor IGF
- mTOR (target of rapamycin) activated by PKB

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Credits:

Prof. Michele Di Foggia

Dipartimento di Scienze Biomediche e Neuromotorie – Sezione di Biochimica
via Irnerio 48
Telephone: +39 051 2094281

michele.difoggia2@unibo.it