
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

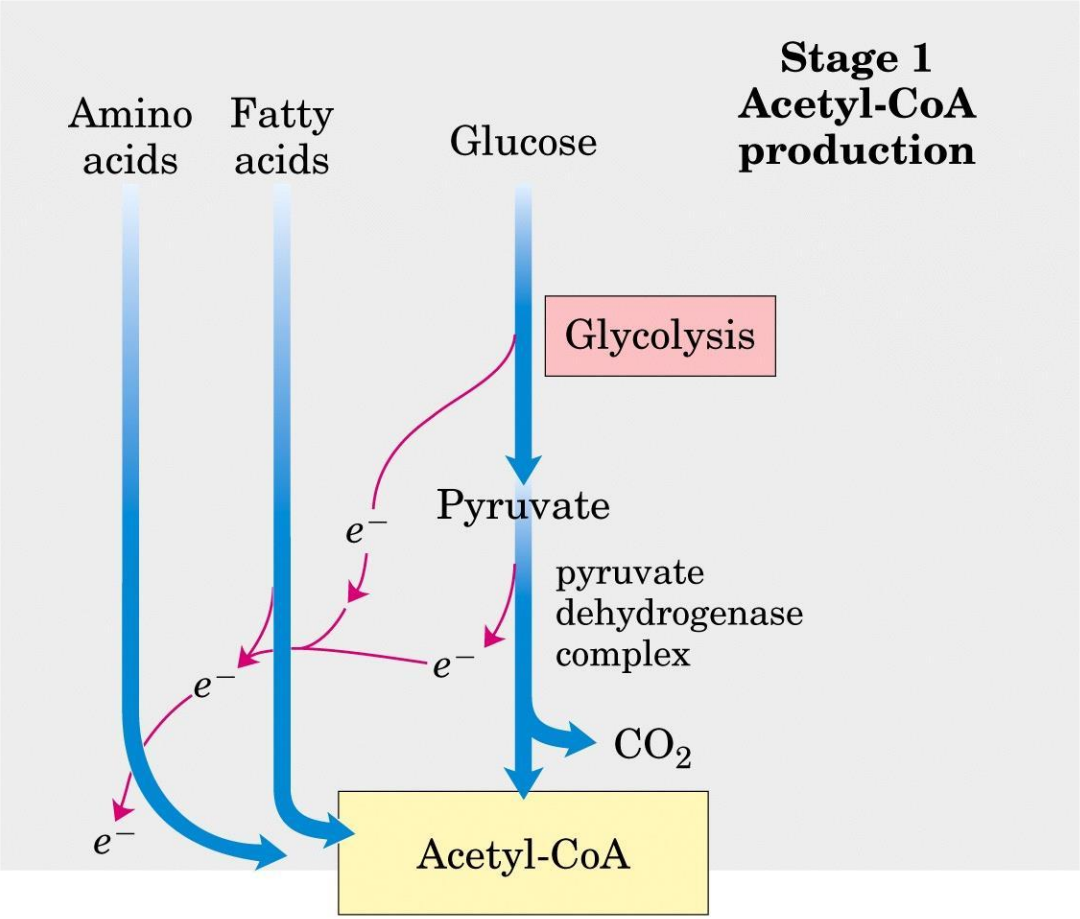
# CARBOHYDRATE METABOLISM – PYRUVATE OXIDATION AND TCA CYCLE

**Prof. Michele Di Foggia**

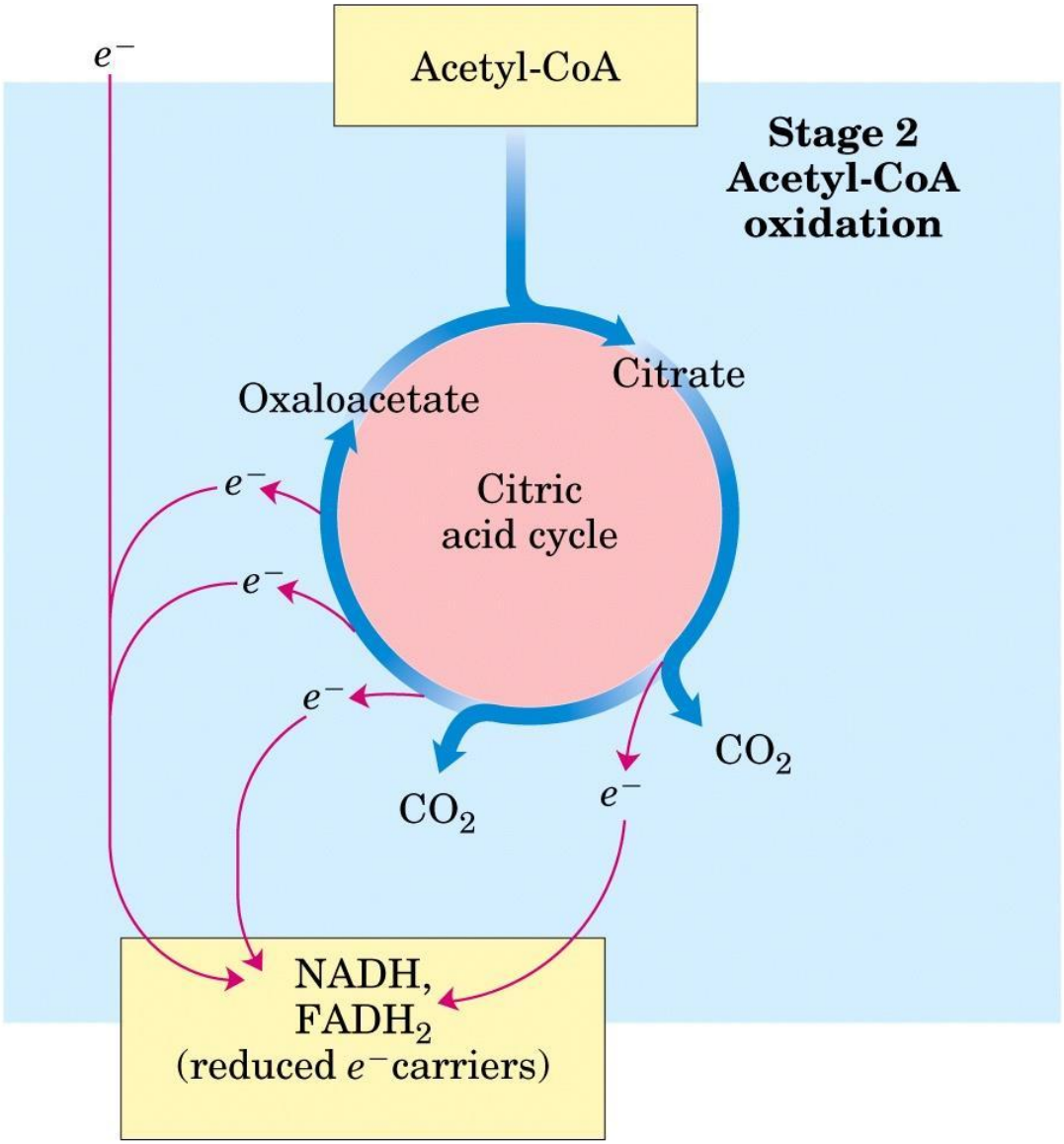
Dipartimento di Scienze Biomediche e Neuromotorie – DIBINEM – via Irnerio 48, Bologna

# AEROBIC PYRUVATE OXIDATION

**Acetyl-CoA** is a key molecule that connects the final stages of carbohydrate, lipid, and amino acid metabolism.



# AEROBIC PYRUVATE OXIDATION



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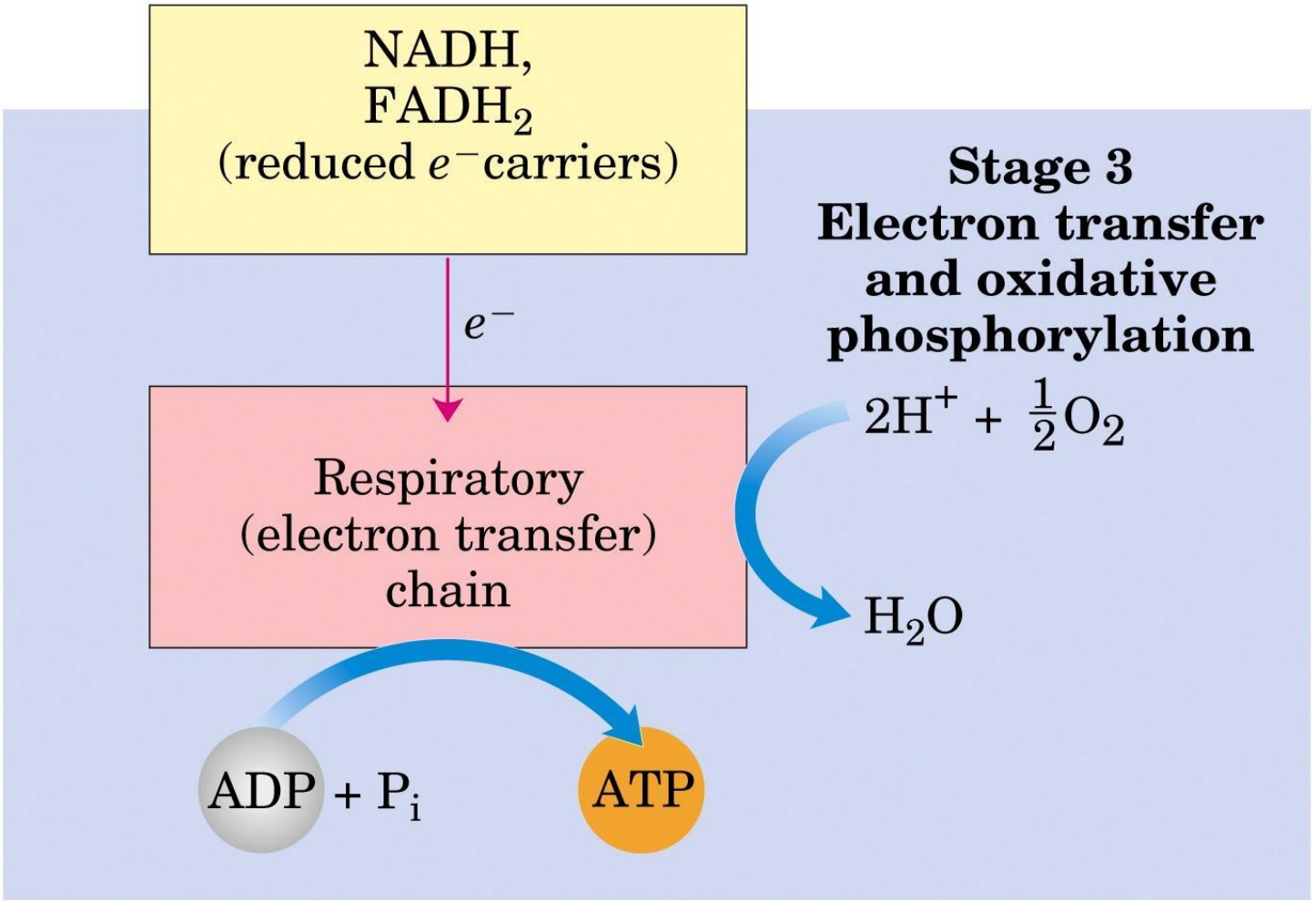
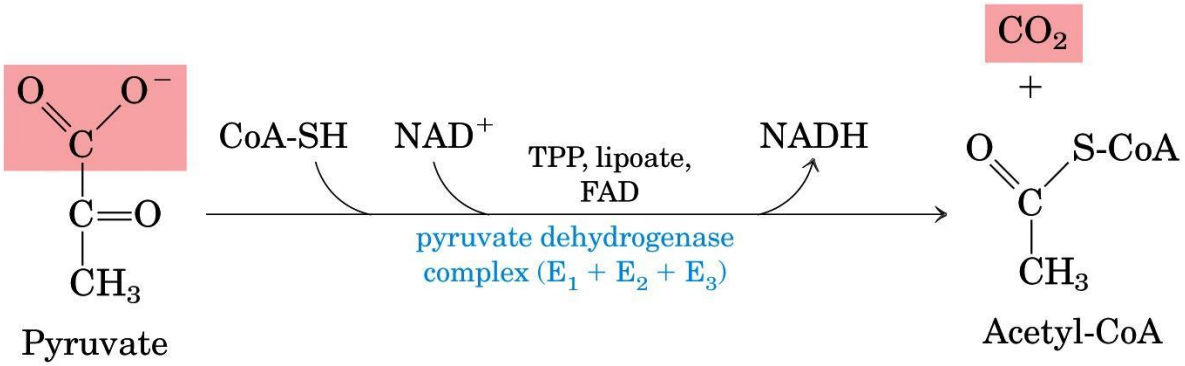


# PYRUVATE DEHYDROGENASE

Pyruvate Dehydrogenase Complex (PDH): oxidative decarboxylation

pyruvate + $NAD^+$ + CoASH → Acetyl-CoA + $CO_2$ + NADH + $H^+$

Formed by 3 enzymes (E1, E2, E3) and 3 prosthetic groups (TPP, Lipoic acid, FAD).



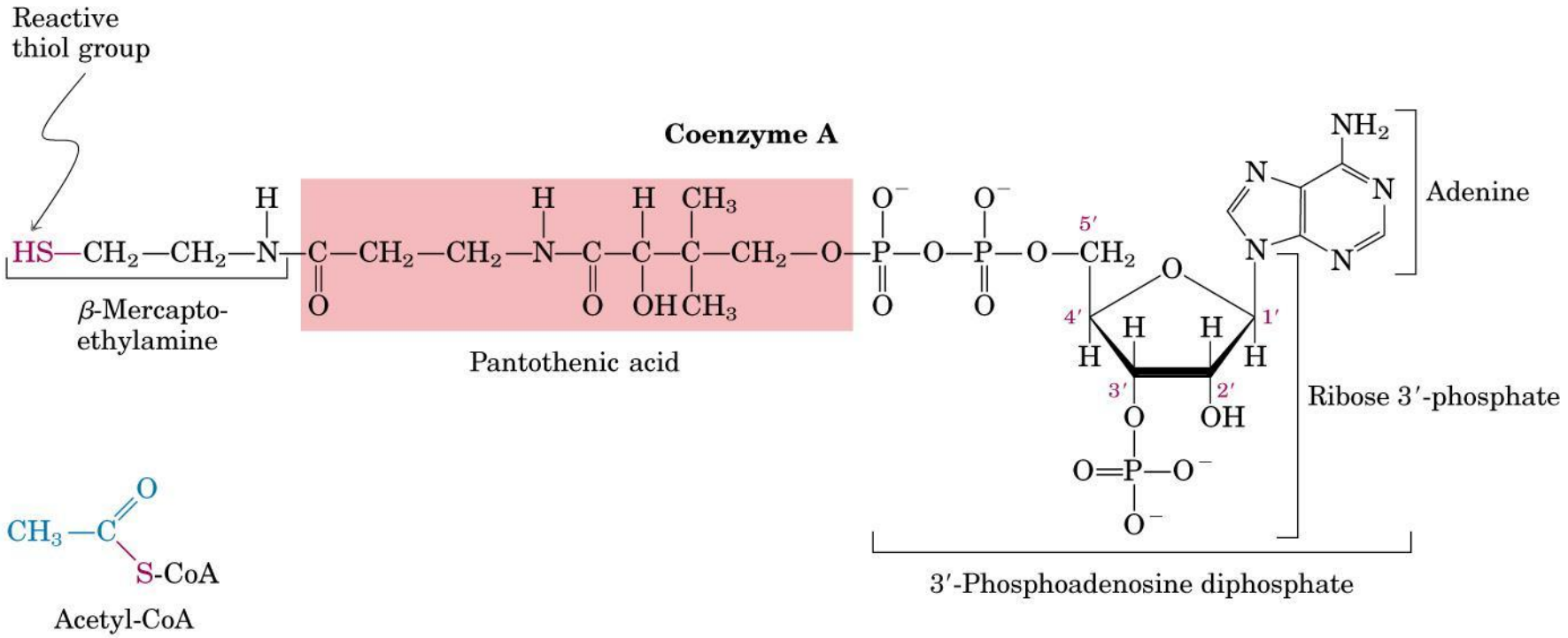

ABSOLUTELY IRREVERSIBLE!

$\Delta G'^\circ = -33.4$ kJ/mol

This is the reason why lipids cannot be converted to carbohydrates.

# PYRUVATE DEHYDROGENASE

Pantothenic acid is a water-soluble vitamin (Vit B5).



# PYRUVATE DEHYDROGENASE

Thiamine is Vitamin B1. Thiamine deficiency induces a severe myocarditis (Beri-beri).

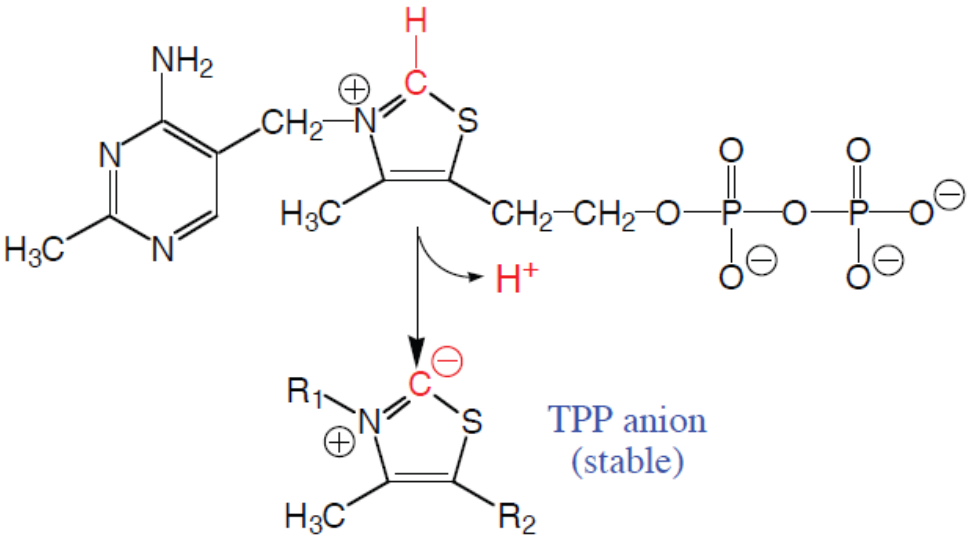
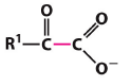
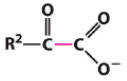
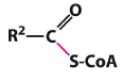
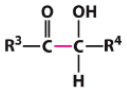
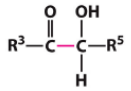
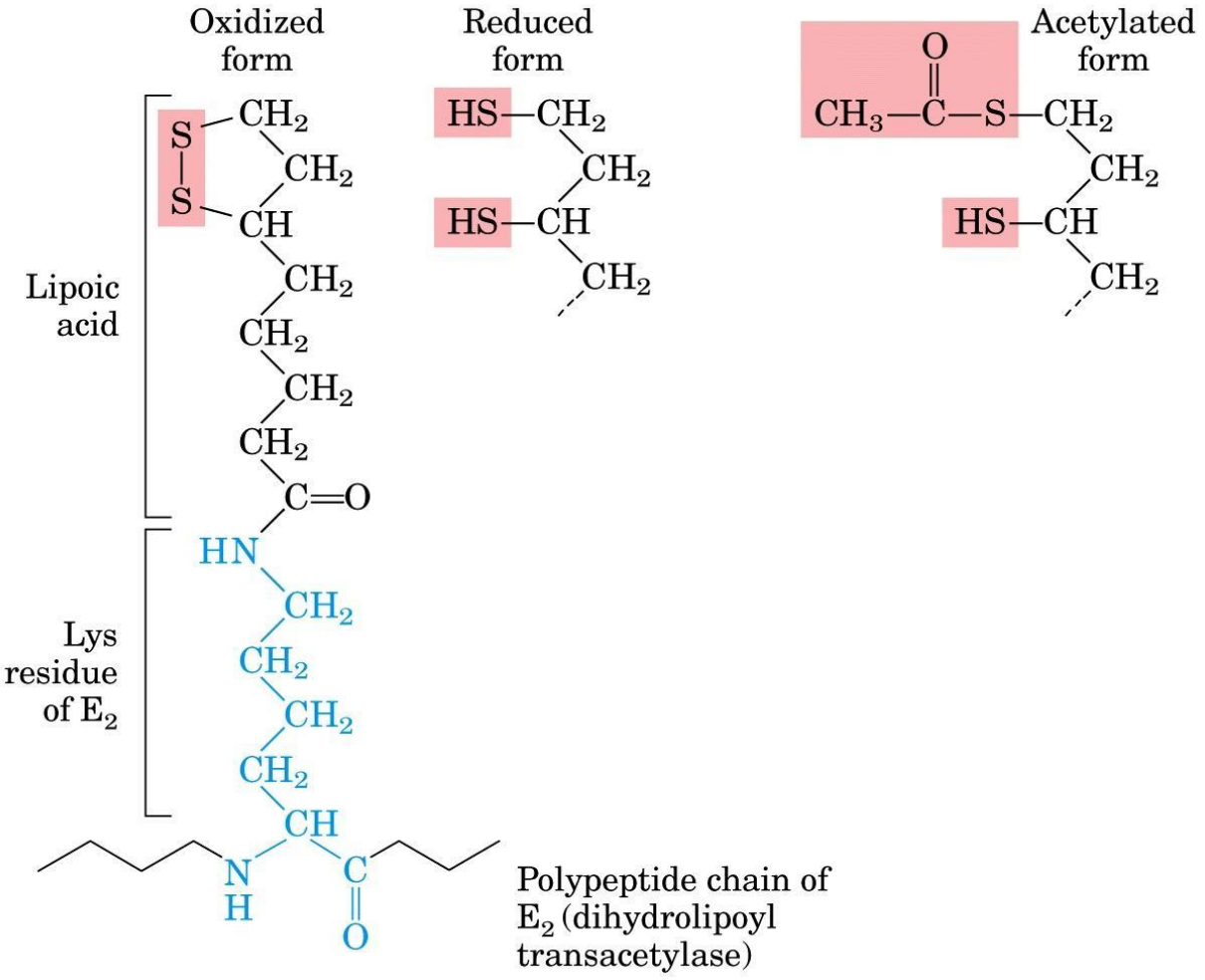



