MACQUARIE University

Regulation of Fuel metabolism Pentose Phosphate Pathway and Citric Acid Cycle

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Objectives

- Control of Fuel Metabolism in Mammals
  - Hormonal regulation of glycolysis, gluconeogenesis and glycogen metabolism
- Diversion from glycolysis - **pentose phosphate pathway (PPP)**
  - Makes NADPH (reducing agent, similar to NADH) for cells
  - Makes 5C (pentose) sugars for DNA/RNA
- Introduction to Citric Acid Cycle, where pyruvate from glycolysis is burnt to CO_2

- Textbook Chaps. 12 and 13

Control of Fuel Metabolism in Mammals

- Maintenance of blood glucose levels is particularly critical to brain function
- Normal hormone regulation
  - Insulin
  - Glucagon
- Epinephrine (special circumstances due to low glucose levels) reacts with second messenger systems:
  - Muscle – activates adenylate cyclase and glycogenolysis (next section)
  - Adipose tissue – simulates breakdown of TAG
  - Pancreas – inhibits insulin secretion, stimulates glucagon secretion
- Unlike glucagon, epinephrine effects are short-lived

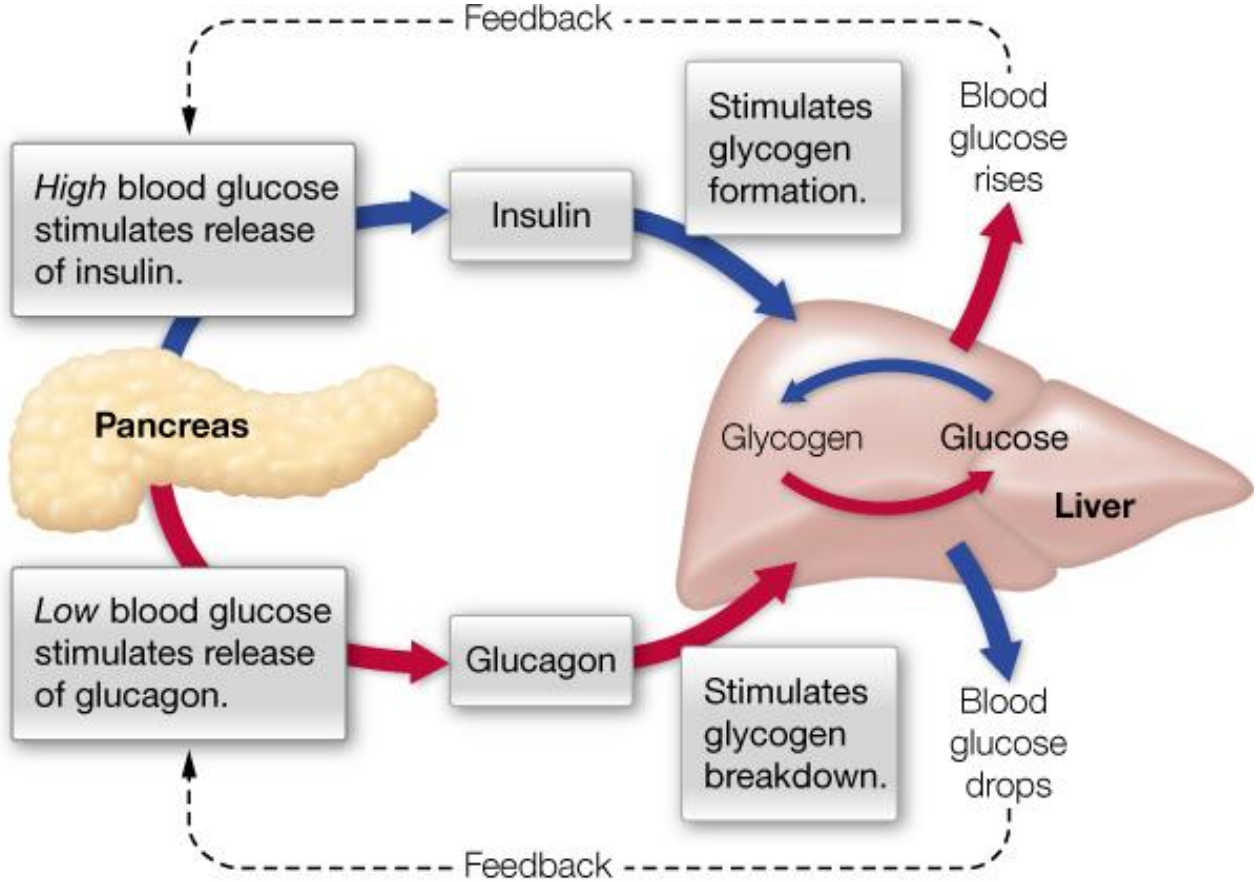


FIGURE 17.2 Aspects of the control of blood glucose levels by pancreatic secretion of insulin and glucagon.

Actions of Glucagon That Lead to an Increase in Blood Glucose Levels

- Glucagon acts primarily on the liver, via the glucagon receptor, a GPCR.
- Second messenger is cAMP
- Downstream phosphorylation events lead to:
  1. Decreased glycolysis
  2. Increased gluconeogenesis
  3. Breakdown of glycogen and suppression of glycogen synthesis

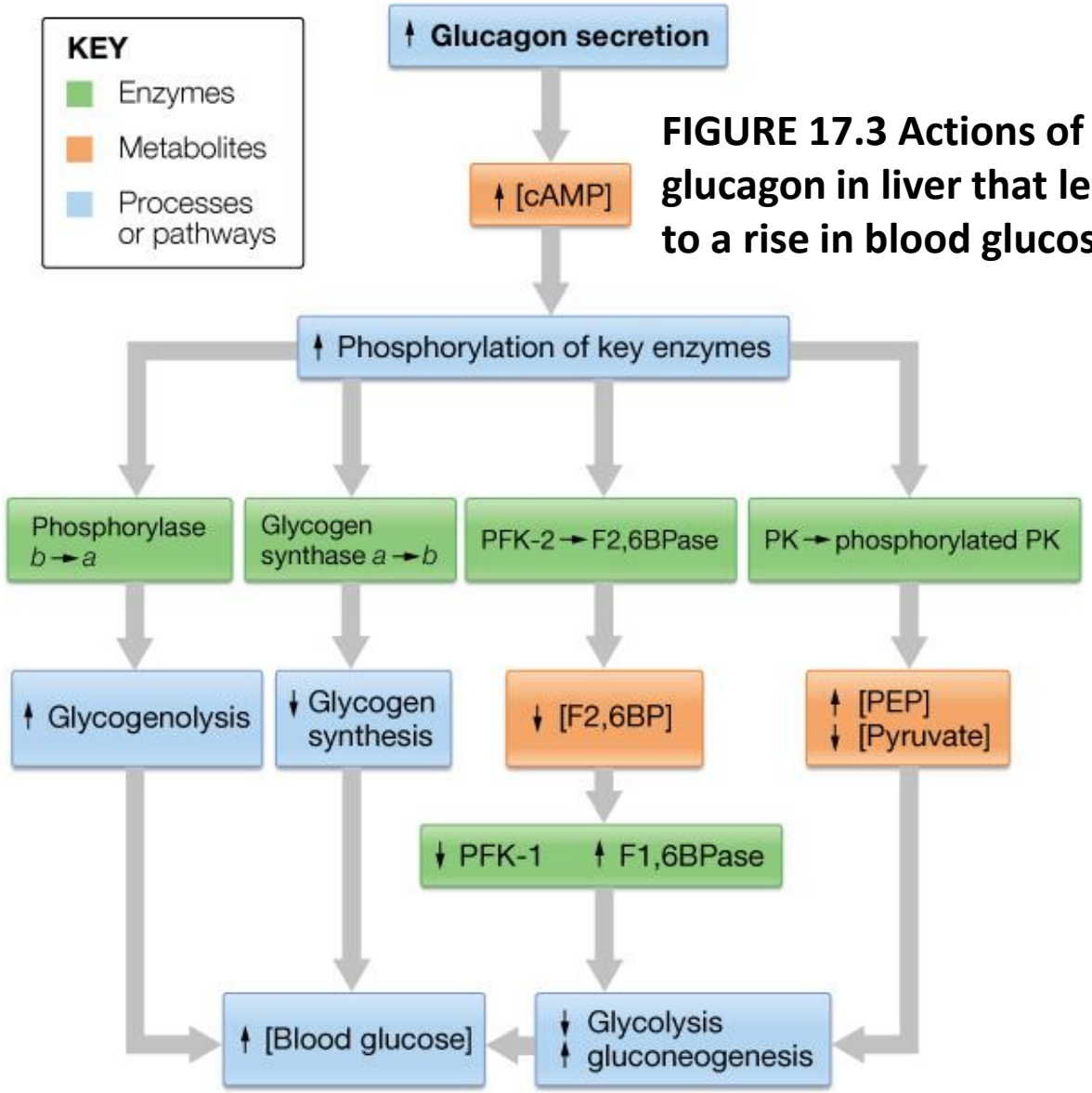
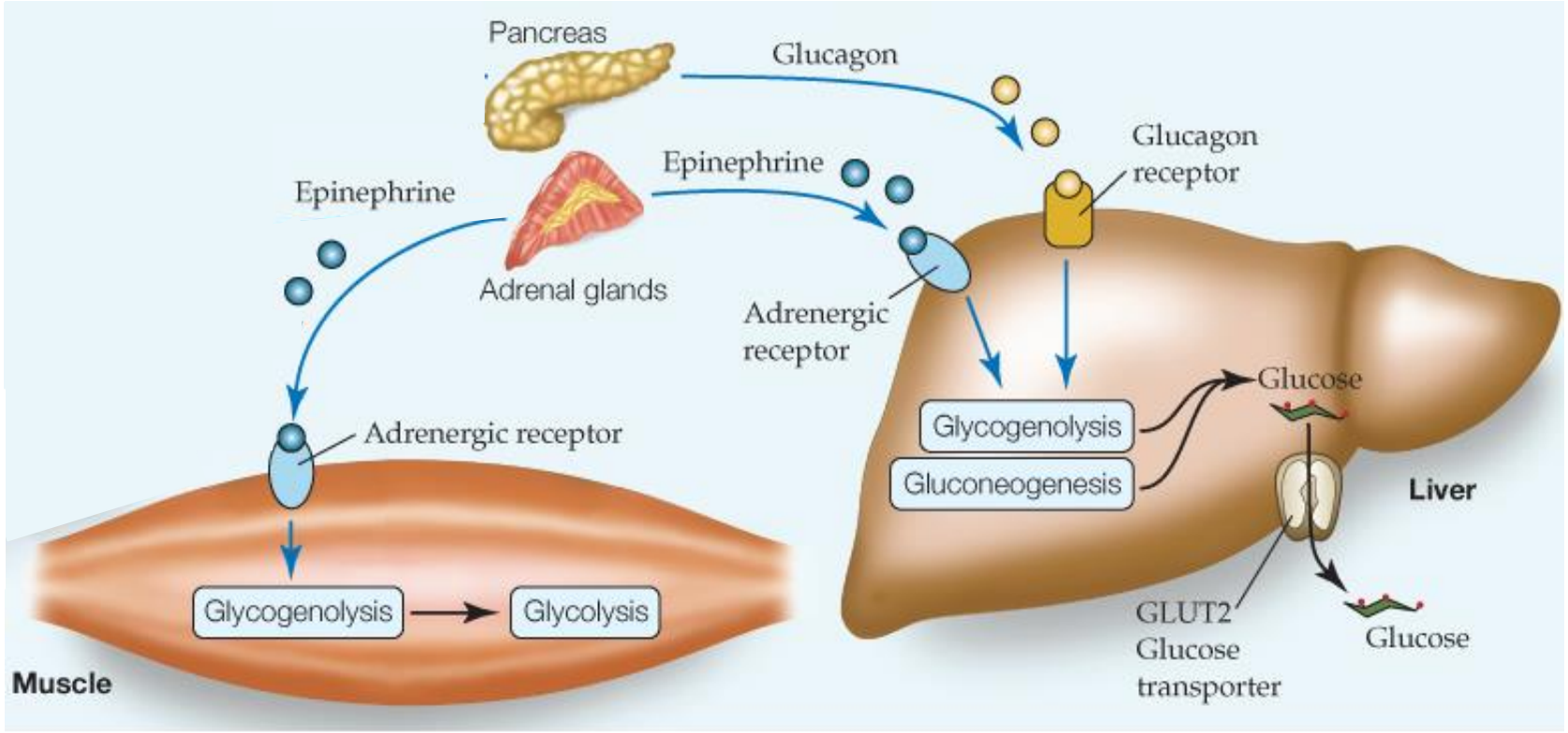