**TABLE 14-1** Some TPP-Dependent Reactions

| Enzyme | Pathway(s) | Bond cleaved | Bond formed |
|---|---|---|---|
| Pyruvate decarboxylase | Ethanol fermentation |  |  |
| Pyruvate dehydrogenase<br>$\alpha$-Ketoglutarate dehydrogenase | Synthesis of acetyl-CoA<br>Citric acid cycle |  |  |
| Transketolase | Carbon-assimilation reactions<br>Pentose phosphate pathway |  |  |

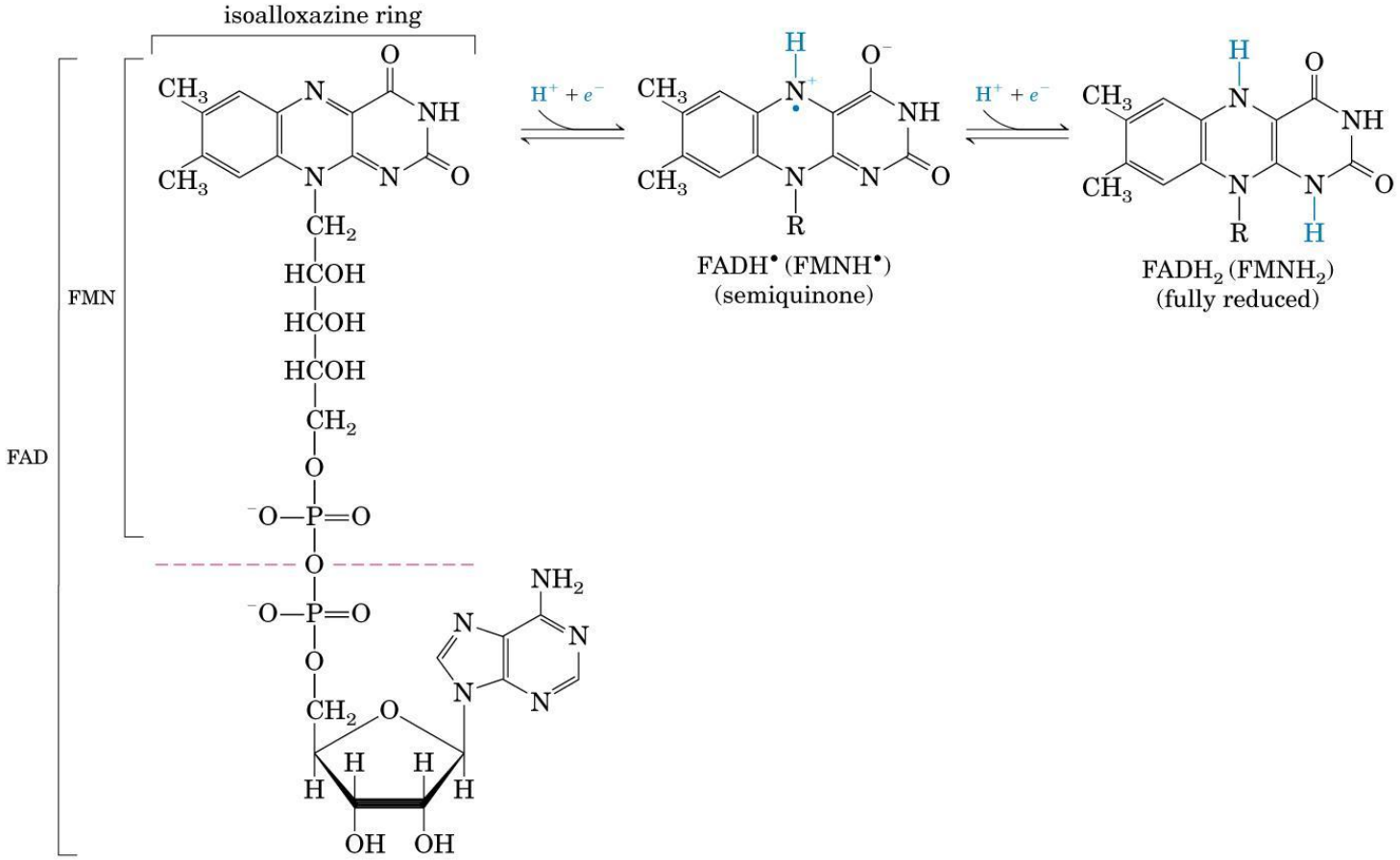
# PYRUVATE DEHYDROGENASE

Lipoic acid is not a Vitamin.



# PYRUVATE DEHYDROGENASE

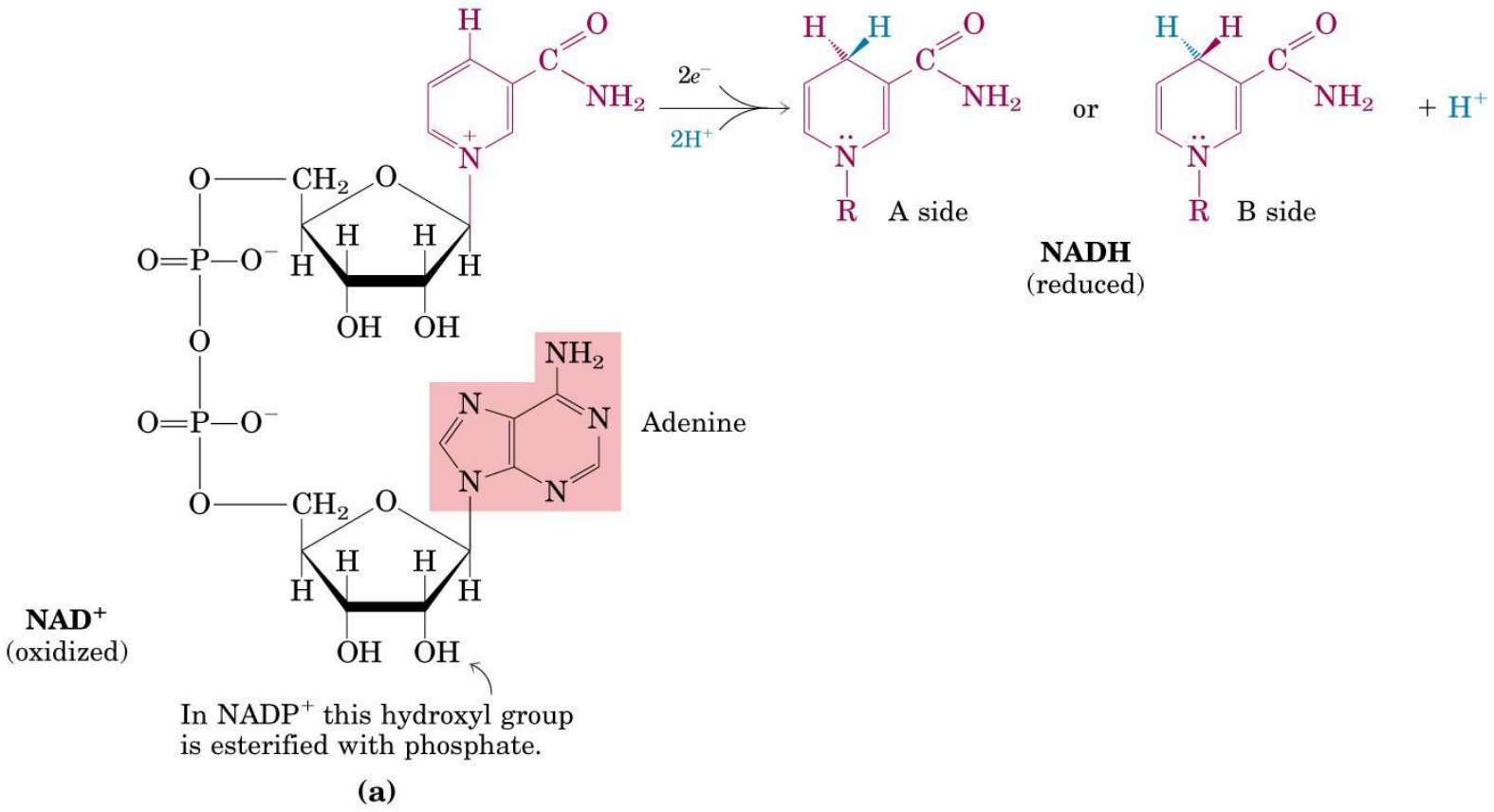
Riboflavin (Vitamin B2).




Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

# PYRUVATE DEHYDROGENASE

Nicotinamide (Vitamin PP or B3)



# PYRUVATE DEHYDROGENASE

Intermediate Reactions of Pyruvate Dehydrogenase

$$CH_3COCOOH + E1\text{-}TPP \rightarrow CO_2 + E1\text{-}TPP\text{-}[CH_3CHO]$$

$$E1\text{-}TPP\text{-}[CH_3CHO] + E2\text{-}Lip\ (S\text{-}S) \rightarrow E1\text{-}TPP + E2\ Lip\text{-}SCO\text{-}CH_3\text{-}SH$$

$$E2\ Lip\text{-}SCO\text{-}CH_3\text{-}SH + CoASH \rightarrow CH_3COSCoA + E2\text{-}Lip\ (SH)_2$$

$$E2\text{-}Lip\ (SH)_2 + E3\text{-}FAD \rightarrow E2\ Lip\ (S\text{-}S) + E3\text{-}FADH_2$$

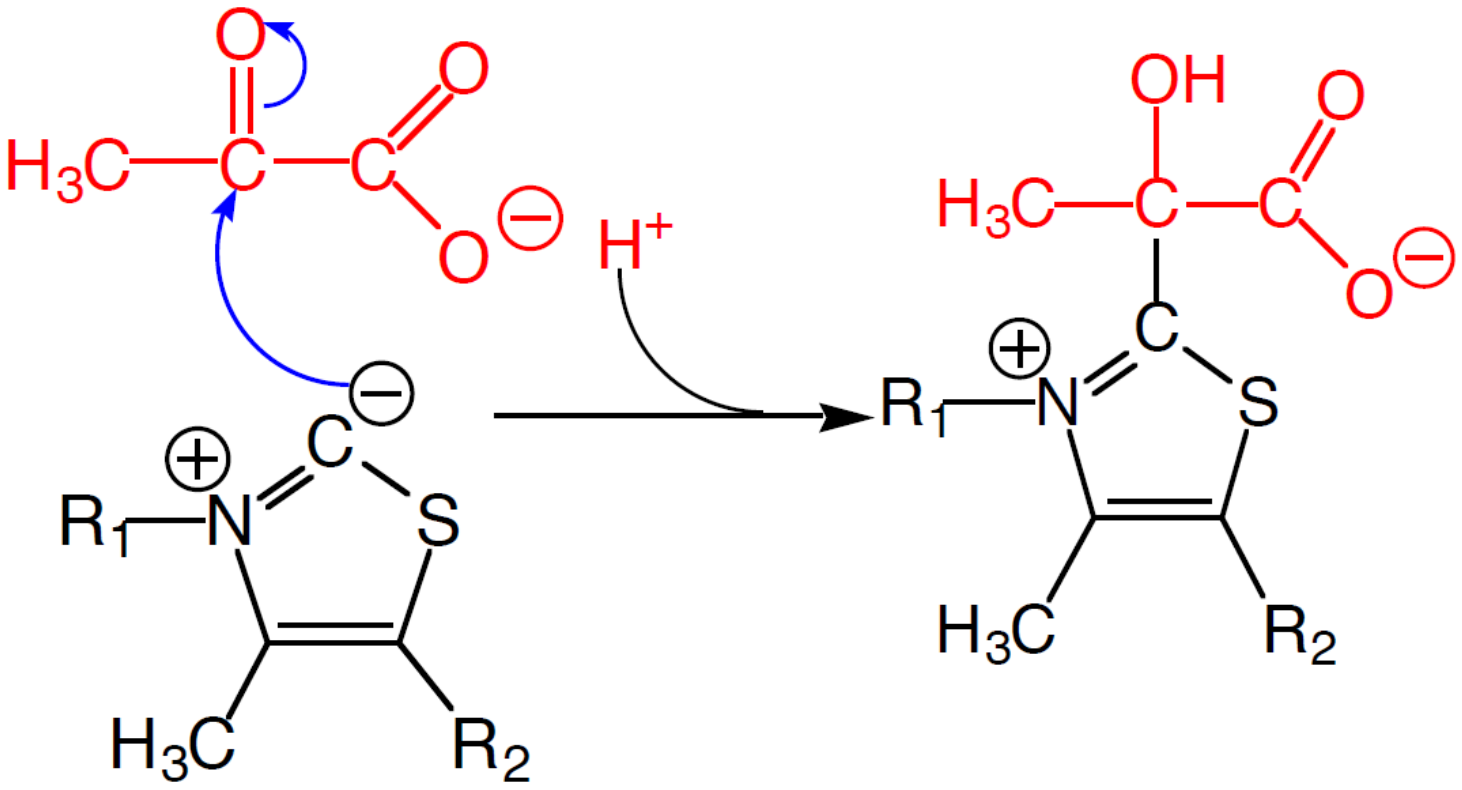
$$E3\text{-}FADH_2 + NAD^+ \rightarrow E3\text{-}FAD + NADH + H^+$$

# PYRUVATE DEHYDROGENASE

Reaction in E1 of Pyruvate Dehydrogenase:

1) decarboxylation

α-keto acid



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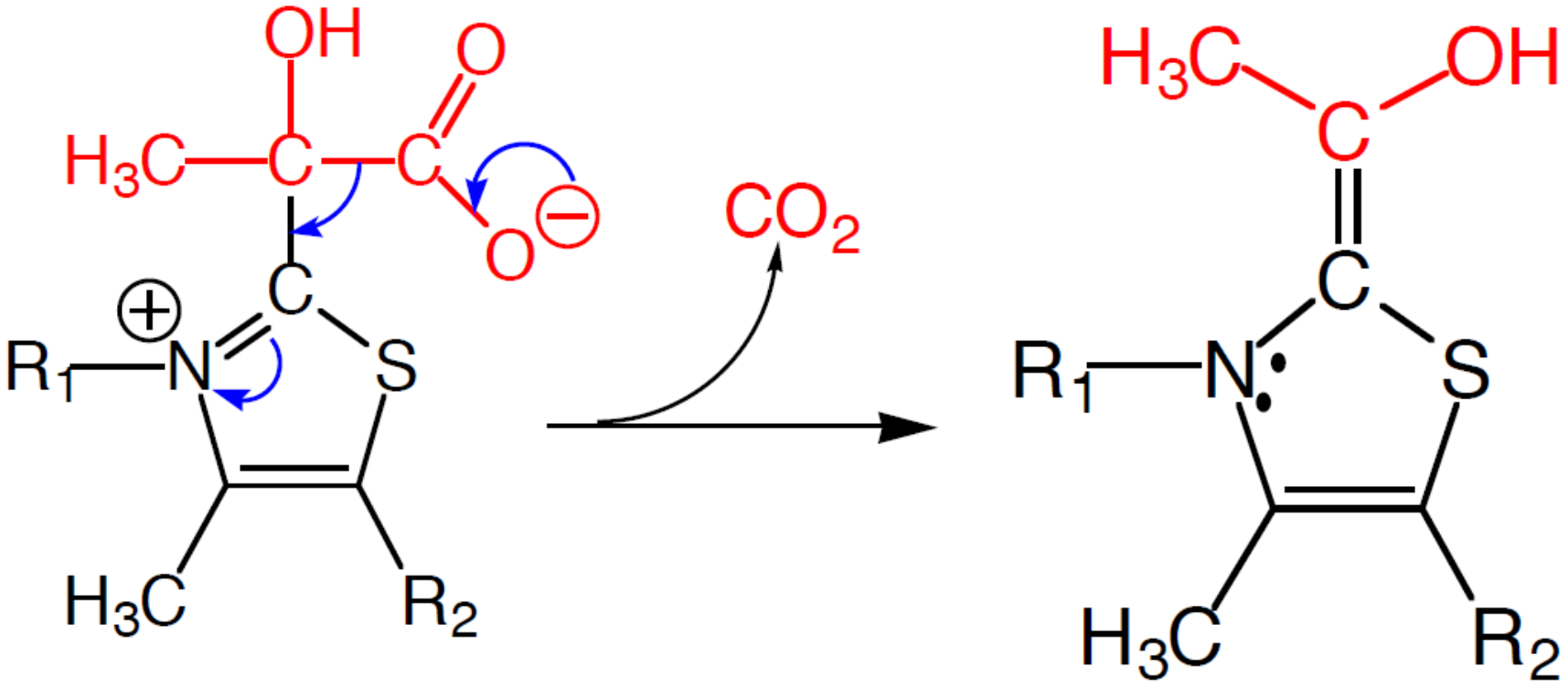

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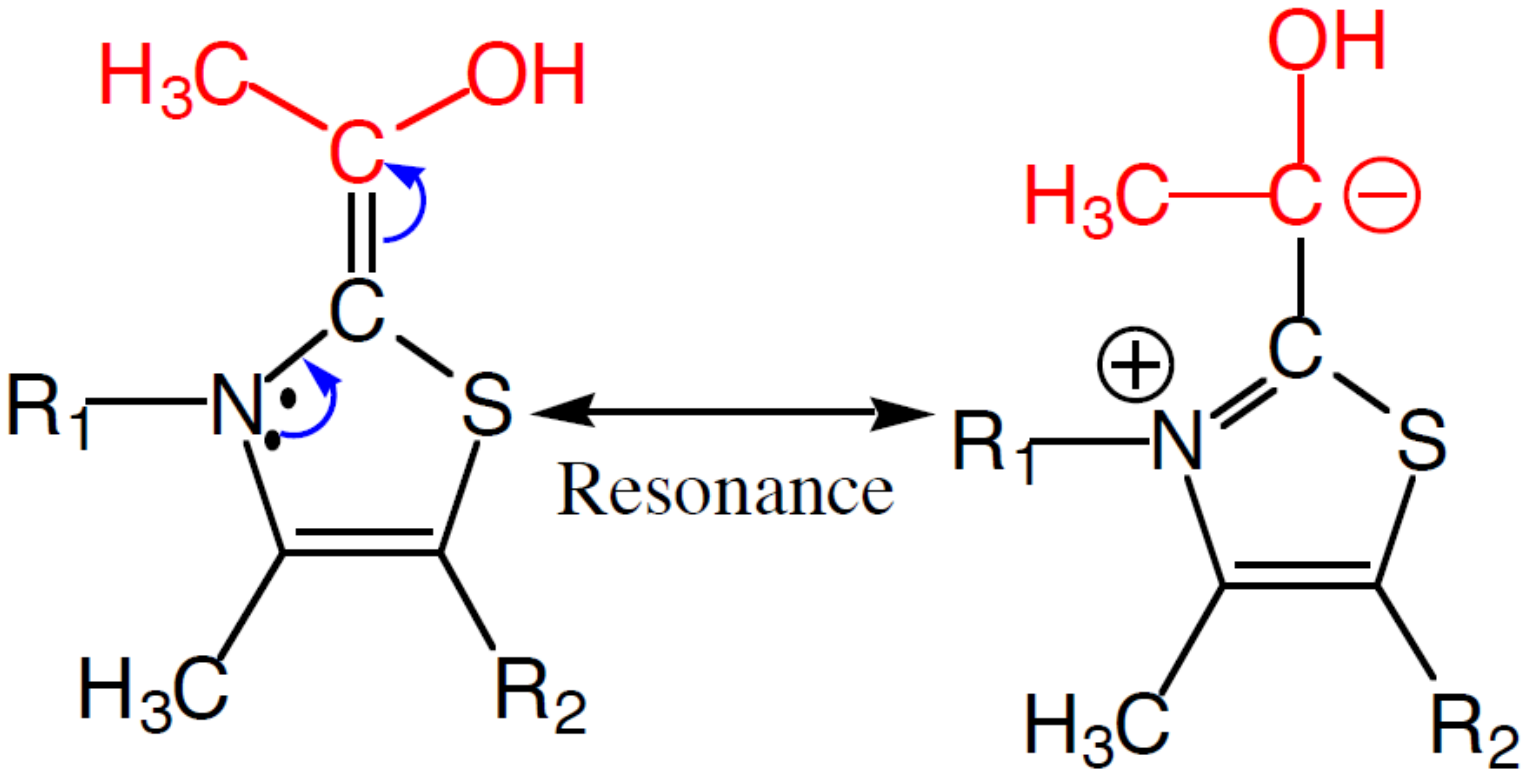