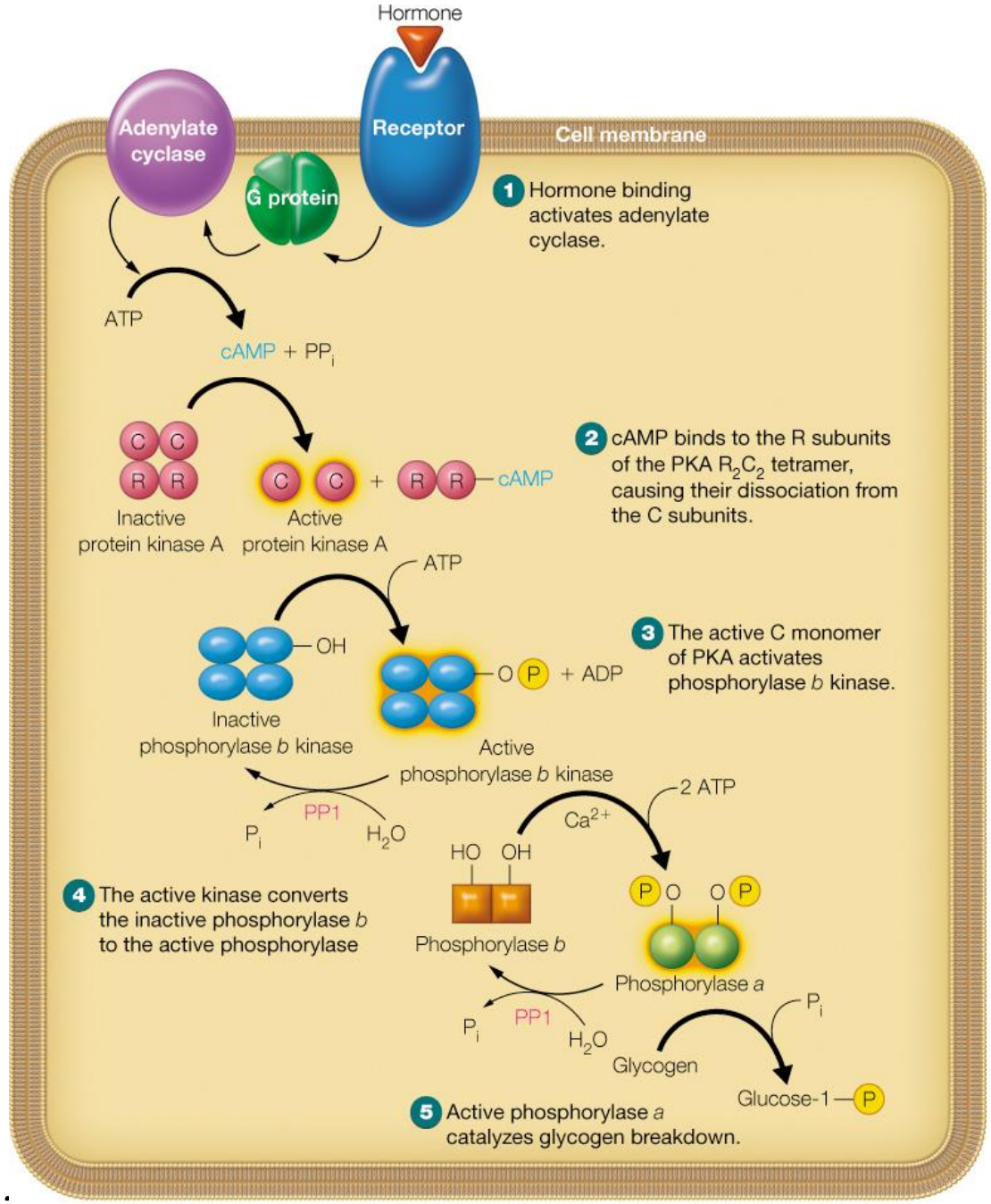
FIGURE 17.3 Actions of glucagon in liver that lead to a rise in blood glucose.