# PYRUVATE DEHYDROGENASE

Aldehyde R-CHO,

$CH_3CHO + Lip\ (S\text{-}S) \rightarrow CH_3COOH + Lip\ (SH)_2$

but oxidised and reduced compounds join together, exploiting the energy of the oxidative reaction:

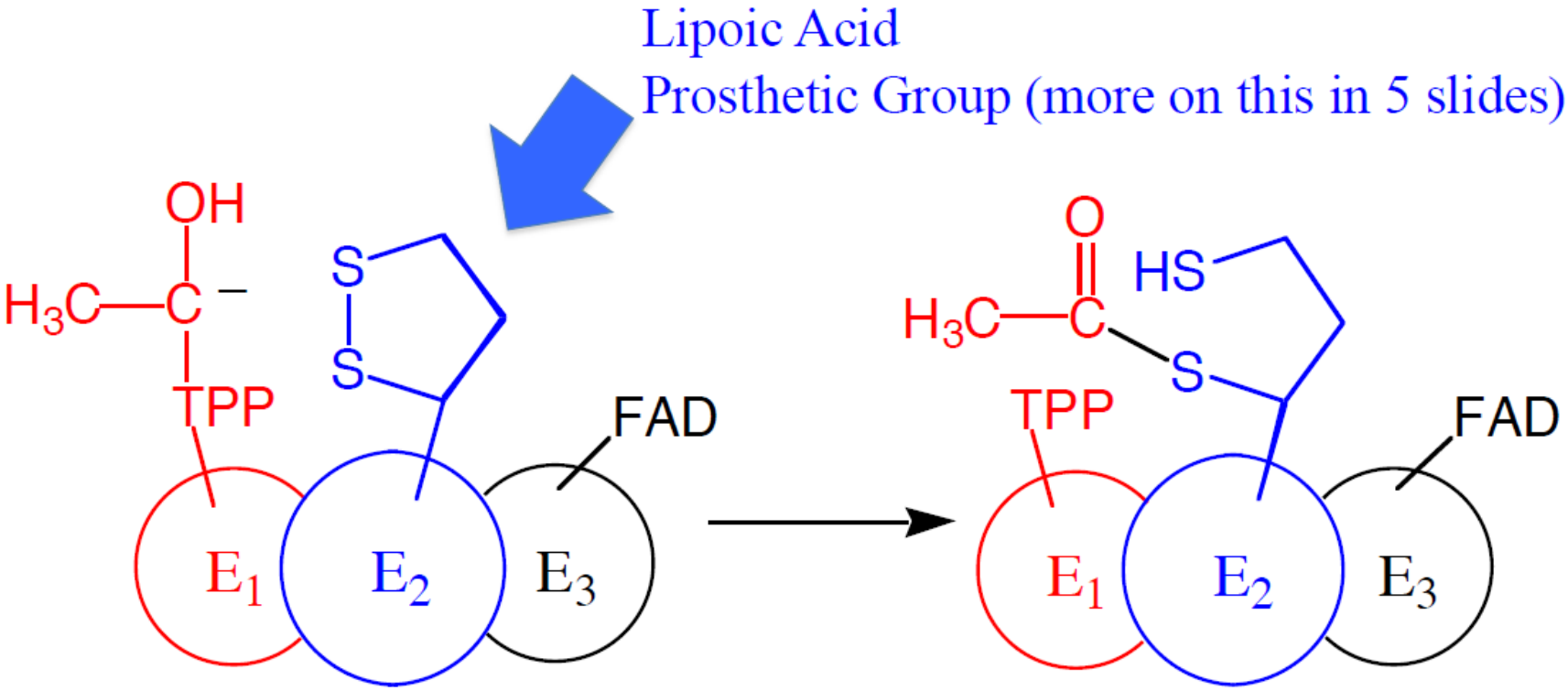
$Lip\text{-}S\text{-}COCH_3$

$-SH$

# PYRUVATE DEHYDROGENASE

Reaction in E2 of Pyruvate Dehydrogenase:

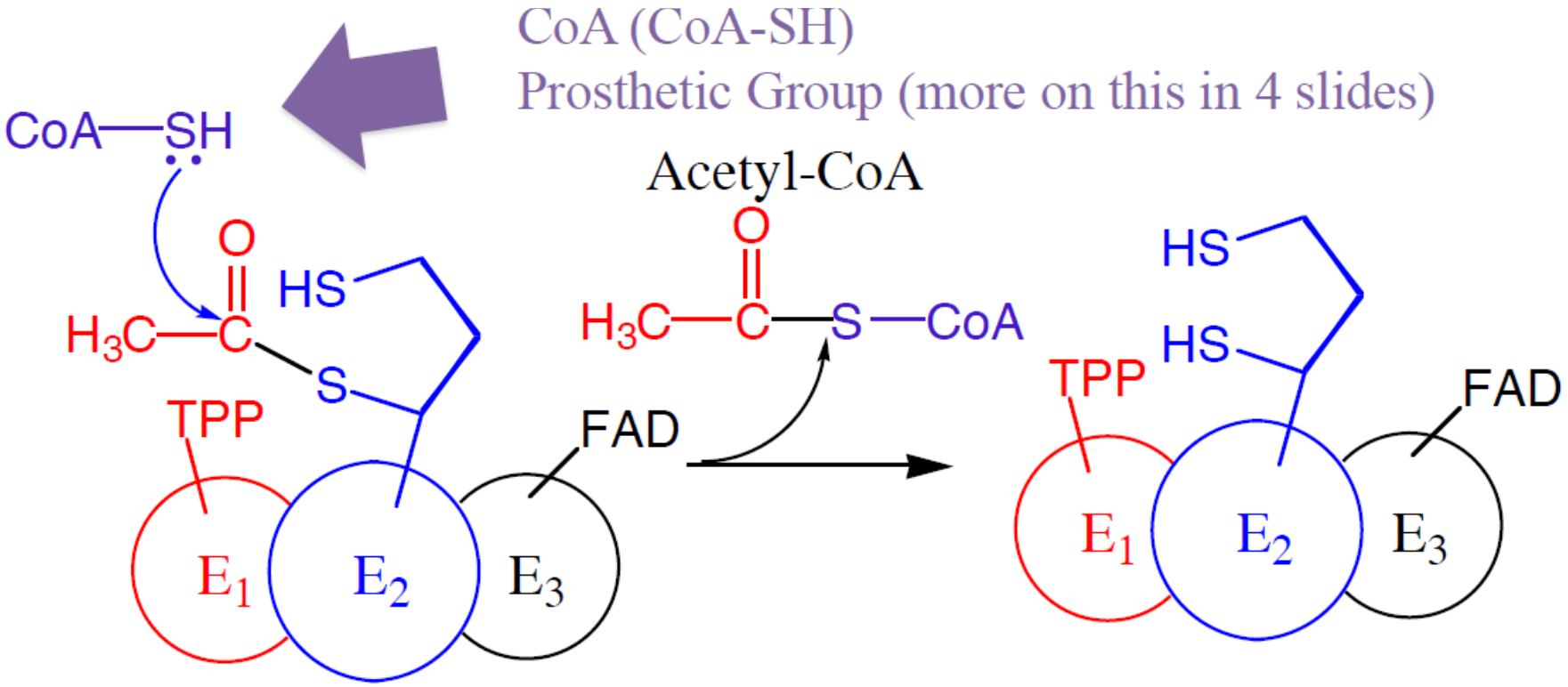
2) oxidation



# PYRUVATE DEHYDROGENASE

Reaction in E2 of Pyruvate Dehydrogenase:

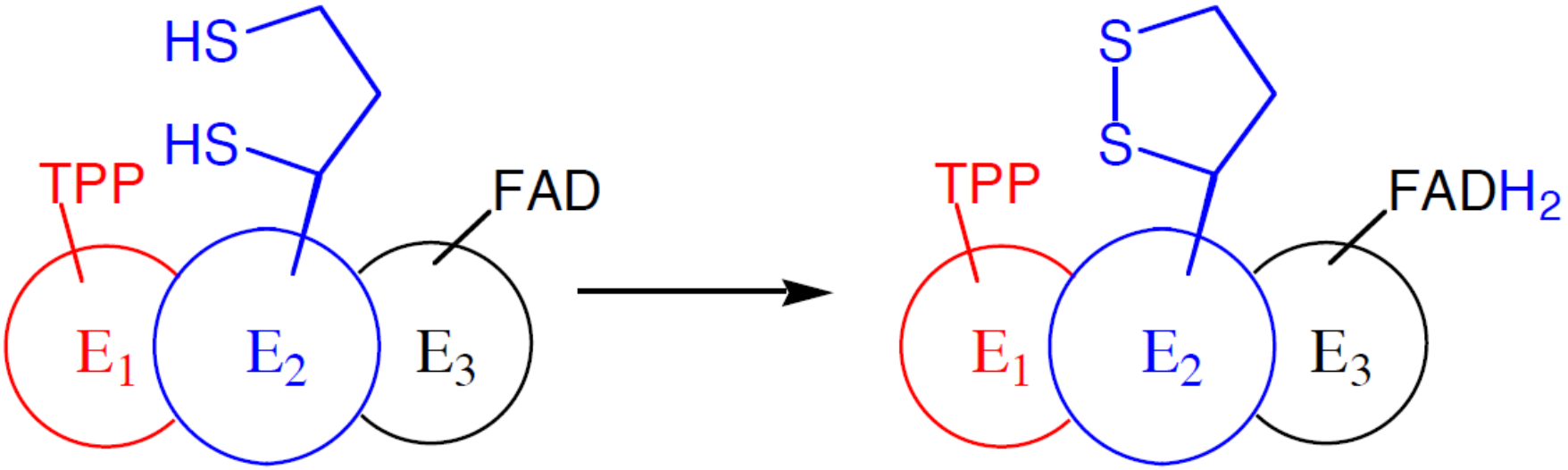
2) oxidation



# PYRUVATE DEHYDROGENASE

Reaction in E3 of Pyruvate Dehydrogenase:

3) shuttling electrons to a carrier ($NAD^+$) and enabling PDH to repeat the cycle



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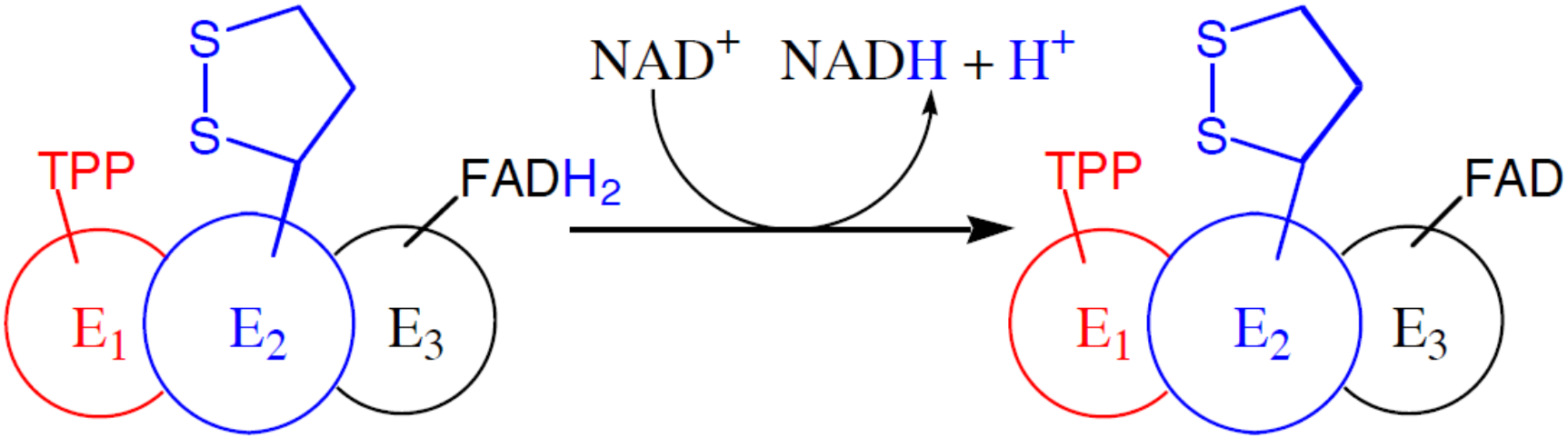
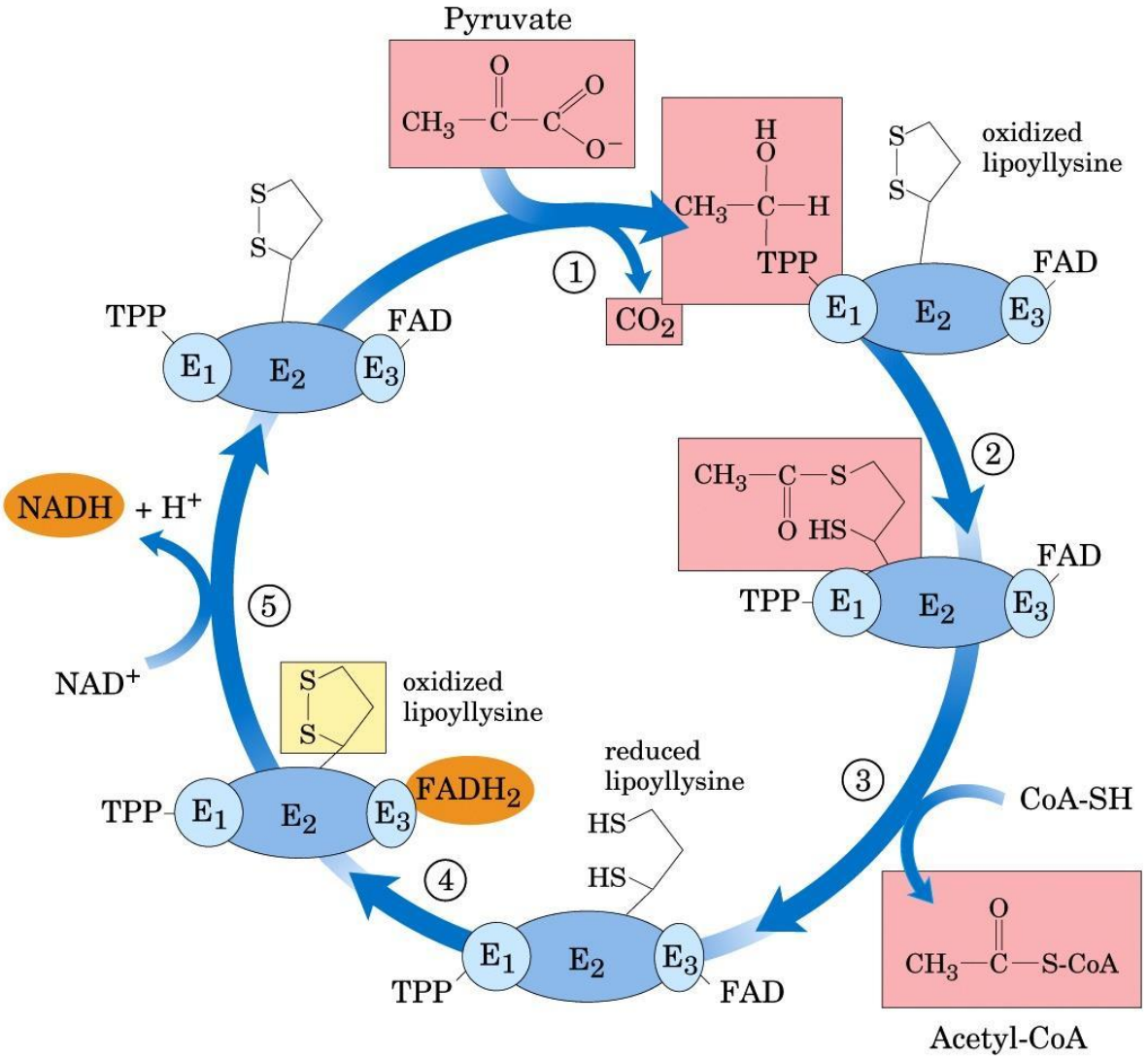
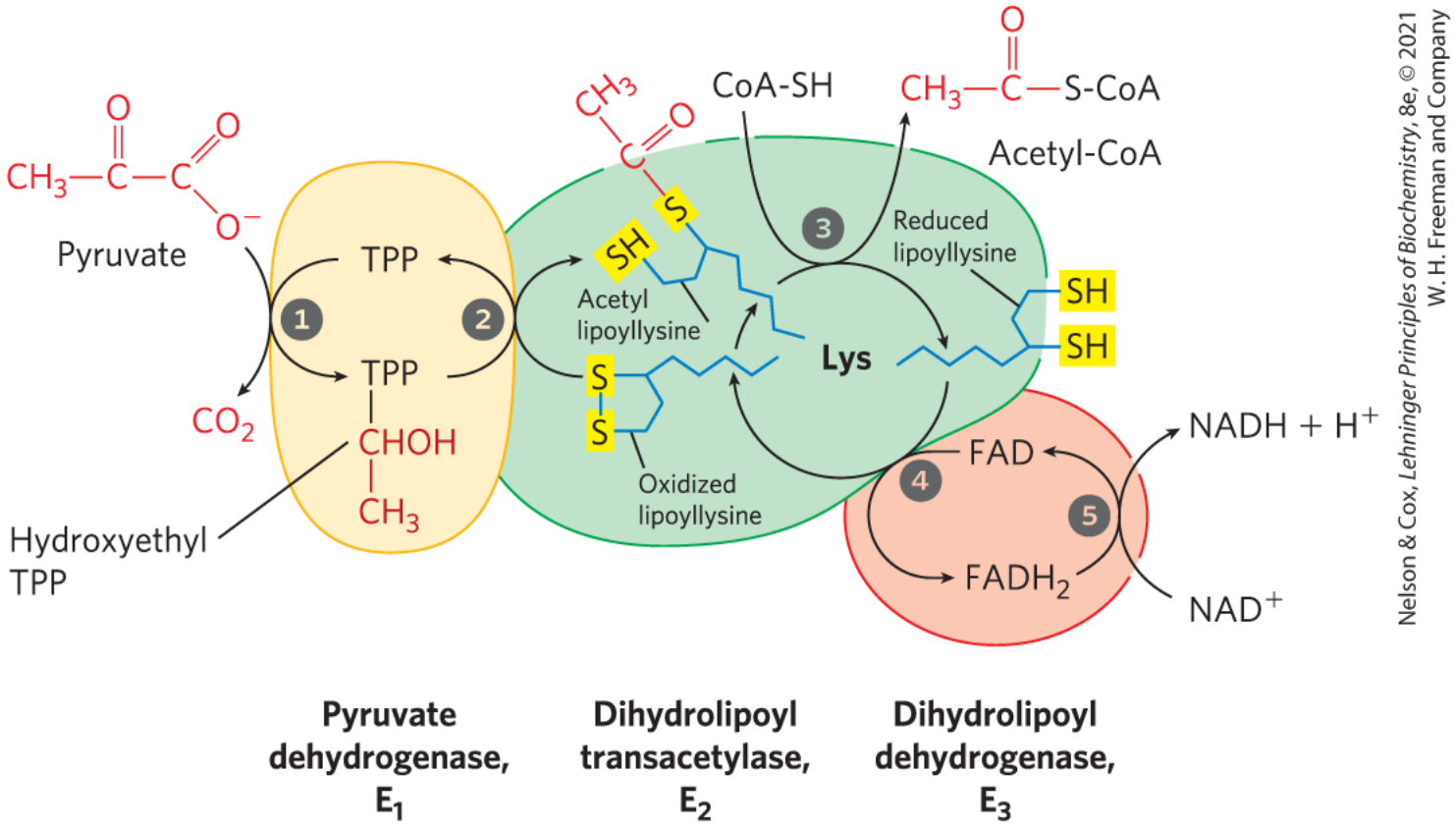


# PYRUVATE DEHYDROGENASE



# PYRUVATE DEHYDROGENASE

**Substrate channeling**: intermediates pass directly from an enzyme to the next one without release.




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

# REGULATION OF PYRUVATE DEHYDROGENASE

**Inhibited** by NADH, ATP, Acetyl-CoA.

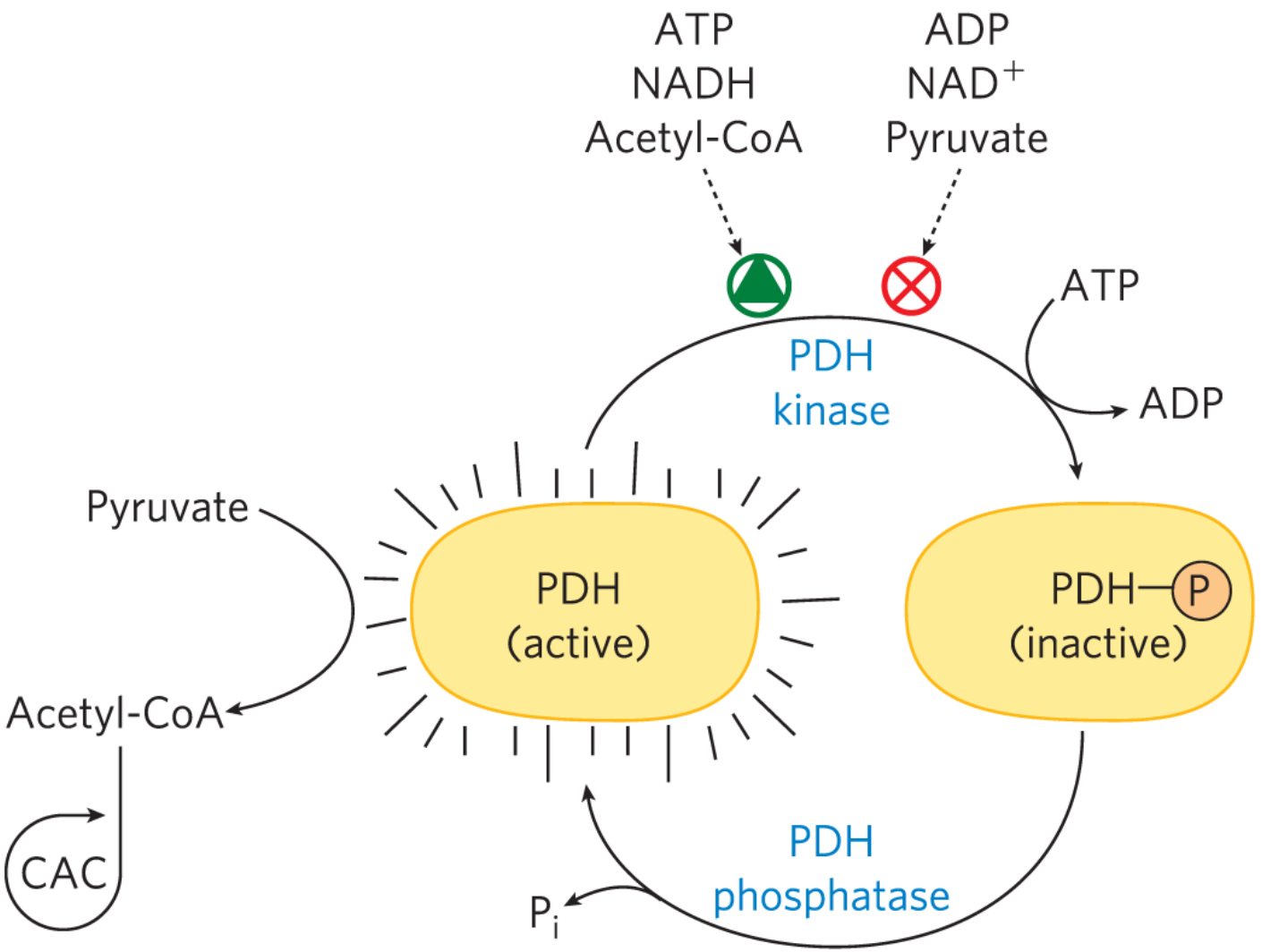
**Stimulated** by $NAD^+$, AMP, CoA.

Inhibited by phosphorylation of E1 (PDH kinase, stimulated by ATP) and activated by dephosphorylation (PDH phosphatase).

**Insulin** enhances **PDH phosphatase** activity, thereby activating the enzyme. This favors formation of acetyl-CoA as a precursor of lipogenesis.

In tumor cells, PDH is inactive because PDHK is activated: this favors glycolysis with formation of lactate (Warburg effect).

# REGULATION OF PYRUVATE DEHYDROGENASE

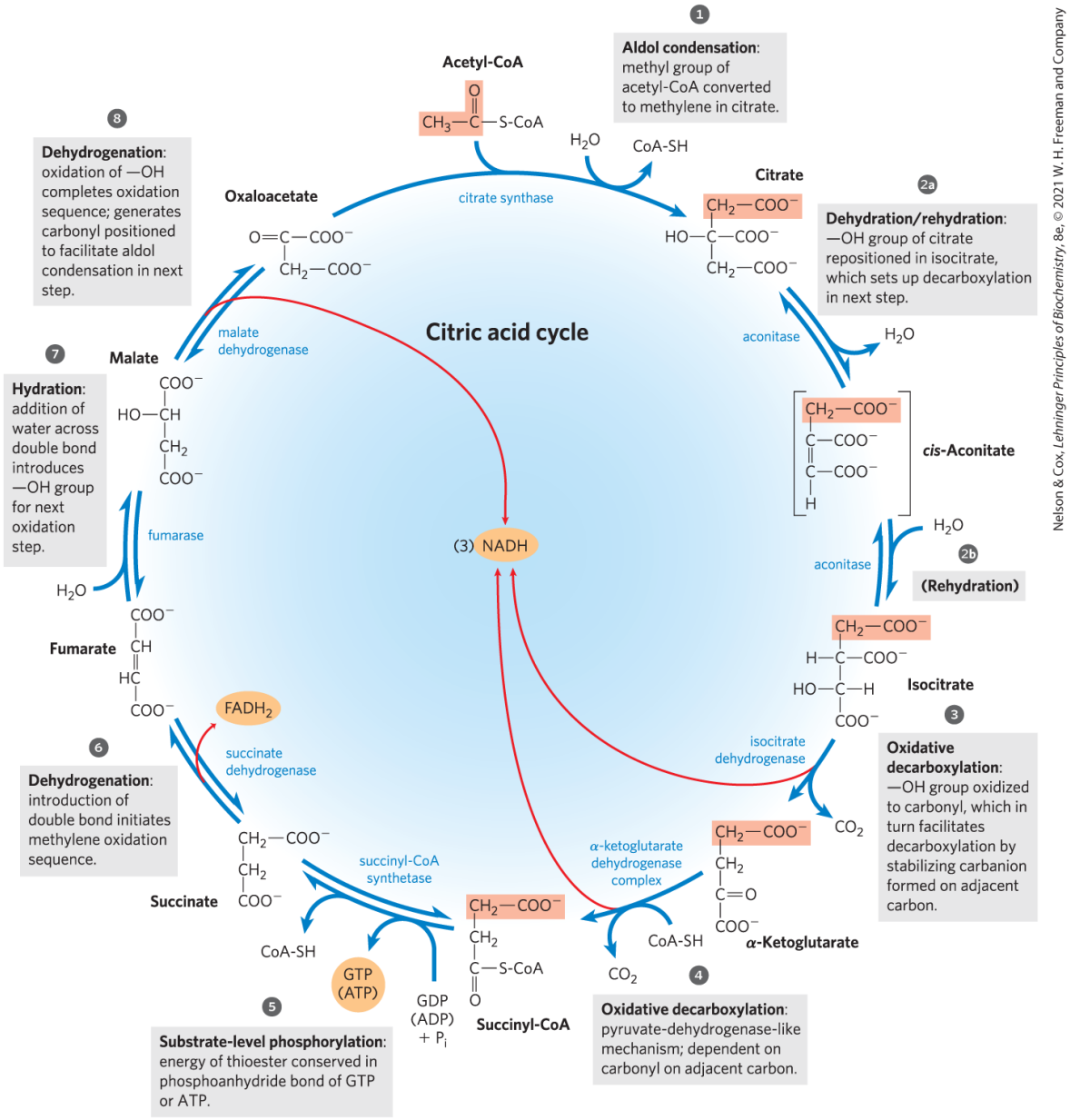


# TCA CYCLE (Krebs, Citric acid)

**Importance of the Citric acid cycle:**

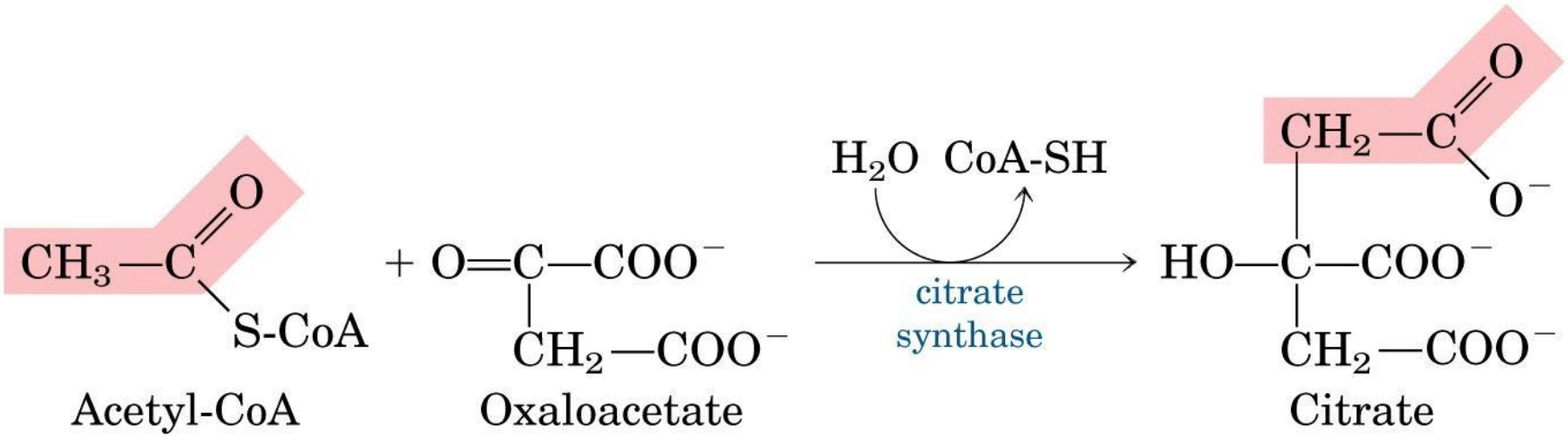
- Central energy-yielding path: point of convergence of catabolism of fats, carbohydrates and proteins;
- In mitochondria;
- Intermediates are recycled (cycle);
- Source of precursor for biosynthesis.

# TCA CYCLE (Krebs, Citric acid)



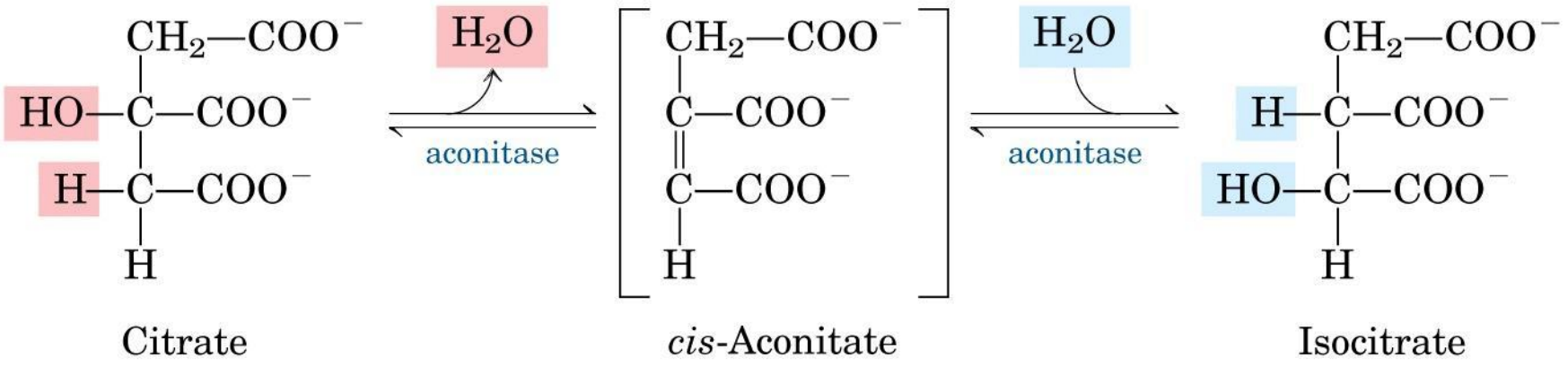
# TCA CYCLE (Krebs, Citric acid)

This reaction is irreversible *in vivo* ([oxaloacetate] low).



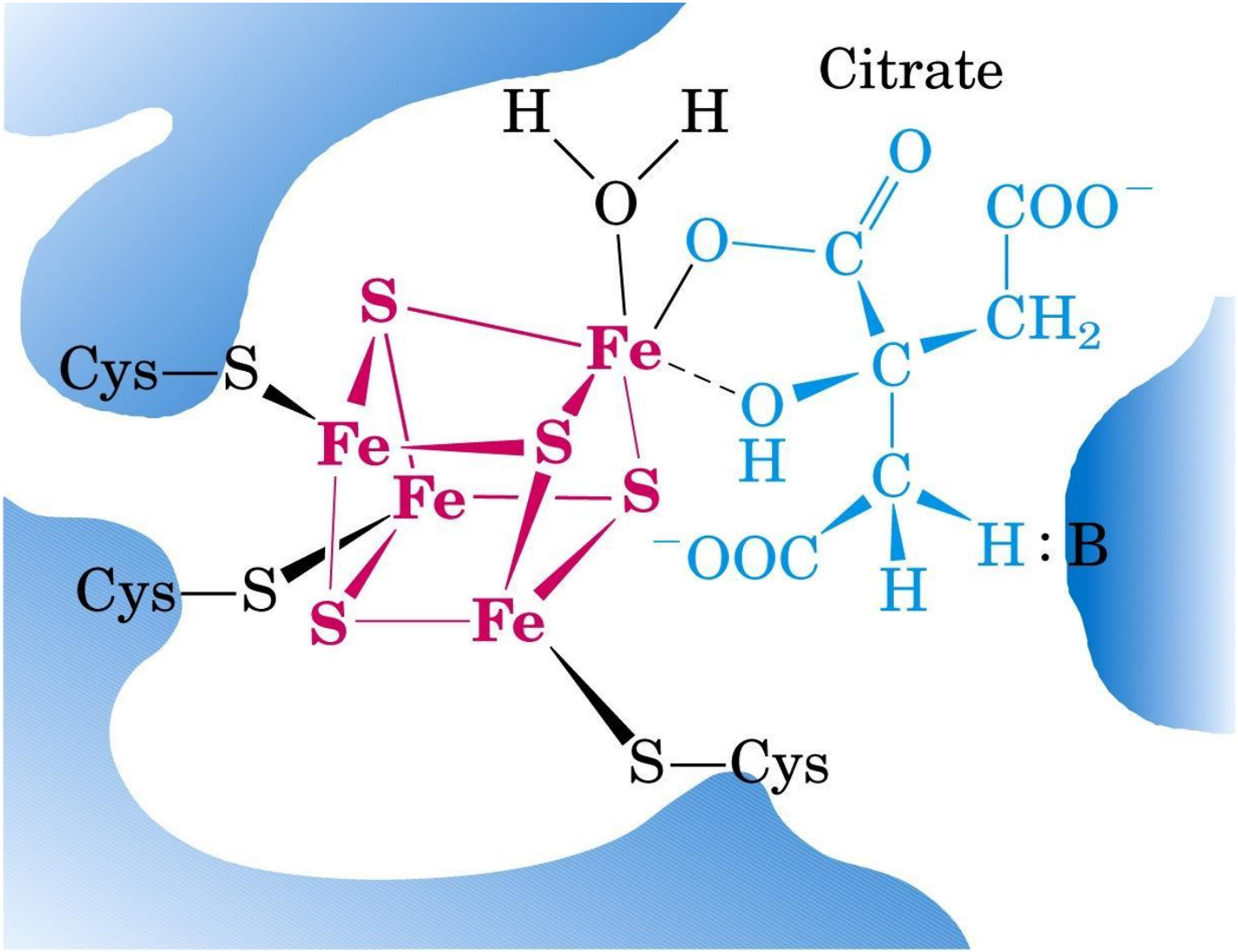

$$\Delta G'^{\circ} = -32.2 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)




$\Delta G'^{\circ} = 13.3$ kJ/mol

# TCA CYCLE (Krebs, Citric acid)

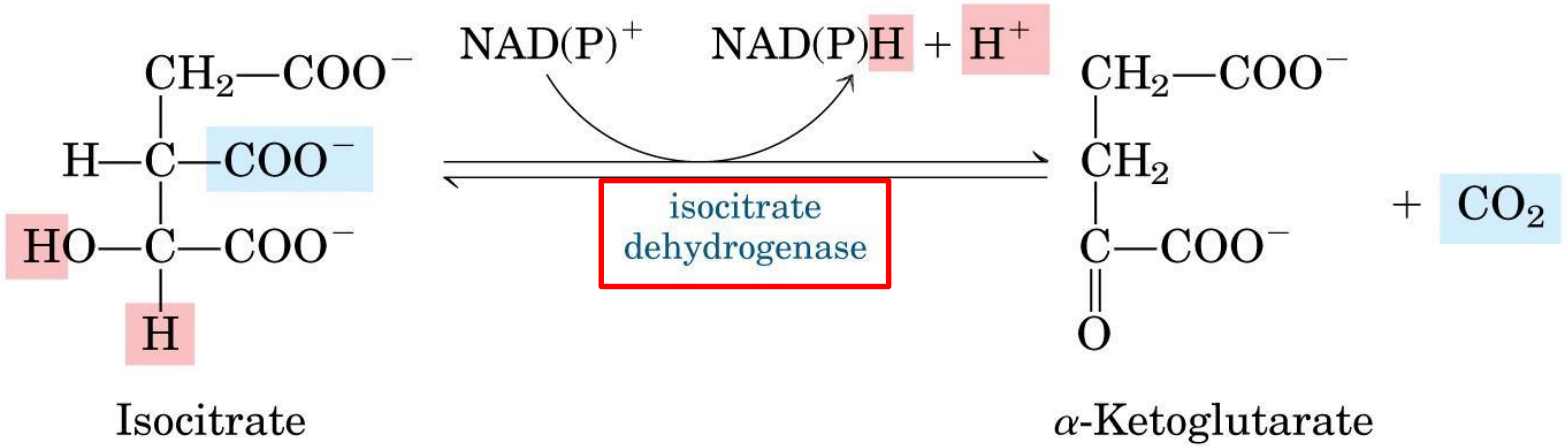


# TCA CYCLE (Krebs, Citric acid)

**Regulatory enzyme**: inhibited by ATP and NADH, activated by ADP and $NAD^+$.

Specific isozymes for $NADP^+$ (cytosolic and mitochondrial) or $NAD^+$ (mitochondrial).

The reaction is **reversible**.