Hormonal control of gluconeogenesis in the liver: increase only by epinephrine during fasting/stress



Regulation of Glycogen Breakdown

1) Hormone (glucagon or epinephrine) binds a cell surface receptor, releasing a G-protein which activates adenylate cyclase
2) Adenylate cyclase synthesizes cAMP, which binds the regulatory subunit of protein kinase A (PKA), releasing the active C subunit of PKA
3) Active PKA phosphorylates phosphorylase *b* kinase, activating it
4) Active kinase converts inactive phosphorylase *b* to the active phosphorylase *a,* catalyzing **glycogen breakdown**



Hormonal Control of Glycolysis, Gluconeogenesis and Glycogen Metabolism in Mammals

TABLE 17.2 Major hormones controlling fuel metabolism in mammals

Hormone	Biochemical Actions	Enzyme Target	Physiological Actions
Insulin	↑ Glucose uptake (muscle, adipose tissue)	GLUT4	Signals fed state:
	↑ Glycolysis (liver, muscle)	PFK-1 (via PFK-2/FBPase-2)	↓ Blood glucose level
	↓ Gluconeogenesis (liver)	FBPase-1 (via PFK-2/FBPase-2)	↑ Fuel storage
	↑ Glycogen synthesis (liver, muscle)	Glycogen synthase	
Glucagon	↑ cAMP level (liver, adipose tissue)		Signals fasting state:
	↑ Glycogenolysis (liver)	Glycogen phosphorylase	↑ Glucose release from liver
	↓ Glycogen synthesis (liver)	Glycogen synthase	↑ Blood glucose level
	↑ Gluconeogenesis (liver)	FBPase-1 (via PFK-2/FBPase-2), pyruvate kinase, PEPCK	
	↓ Glycolysis (liver)	PFK-1 (via PFK-2/FBPase-2)	
Epinephrine	↑ cAMP level (muscle)		Signals stress:
	↑ Glycogenolysis (liver, muscle)	Glycogen phosphorylase	↑ Glucose release from liver
	↓ Glycogen synthesis (liver, muscle)	Glycogen synthase	↑ Blood glucose level
	↑ Glycolysis (muscle)	Glycogen phosphorylase, providing increased glucose	