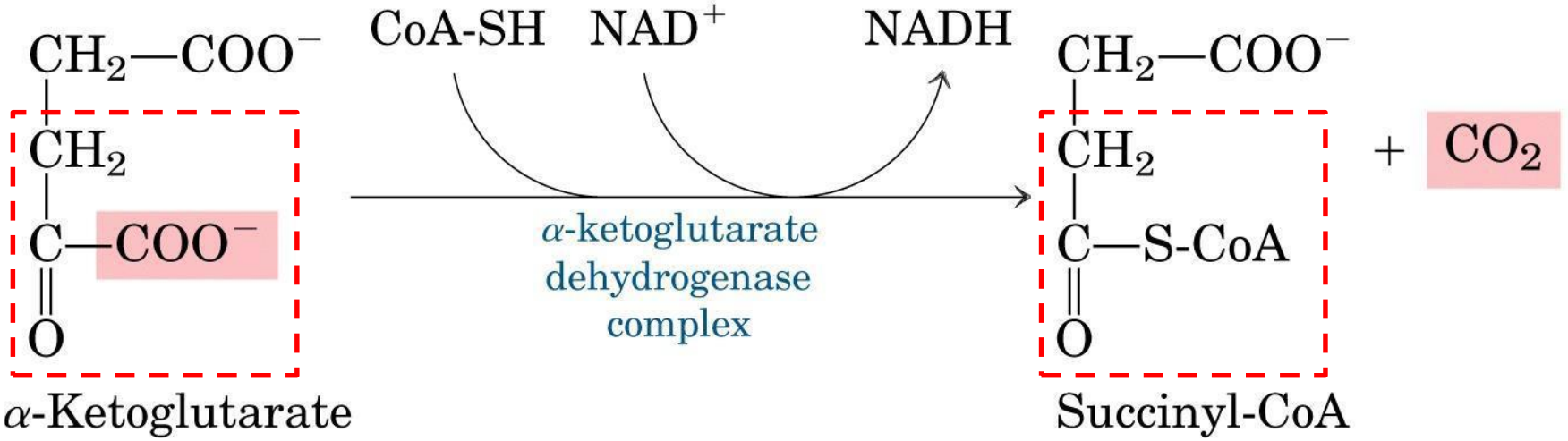



$$\Delta G'^{\circ} = -20.9 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)

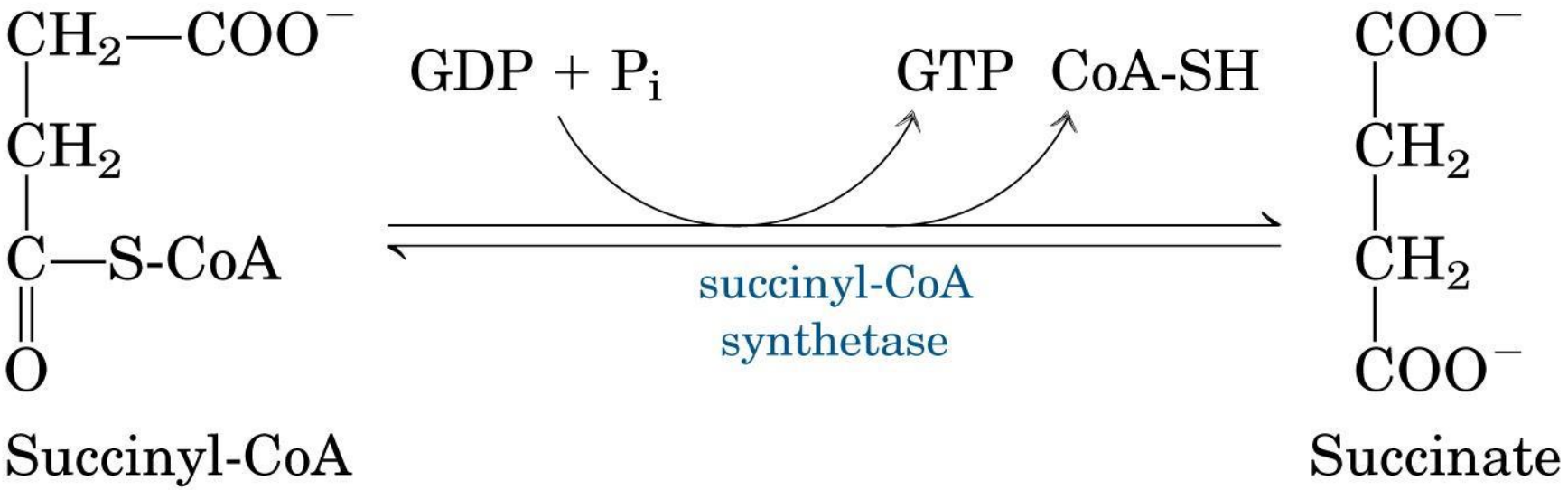
As in PDH, the KGDH complex consists of E1 (with TPP), E2 (with lipoamide), and E3 (with FAD) and follows the same steps.

Reaction is **irreversible**.



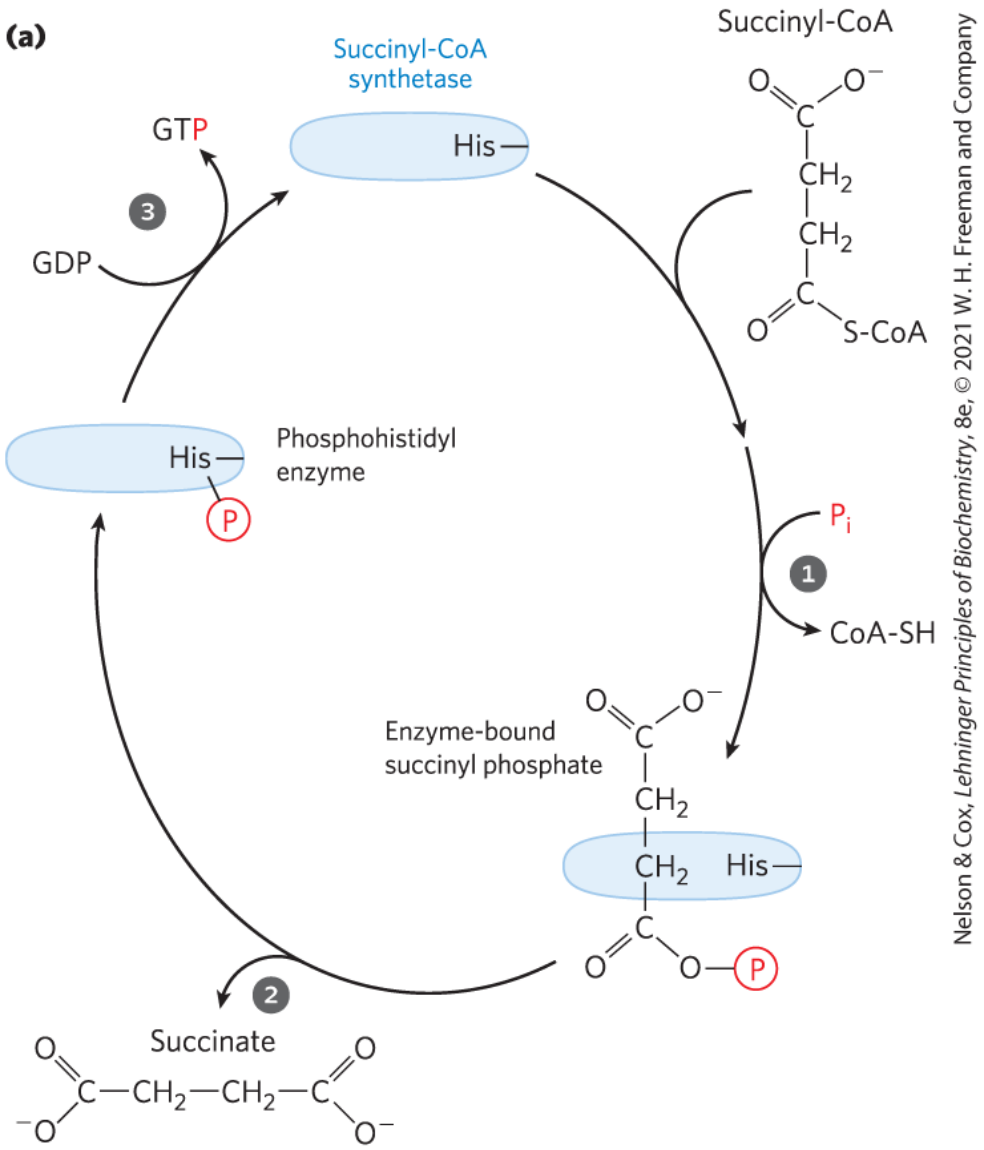

$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)



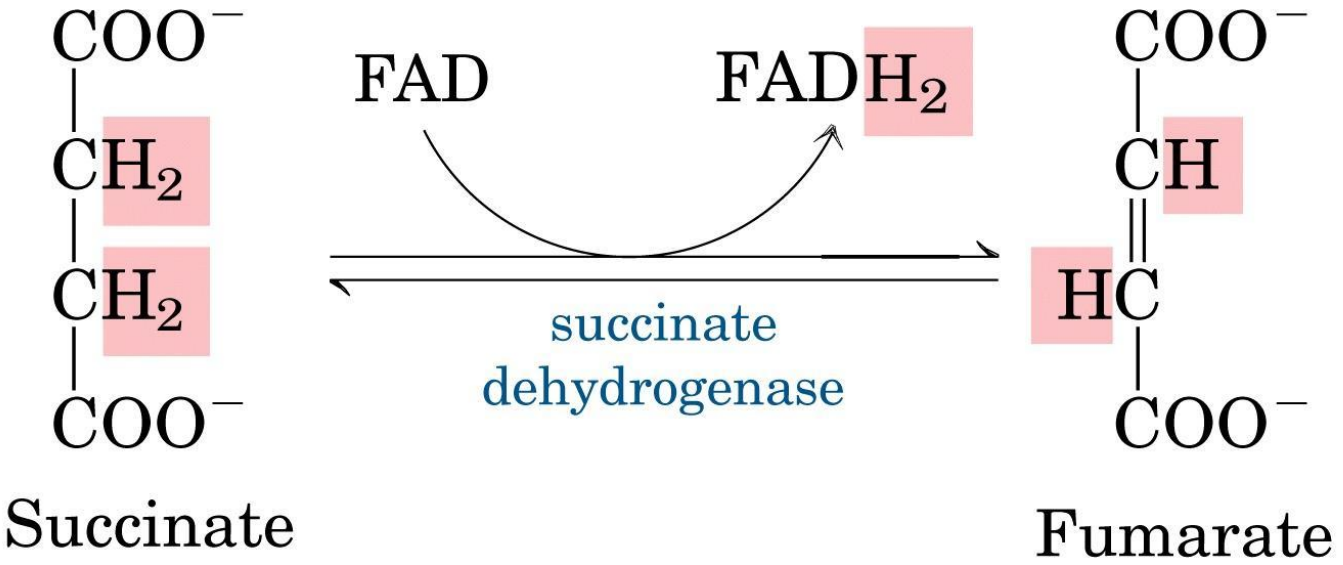

$$\Delta G'^{\circ} = -2.9 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)



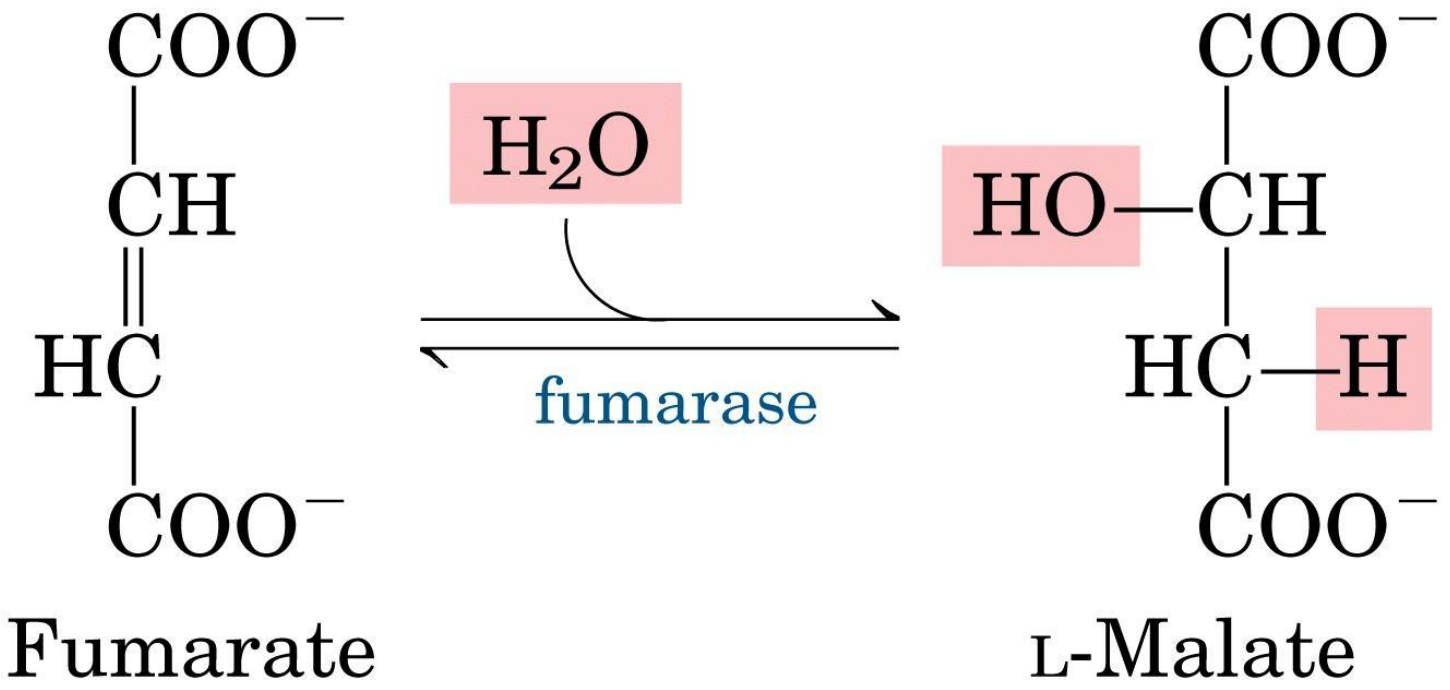
# TCA CYCLE (Krebs, Citric acid)

Natural acceptor is CoQ: succinate CoQ reductase is Complex II. The only membrane-bound enzyme of TCA cycle (mitochondrial inner membrane).




$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)




Fumarate

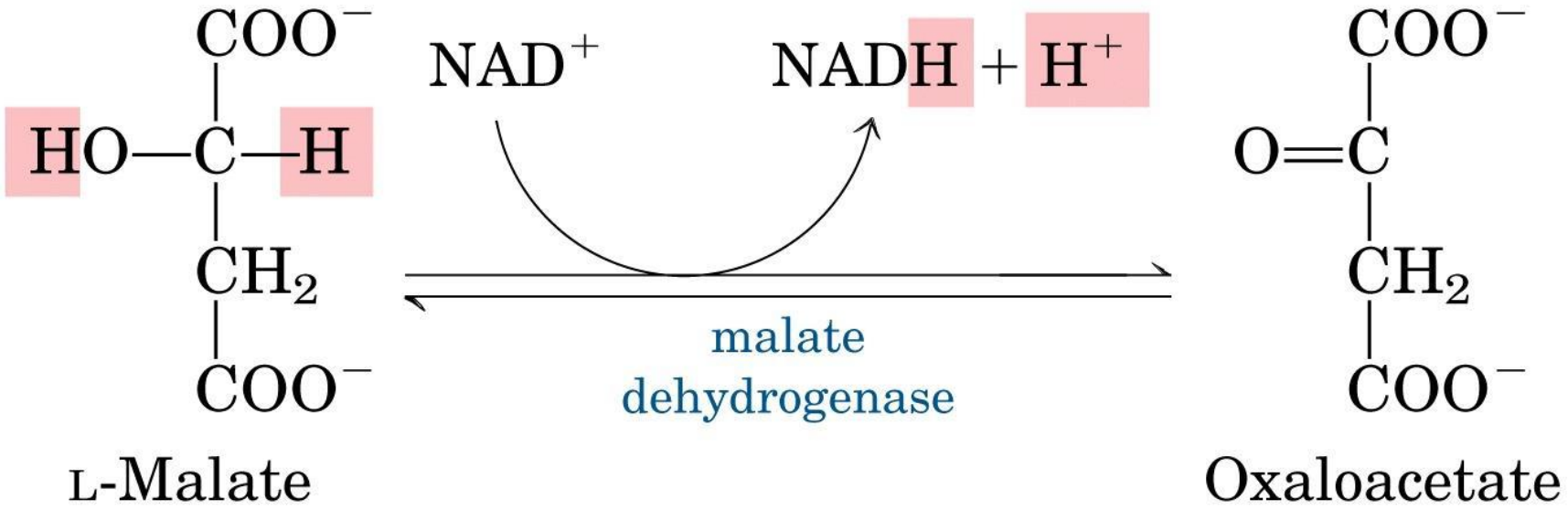
L-Malate

$$\Delta G'^{\circ} = -3.8 \text{ kJ/mol}$$

# TCA CYCLE (Krebs, Citric acid)

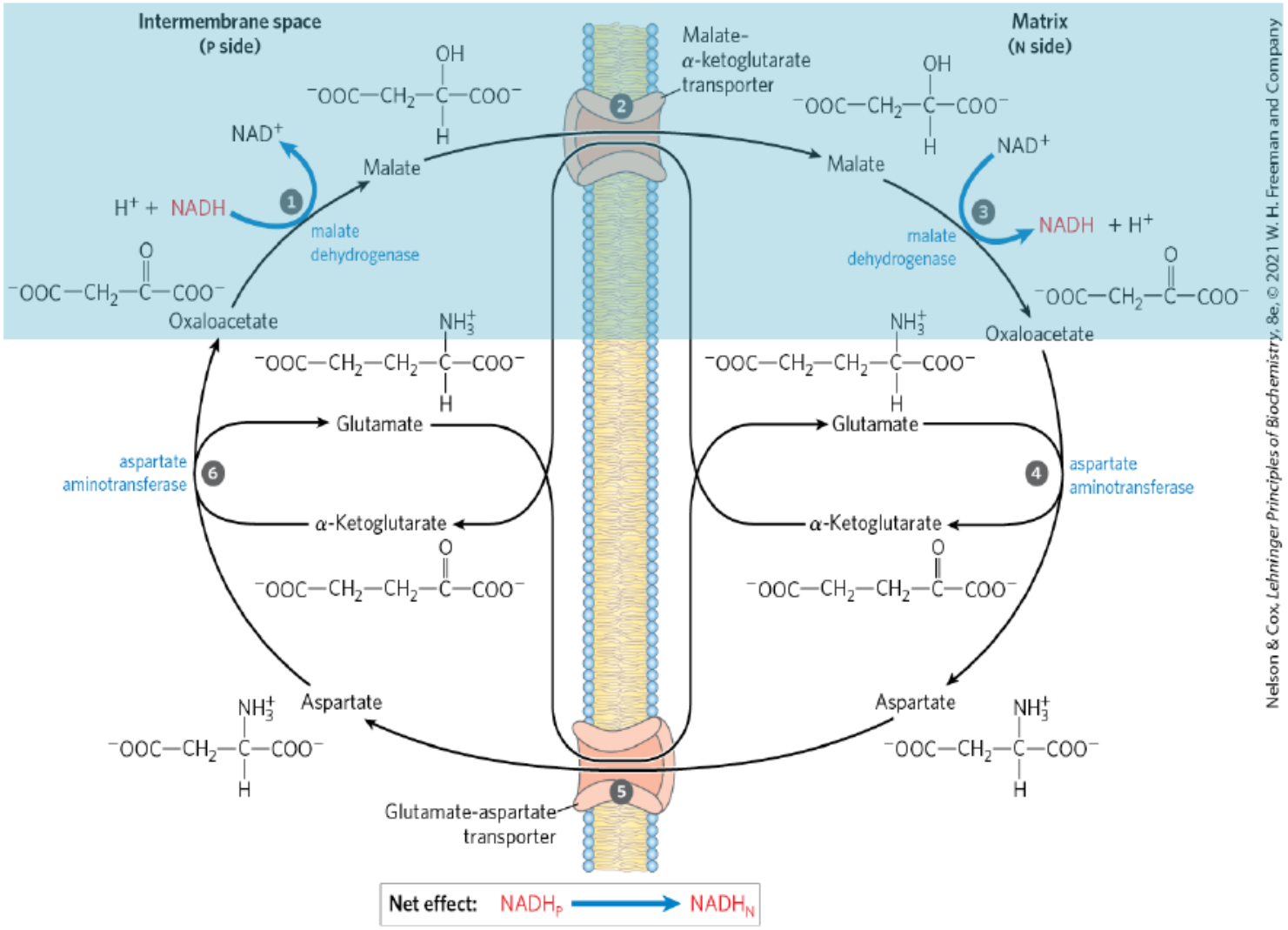
Low oxaloacetate levels pull the reaction forward.

Regenerates oxaloacetate for citrate synthesis.

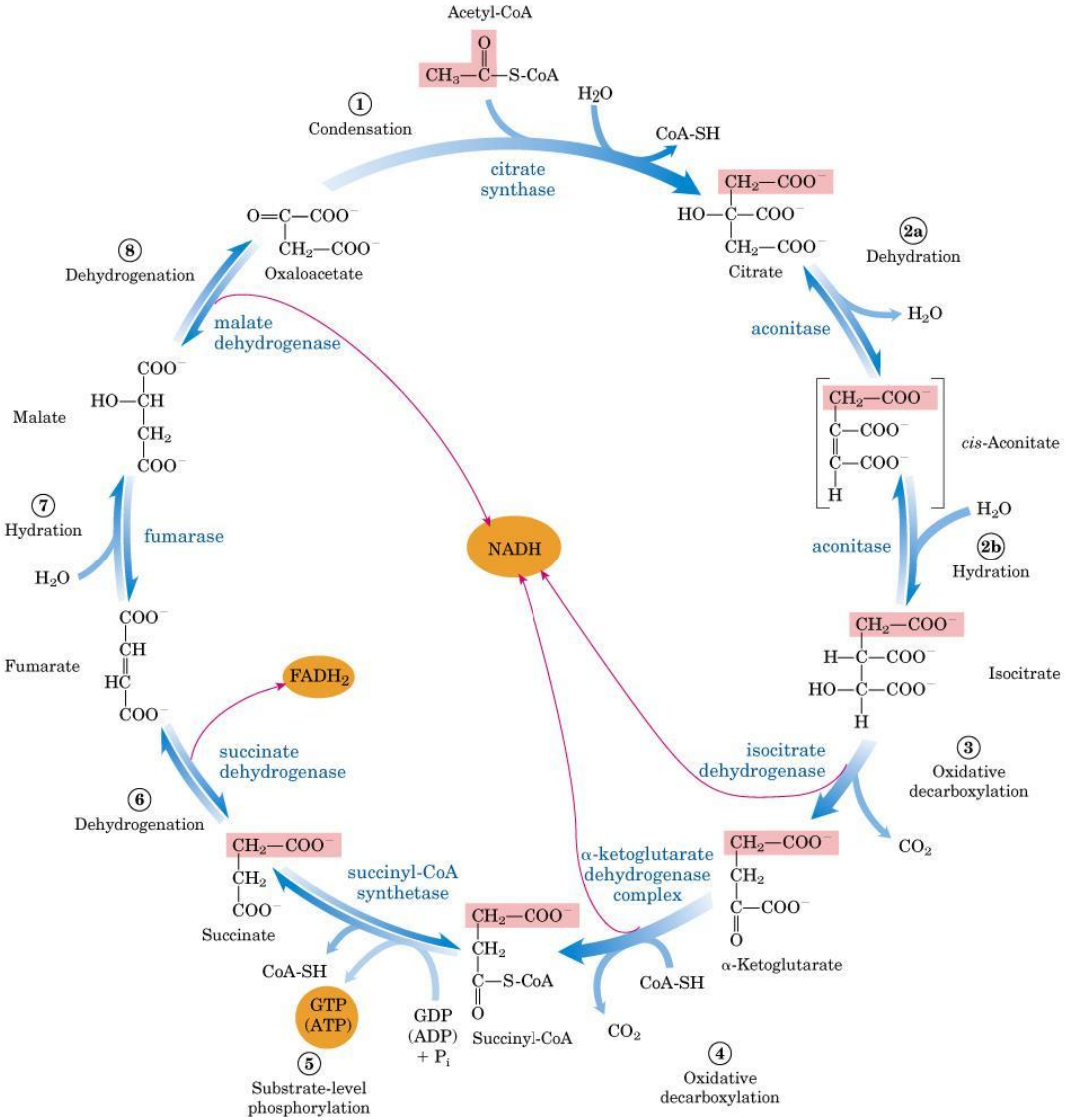



$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$

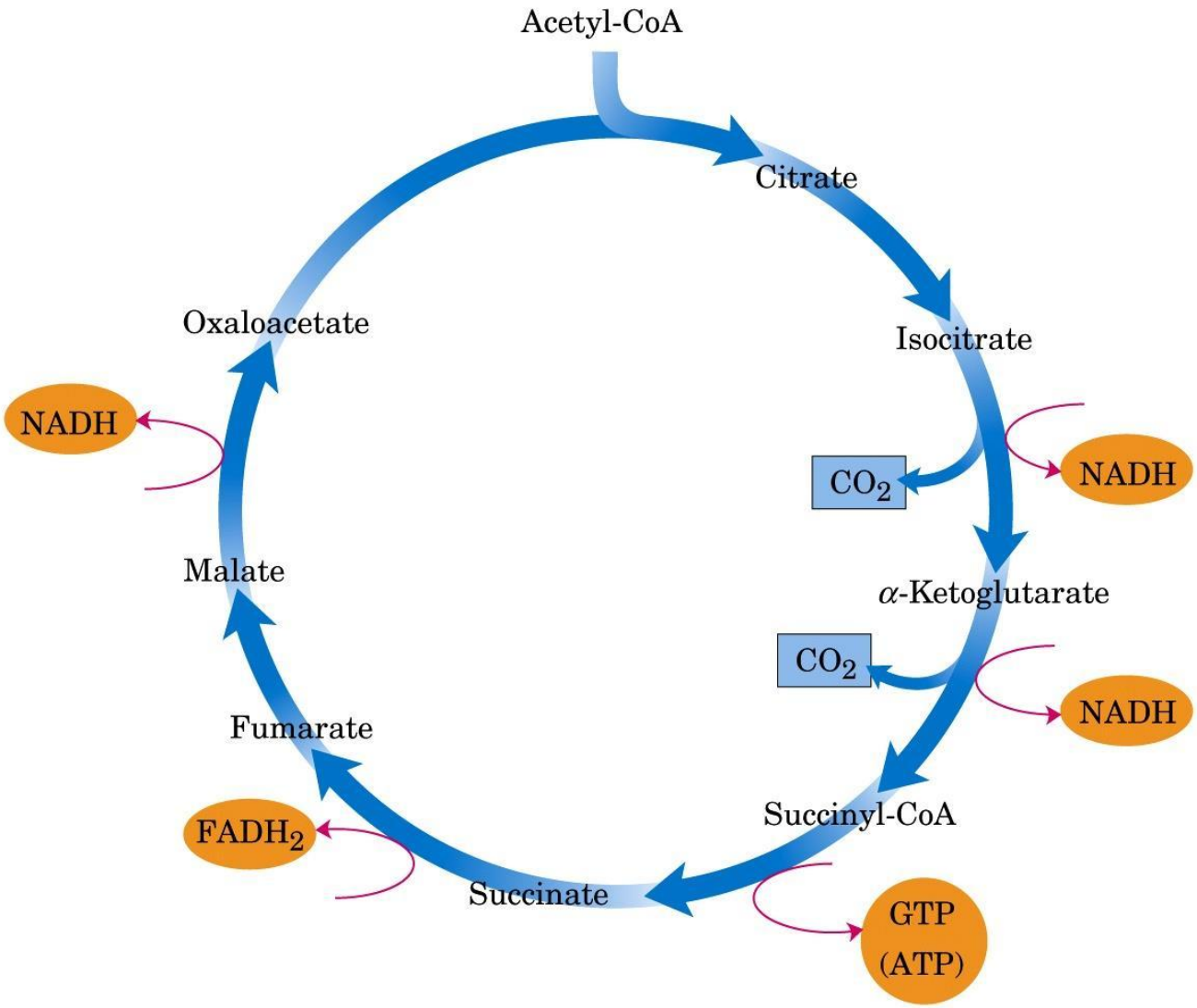
# MECHANISMS OF NADH TRANSPORT



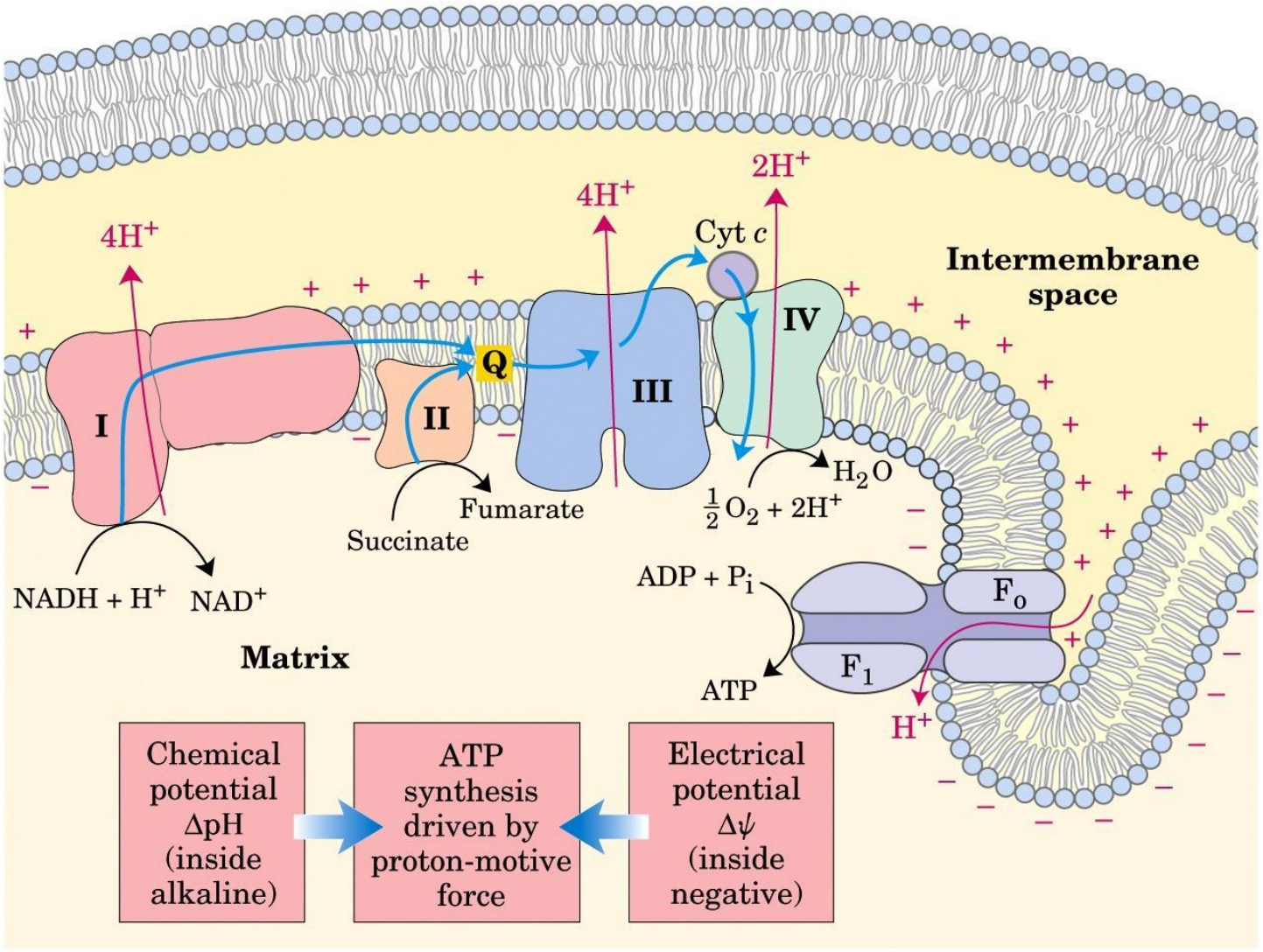
# TCA CYCLE (Krebs, Citric acid)



# TCA CYCLE (Krebs, Citric acid)



# TCA CYCLE (Krebs, Citric acid)



# TCA CYCLE (Krebs, Citric acid)

**Stoichiometry of TCA cycle**

$$CH_3COSCoA + 3\ H2O^* \rightarrow CoASH + 2\ CO_2 + 8\ H$$

$$8\ H + 2\ O_2 \rightarrow 4\ H_2O$$

Total:

$$CH_3COSCoA + 2\ O_2 \rightarrow CoASH + 2\ CO_2 + H_2O$$

* One molecule of $H_2O$ may be considered as derived from GDP and $P_i$ dehydration during succinyl-CoA synthetase reaction:

$$HOOCCH_2CH_2COSCoA + GDPH + POH \rightarrow HOOCCH_2CH_2COOH + CoASH + GTP$$

Real (complete) stoichiometry should be:

$$CH_3COSCoA + 2\ O_2 + GDPH + POH \rightarrow CoASH + 2\ CO_2 + H_2O + GTP$$

# TCA CYCLE (Krebs, Citric acid)

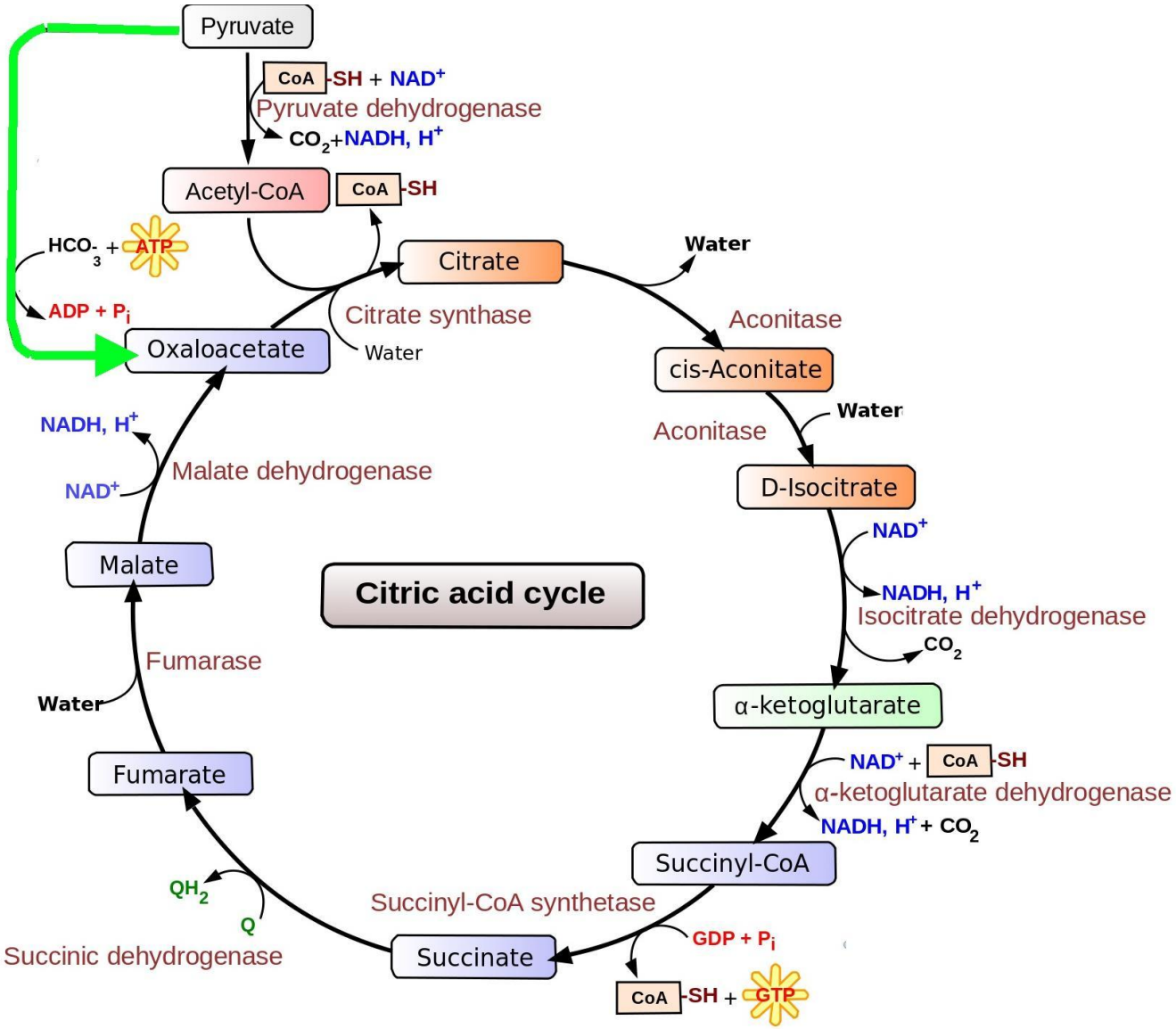
table 16–1

**Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation**

| Reaction | Number of ATP or reduced coenzymes directly formed | Number of ATP ultimately formed* |
|---|---|---|
| Glucose ⟶ glucose 6-phosphate | −1 ATP | −1 |
| Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate | −1 ATP | −1 |
| 2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate | 2 NADH | 3–5 |
| 2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate | 2 ATP | 2 |
| 2 Phosphoenolpyruvate ⟶ 2 pyruvate | 2 ATP | 2 |
| 2 Pyruvate ⟶ 2 acetyl-CoA | 2 NADH | 5 |
| 2 Isocitrate ⟶ 2 $\alpha$-ketoglutarate | 2 NADH | 5 |
| 2 $\alpha$-Ketoglutarate ⟶ 2 succinyl-CoA | 2 NADH | 5 |
| 2 Succinyl-CoA ⟶ 2 succinate | 2 ATP (or 2 GTP) | 2 |
| 2 Succinate ⟶ 2 fumarate | 2 $FADH_2$ | 3 |
| 2 Malate ⟶ 2 oxaloacetate | 2 NADH | 5 |
| Total | | 30–32 |

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

# ANAPLEROTIC REACTIONS



# ANAPLEROTIC REACTIONS

The most important is **pyruvate carboxylase**, an allosteric enzyme activated by acetyl-CoA. Acetyl-CoA by this way acts as a signal to enhance its oxidation in the TCA cycle.