Hormonal Control of Glycolysis, Gluconeogenesis and Glycogen Metabolism

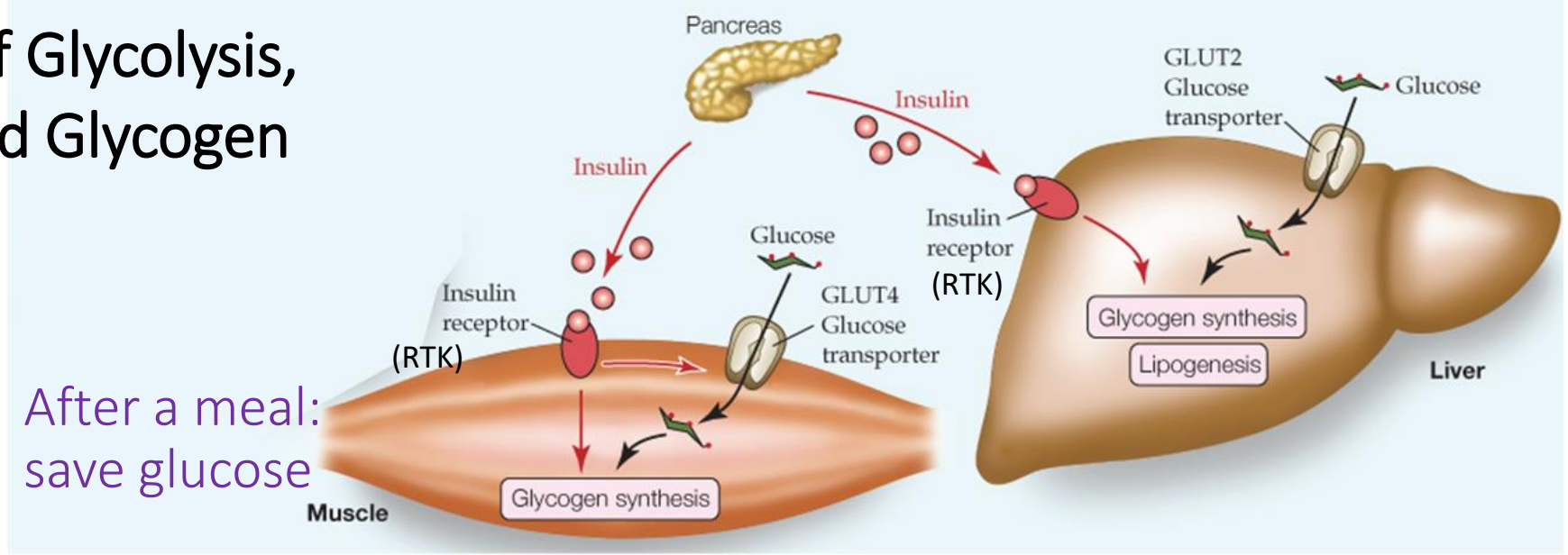
Insulin: glucose level ↑
- Glycogen synthesis ↑;
- Gluconeogenesis (liver) ↓