At fasting, acetyl-CoA from fatty acid oxidation in liver may also be a signal for gluconeogenesis, that requires oxaloacetate.

table 16–2

**Anaplerotic Reactions**

| Reaction | Tissue(s)/organism(s) |
|---|---|
| Pyruvate + $HCO_3^-$ + ATP $\xrightleftharpoons{\text{pyruvate carboxylase}}$ oxaloacetate + ADP + $P_i$ | Liver, kidney |
| Phosphoenolpyruvate + $CO_2$ + GDP $\xrightleftharpoons{\text{PEP carboxykinase}}$ oxaloacetate + GTP | Heart, skeletal muscle |
| Phosphoenolpyruvate + $HCO_3^-$ $\xrightleftharpoons{\text{PEP carboxylase}}$ oxaloacetate + $P_i$ | Higher plants, yeast, bacteria |
| Pyruvate + $HCO_3^-$ + NAD(P)H $\xrightleftharpoons{\text{malic enzyme}}$ malate + $NAD(P)^+$ | Widely distributed in eukaryotes and prokaryotes |

# PYRUVATE CARBOXYLASE

**Pyruvate carboxylase**:

- exclusively mitochondrial
- four subunits:

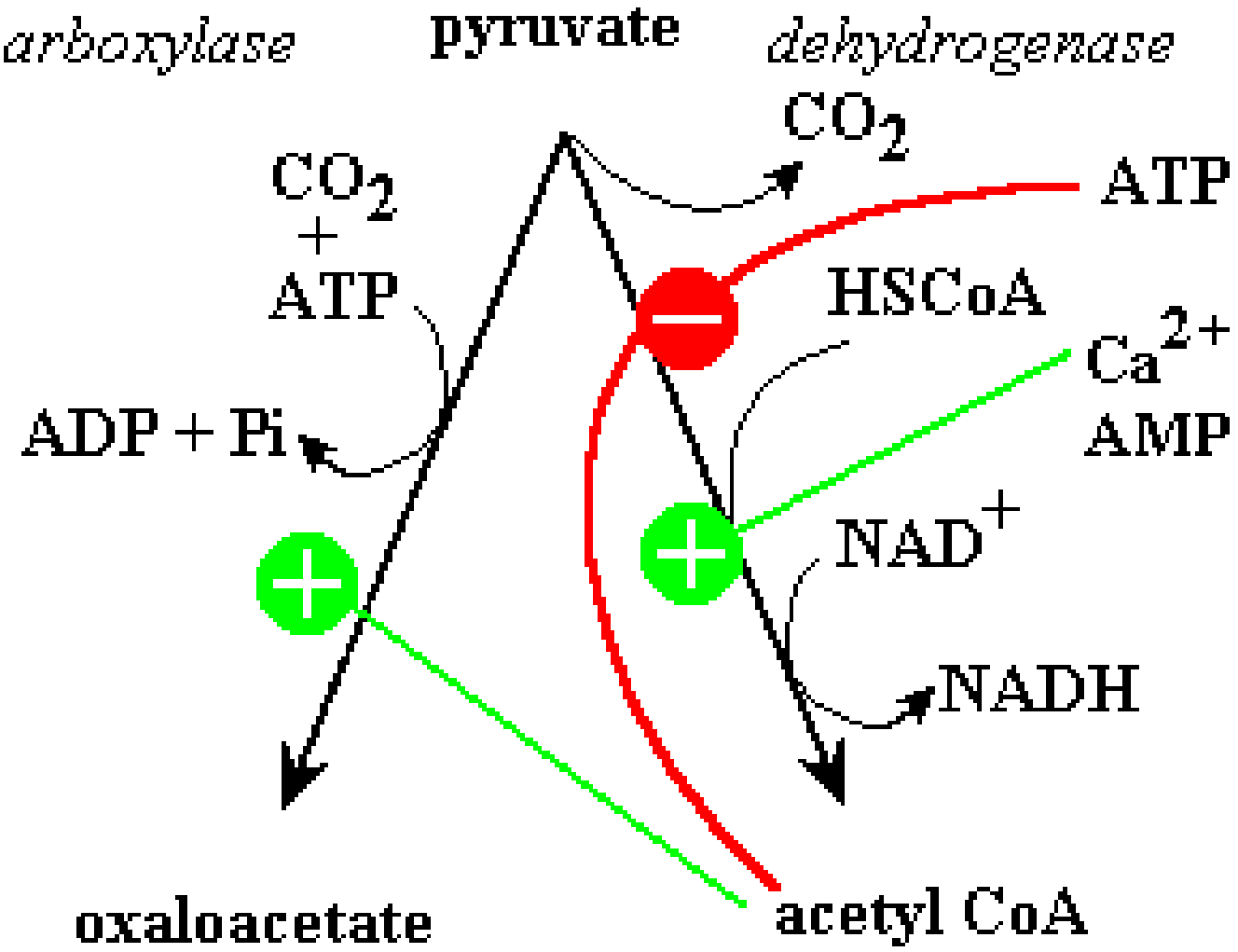
1) ATP + $HCO_3^-$ binding site

2) Biotin-binding site (mobile arm) present in many carboxylation reactions

3) Pyruvate-binding site

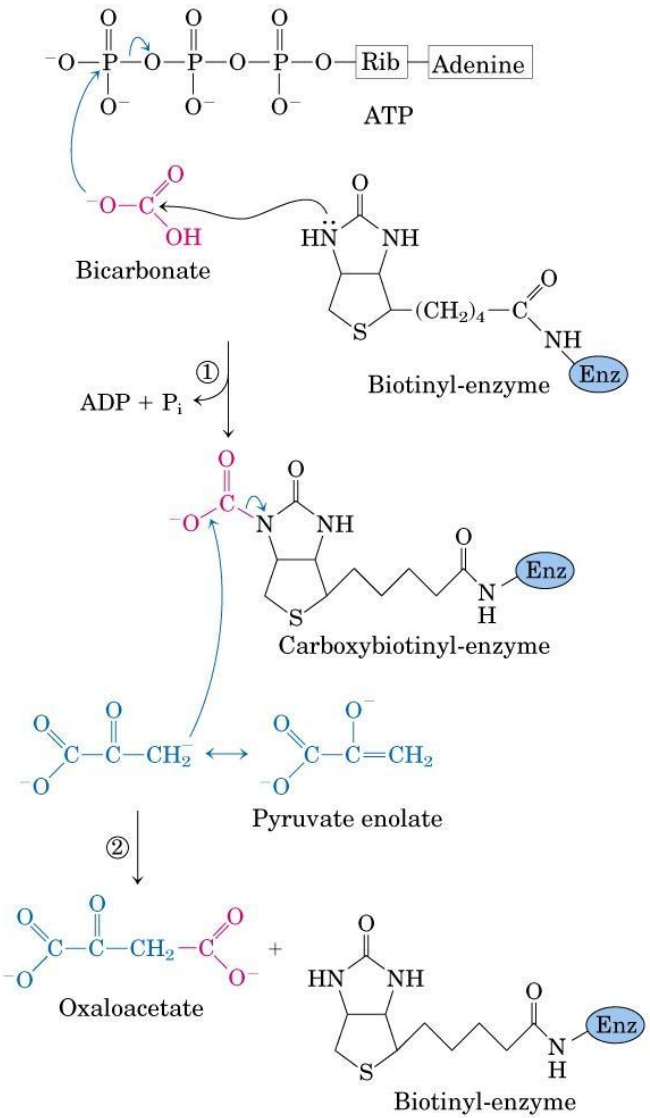
4) Allosteric site for acetyl-CoA (obligatory activator)

# PYRUVATE CARBOXYLASE



# PYRUVATE CARBOXYLASE

Via a carbonyl-P intermediate




**Biotin** is a water-soluble Vitamin (Vit B7)

# REGULATION OF CITRIC ACID CYCLE

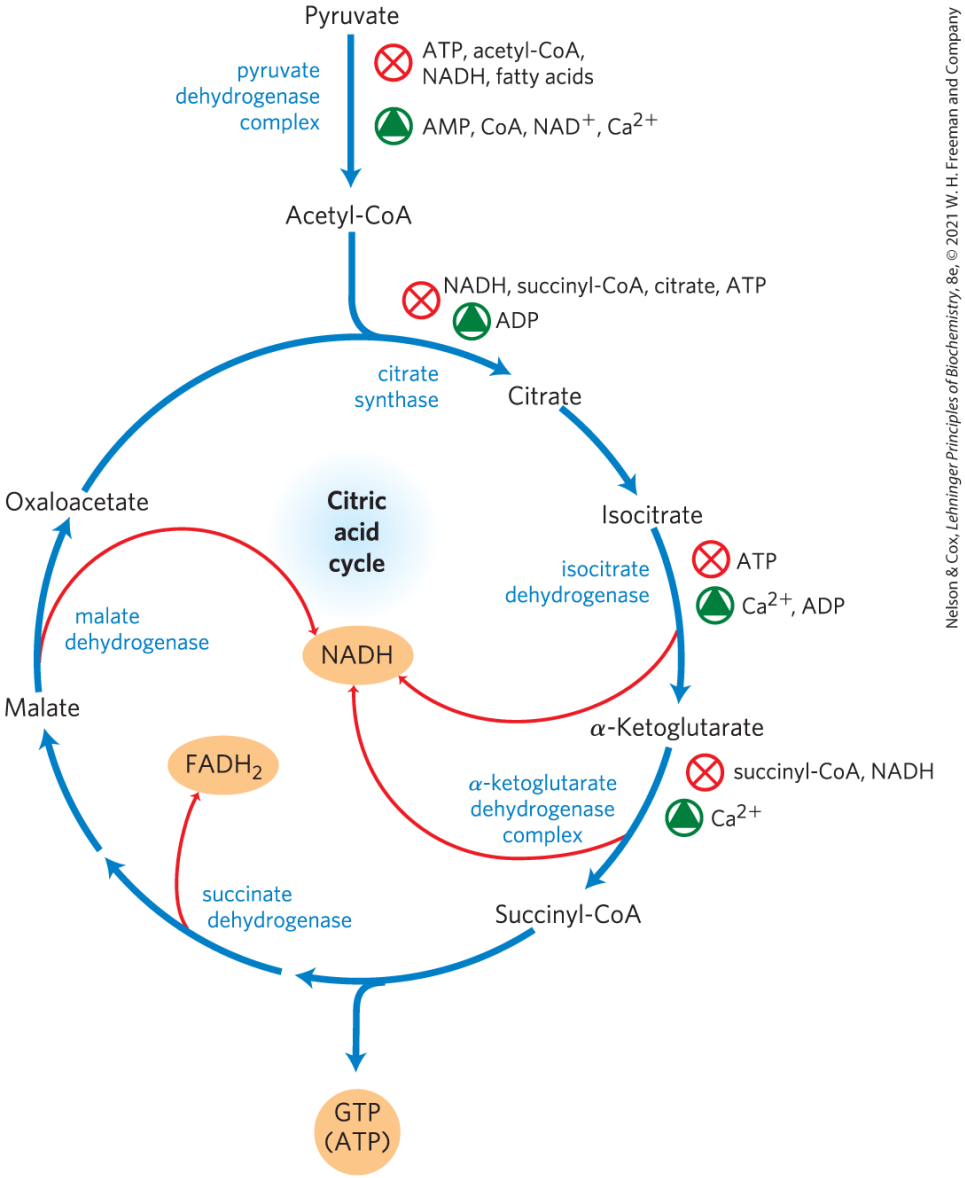
Regulated at highly thermodynamically favorable and **irreversible steps**

- PDH, citrate synthase, IDH, and KDH

General regulatory mechanism:

- activated by substrate availability;
- inhibited by product accumulation;
- Overall products of the pathway are **NADH** and **ATP**.
    - affect all regulated enzymes in the cycle;
    - inhibitors: NADH and ATP;
    - activators: $NAD^+$ and AMP.

# REGULATION OF CITRIC ACID CYCLE



# BIOSYNTHETIC FUNCTIONS IN TCA CYCLE

Intermediates of TCA cycle are starting points for biosynthetic pathways.

**Intermediates**:

Citrate

α-ketoglutarate

Succinyl-CoA

Malate

Oxaloacetate

**Pathways**:

Gluconeogenesis

Fatty acids biosynthesis

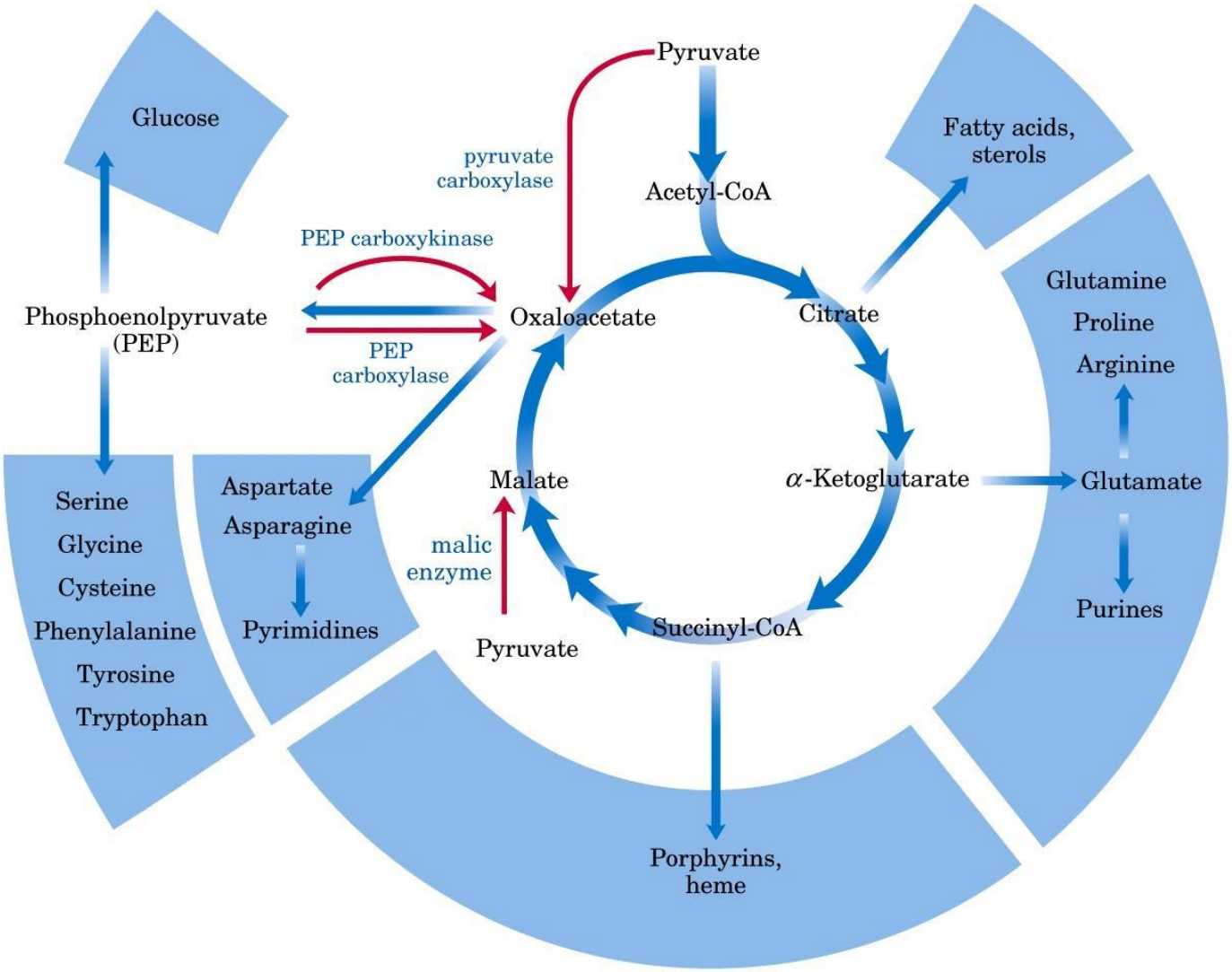
Cholesterol biosynthesis

Non-essential amino acids

Porphyrins

Purine and pyrimidine rings

# BIOSYNTHETIC FUNCTIONS IN TCA CYCLE



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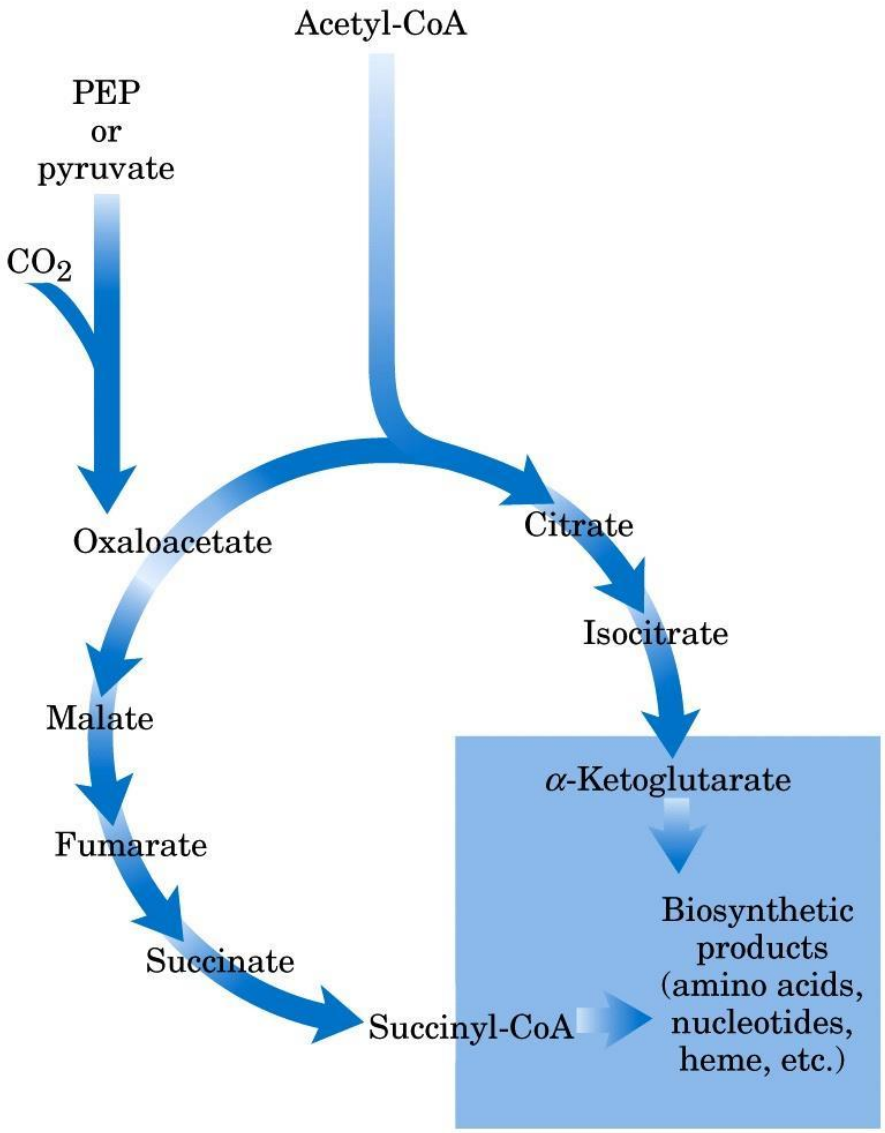


# GLUTAMINOLYSIS

In **neoplastic cells** pyruvate dehydrogenase is inhibited:

Glucose → pyruvate → lactate

Neoplastic cells largely utilize **glutamine**:

glutamine → glutamate → α-ketoglutarate → malate → pyruvate → lactate

also

glutamine → glutamate → α-ketoglutarate → isocitrate → citrate → membrane lipids

# WARBURG EFFECT

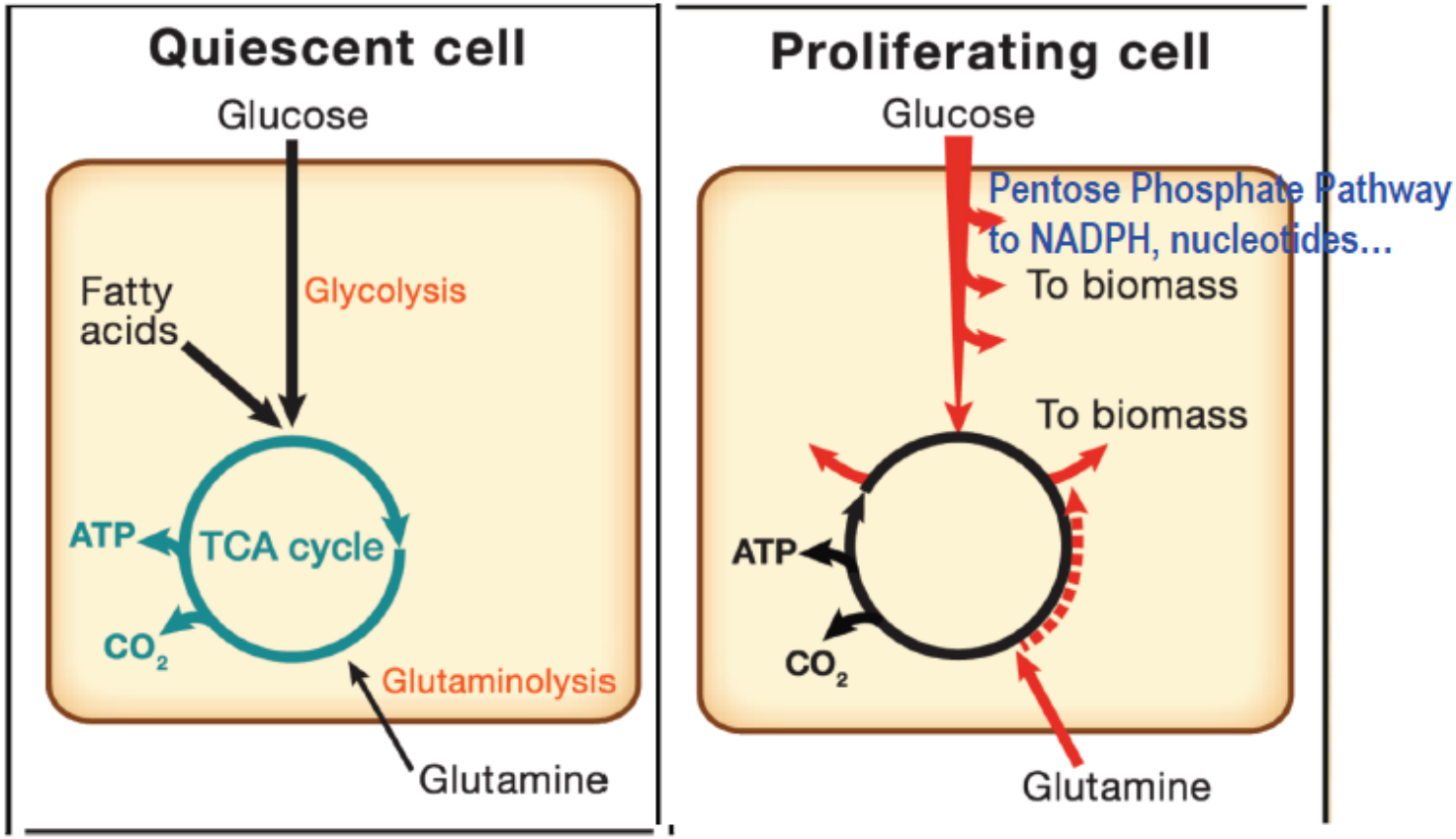
Normal cells produce lactate only in the absence of oxygen, or anaerobic conditions, while **cancer cells** produce lactate in both **anaerobic and aerobic environments**.