Glucagon: glucose level ↓

Liver
- Glycogenolysis ↑;
- Glycolysis ↓;
- Gluconeogenesis ↑

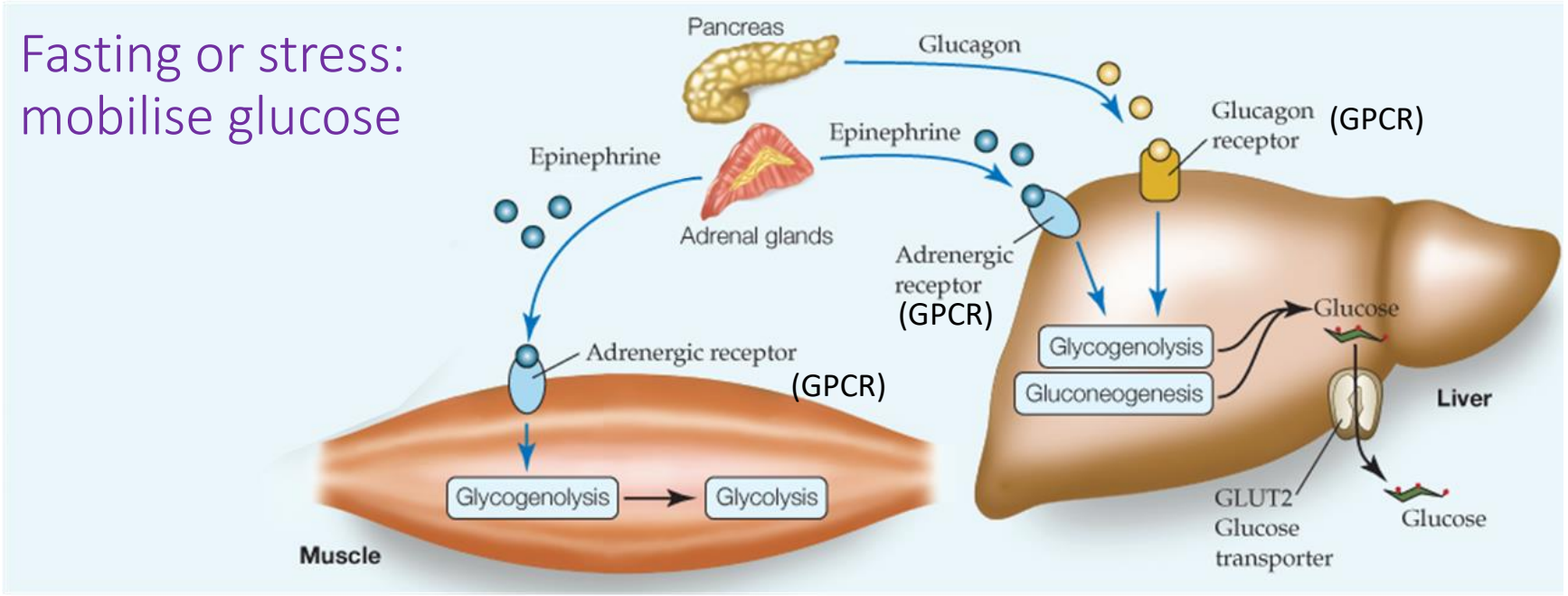
Epinephrine: glucose level ↓
- Glycogenolysis ↑;
- Glycolysis ↑

After a meal: save glucose



(a) Fed state

Fasting or stress: mobilise glucose



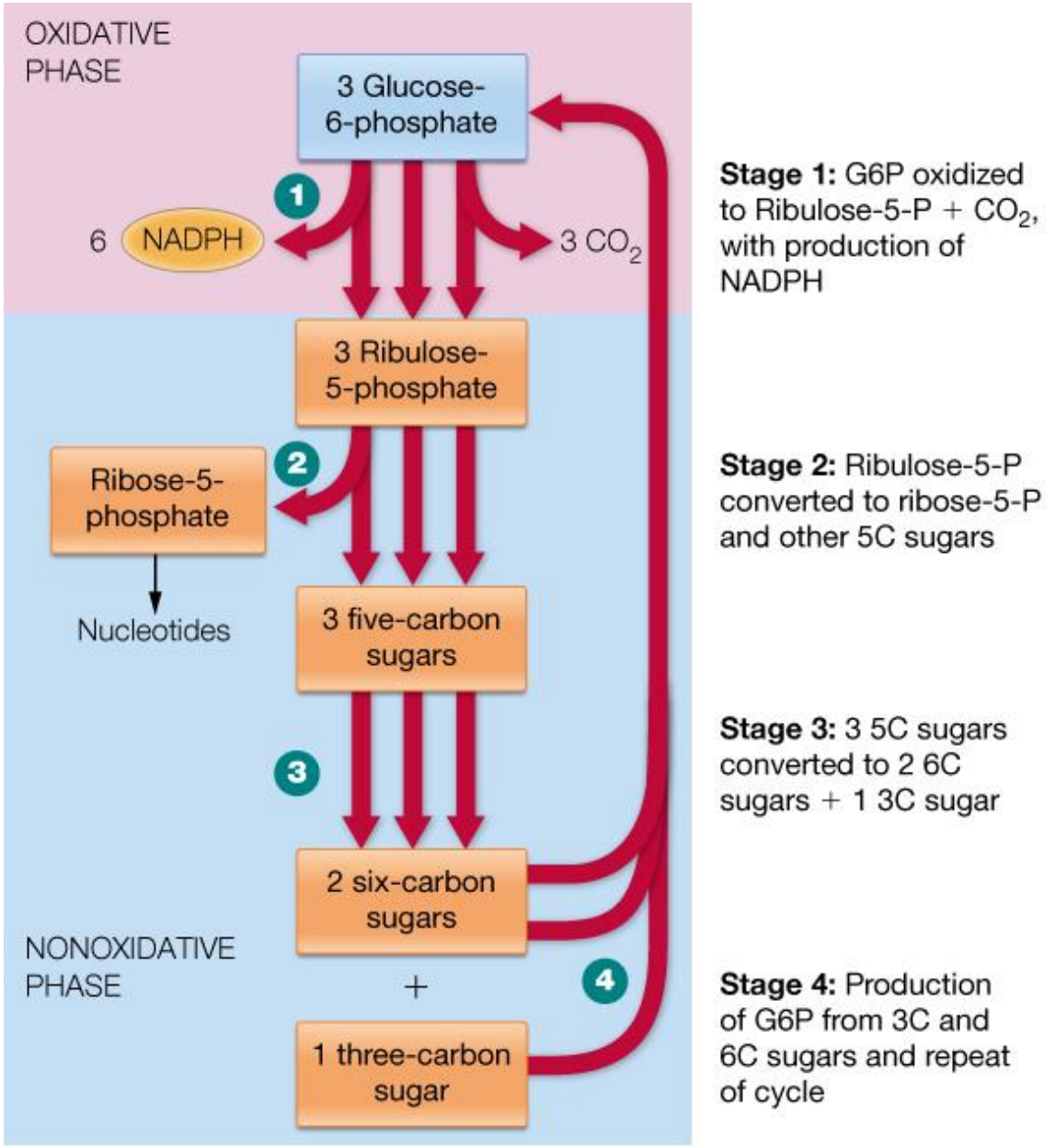
(b) Fasted state/stress

The pentose phosphate pathway (PPP)

- A biosynthetic pathway that oxidizes glucose.
- Also called the **hexose monophosphate shunt** or the **phosphogluconate** pathway
- The PPP is mainly needed to provide:
  - ➢ reducing equivalents (in the form of NADPH) for reductive biosynthesis and for dealing with oxidative stress
  - ➢ ribose-5-phosphate (R5P) for nucleotide and nucleic acid synthesis
- 6C glucose-6-phosphate is converted to 5C ribose (*for nucleic acid synthesis*) and NADPH *(for lipid and steroid synthesis +* CO_2)

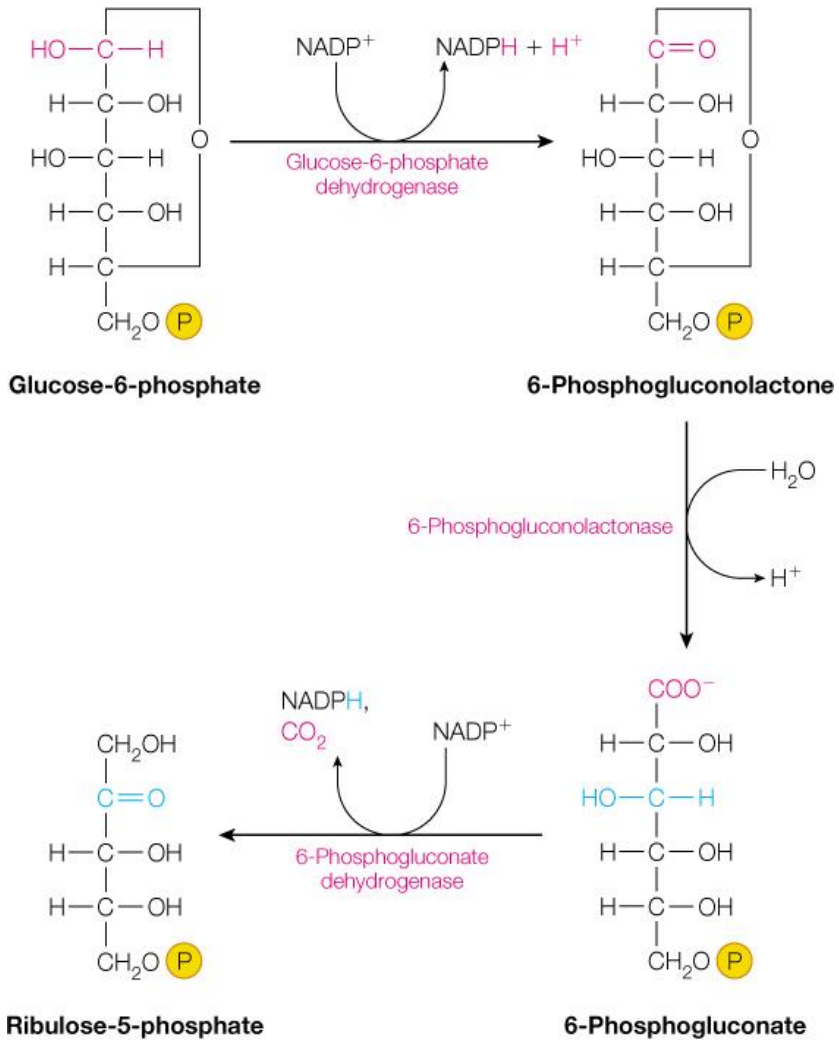
The Four Stages of PPP

- Stage 1: Two oxidations (glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) generate NADPH and CO_2
- Stage 2: produces Ribose 5-phosphate
- Stage 3: interconversion of 5C sugars to 6C and 3C sugars