As a result, in tumor cells, pyruvate is not directed to the citric acid cycle. The increased rate of glycolysis in cancer cells allows for tumor growth even in areas with poor blood supply, as these cells overexpress glucose uptake systems.

Although cancer cells require the same amount of ATP as normal cells, they utilize the **TCA cycle** for the production of **lipids**, **proteins**, and **nucleic acids**.

# WARBURG EFFECT

SnapShot: Cancer Metabolism Pathways; Lydia Finley et al.
DOI: http://dx.doi.org/10.1016/j.cmet.2013.02.016

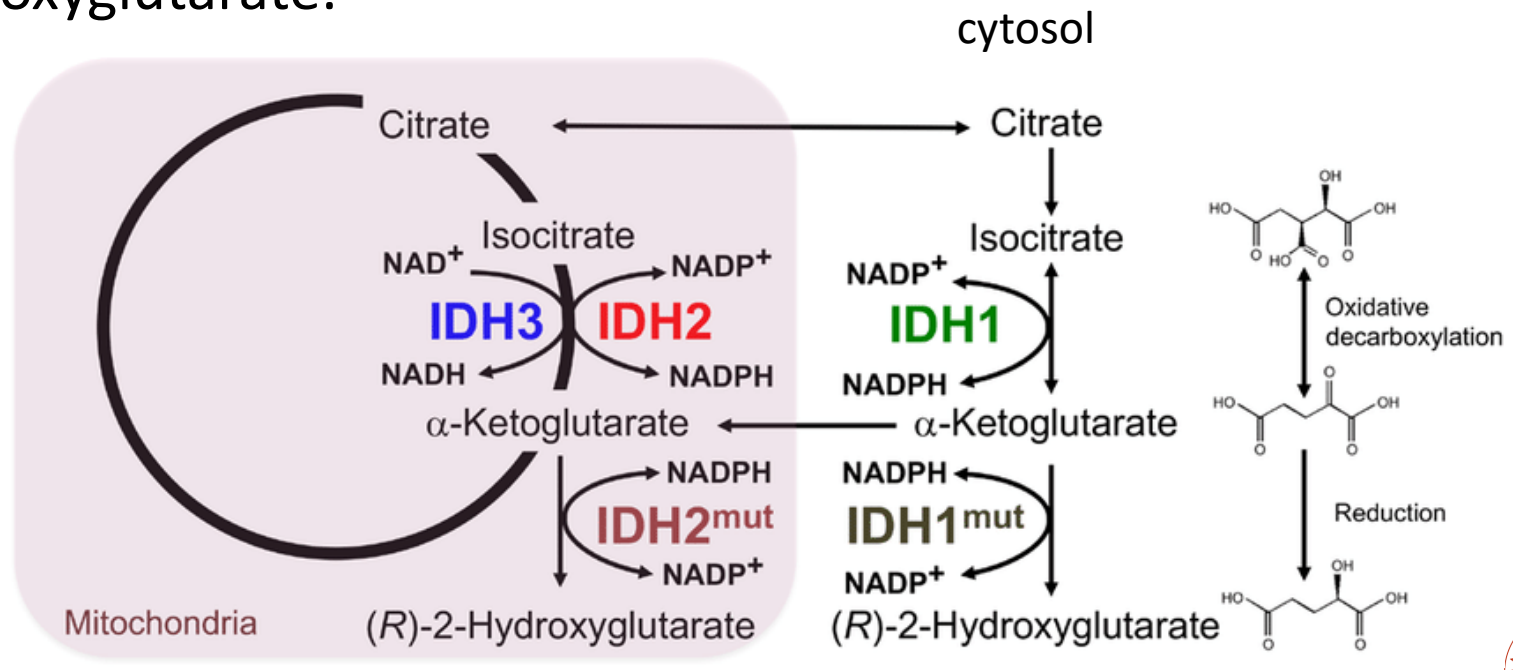



Box 14-1: chemotherapeutic agents that target unique aspects of cancer cell metabolism.

# TUMORS AND ISOCITRATE DEHYDROGENASE

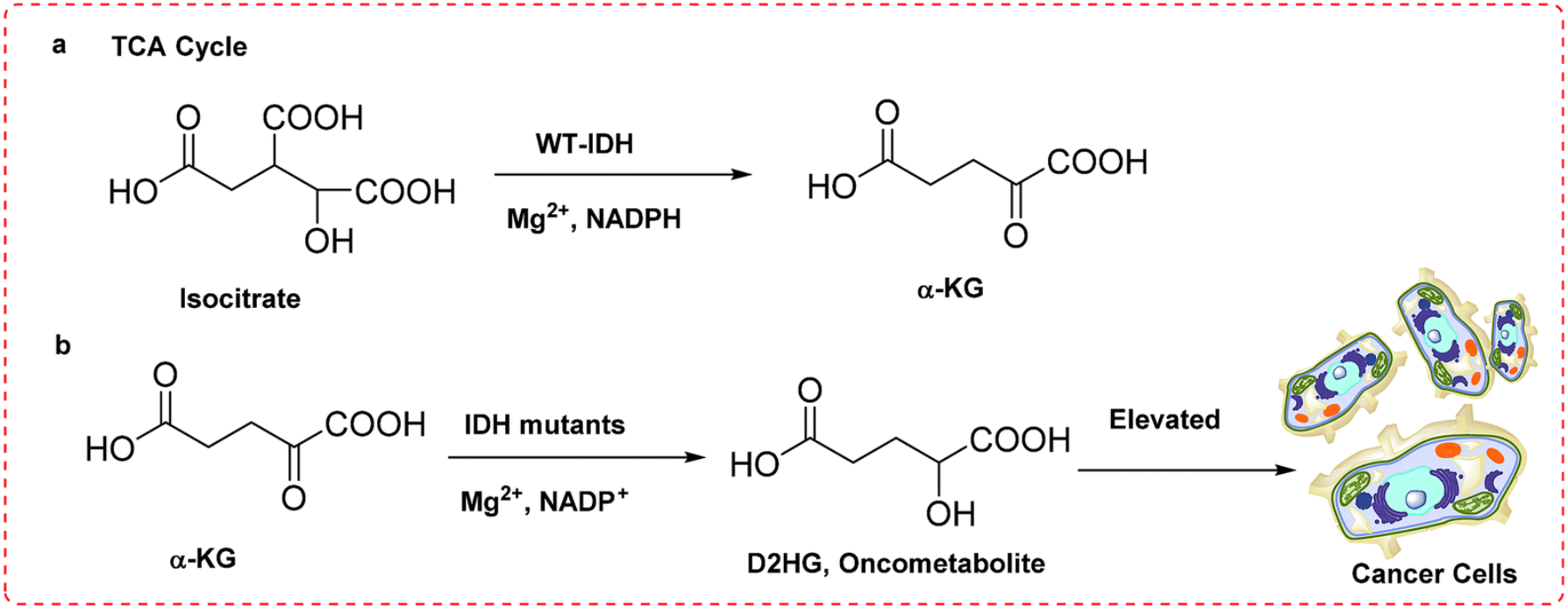
Isoforms of isocitrate dehydrogenase.

Glial cell tumors have mutant NADPH-dependent isocitrate dehydrogenase: they lose the ability to convert isocitrate to α-ketoglutarate, but gain the ability to convert α-ketoglutarate to 2-hydroxyglutarate.



# TUMORS AND ISOCITRATE DEHYDROGENASE

Isoform of isocitrate dehydrogenase mutations promotes cancerogenesis.



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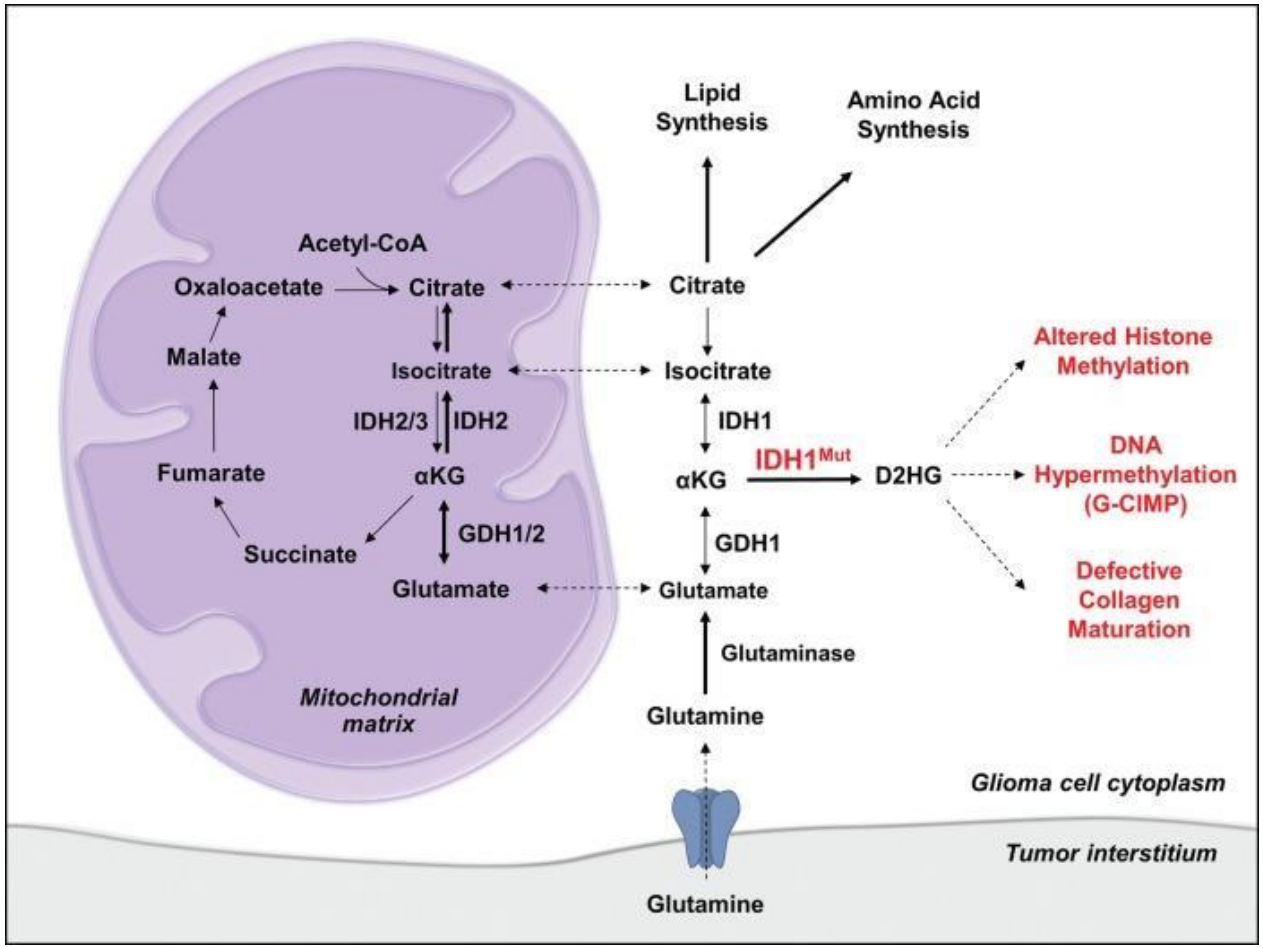

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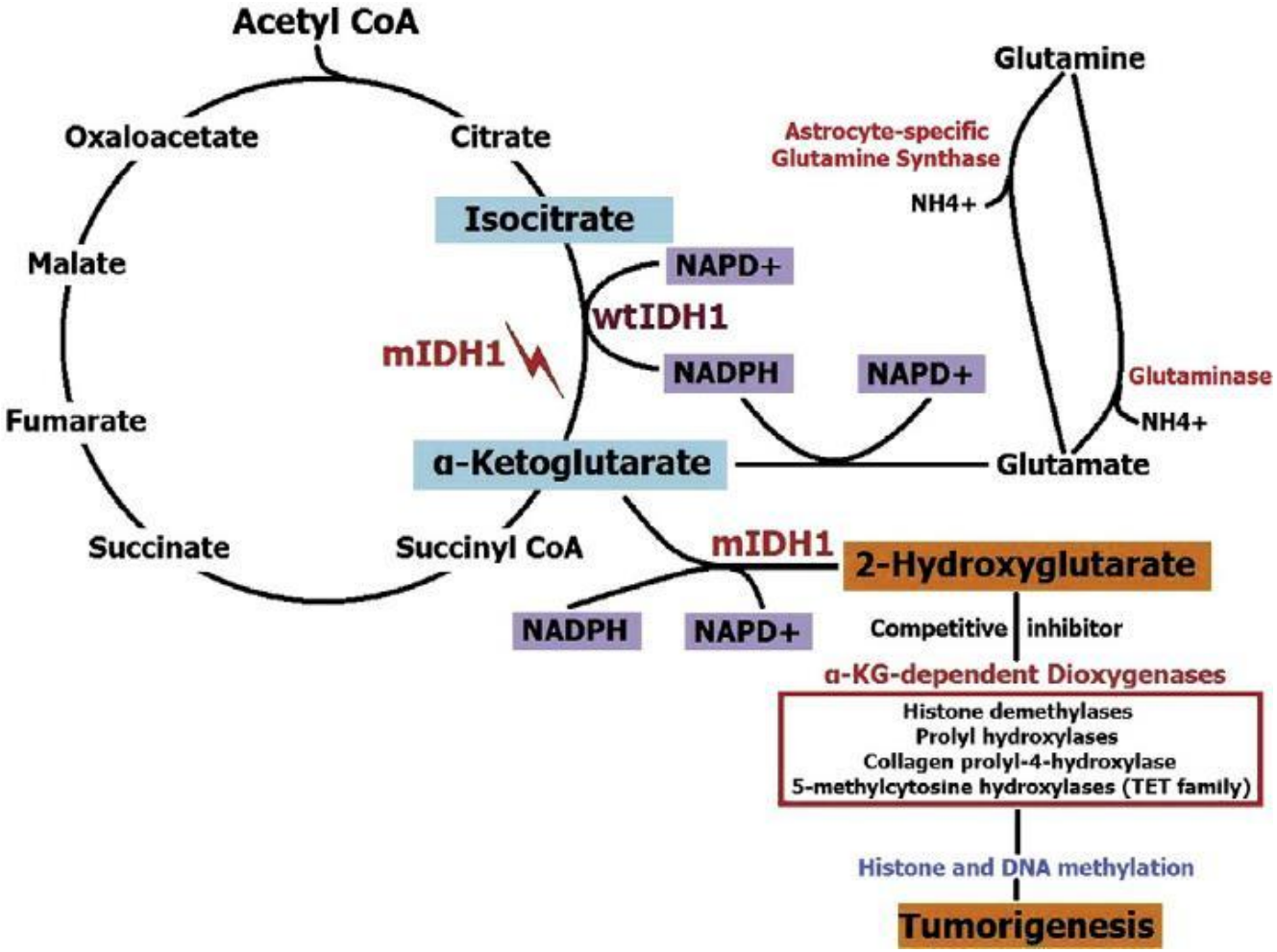
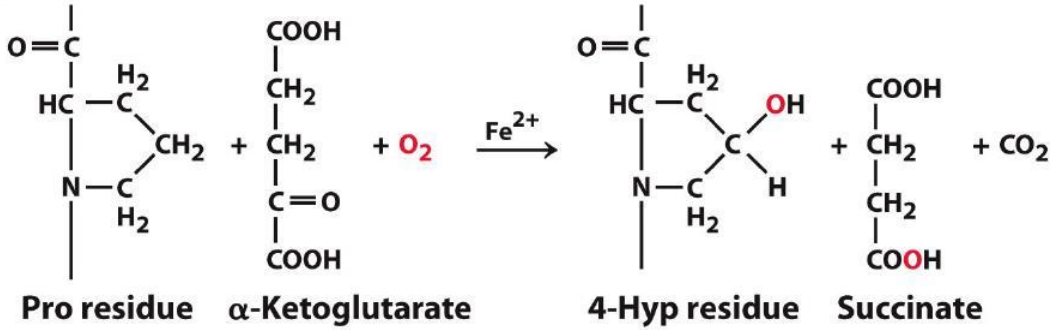


# HYPOXIA INDUCED FACTOR (HIF 1α)

Under normal $O_2$, HIF is hydroxylated on a proline residue and INACTIVE.

α-ketoglutarate + Proline + $O_2$ → succinate + $CO_2$ + proline-OH




Hypoxia prevents proline hydroxylation, thereby activating HIF.

Succinate inhibits proline hydroxylase, thus acting as a hypoxia mimic.

A genetic defect of succinate dehydrogenase acts as an oncogene, promoting inherited tumors as paraganglioma.

Activation of HIF induces metabolic changes, including the activation of glycolysis, inhibition of OXPHOS, and subsequent angiogenesis and metastasis.

# HYPOXIA INDUCED FACTOR (HIF 1α)

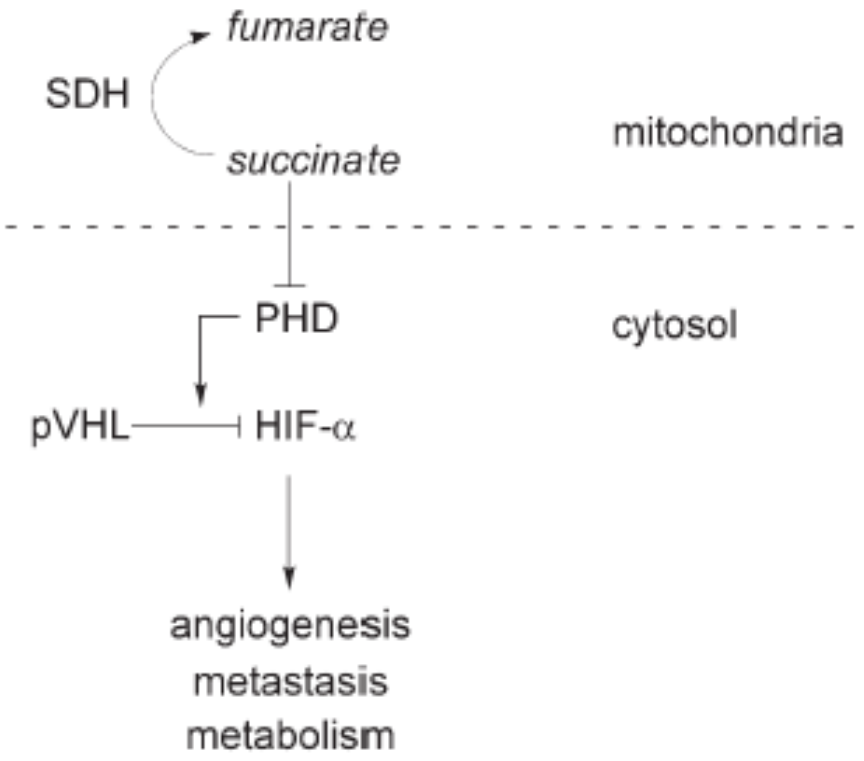



**Figure 7.** A schematic model that summarizes the role of succinate in the mitochondrion-to-cytosol signaling pathway

Succinate accumulated in the mitochondria due to SDH inhibition is transported to the cytosol. Elevated cytosolic succinate inhibits PHD and thereby HIF-α hydroxylation. Consequently, pVHL binding to HIF-α is decreased, and elevated HIF activity induces expression of genes that facilitate angiogenesis, metastasis, and metabolism, leading to more aggressive tumors.

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