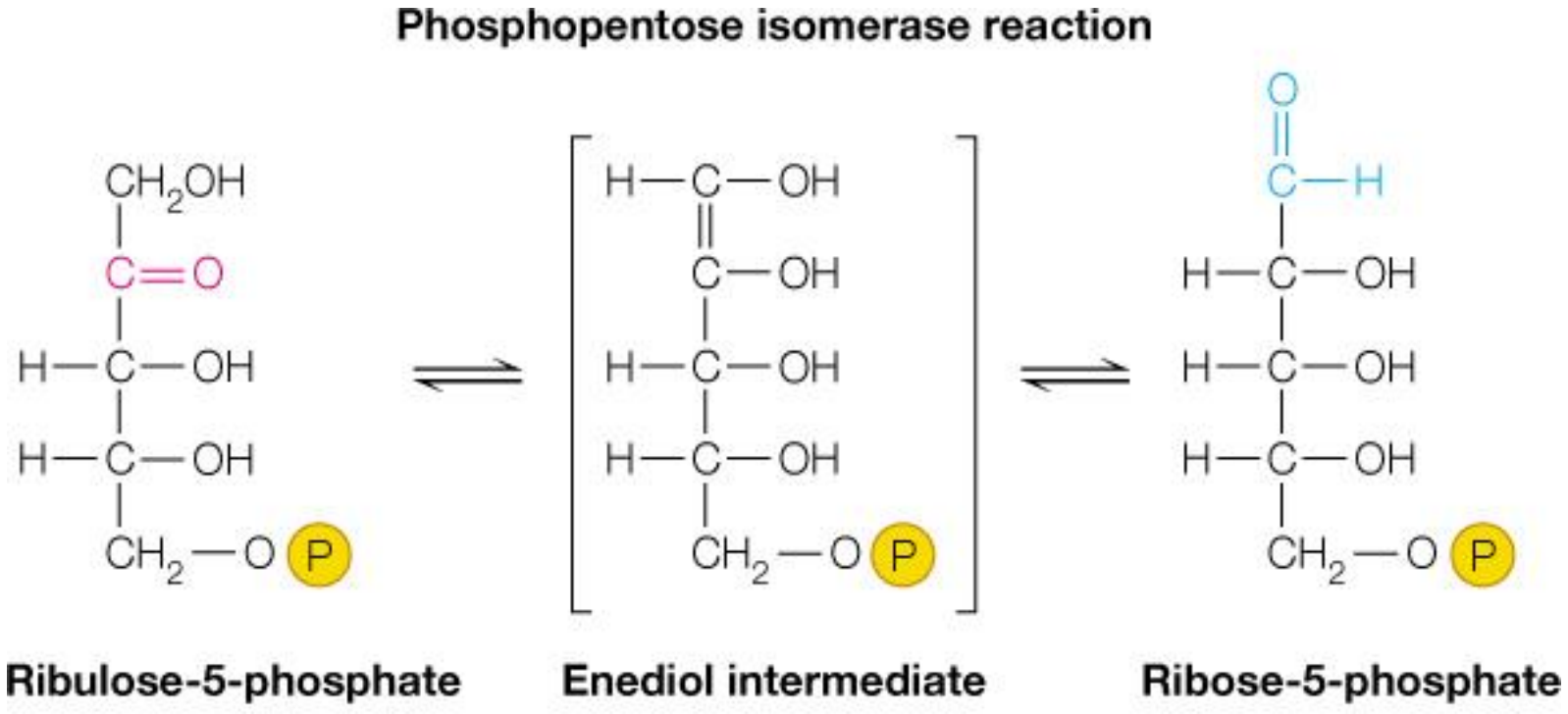
The Oxidative Phase of PPP (Stage 1)

- 2 NADPH + H^+, 1 CO_2, and 1 pentose (5C) phosphate are generated from 1 glucose 6-phosphate and 2 $NADP^+$
- The first reaction is regulated and controls the entire pathway!



The Nonoxidative Phase of PPP (Stage 2)

The phosphopentose isomerase reaction: produces ribose 5-phosphate

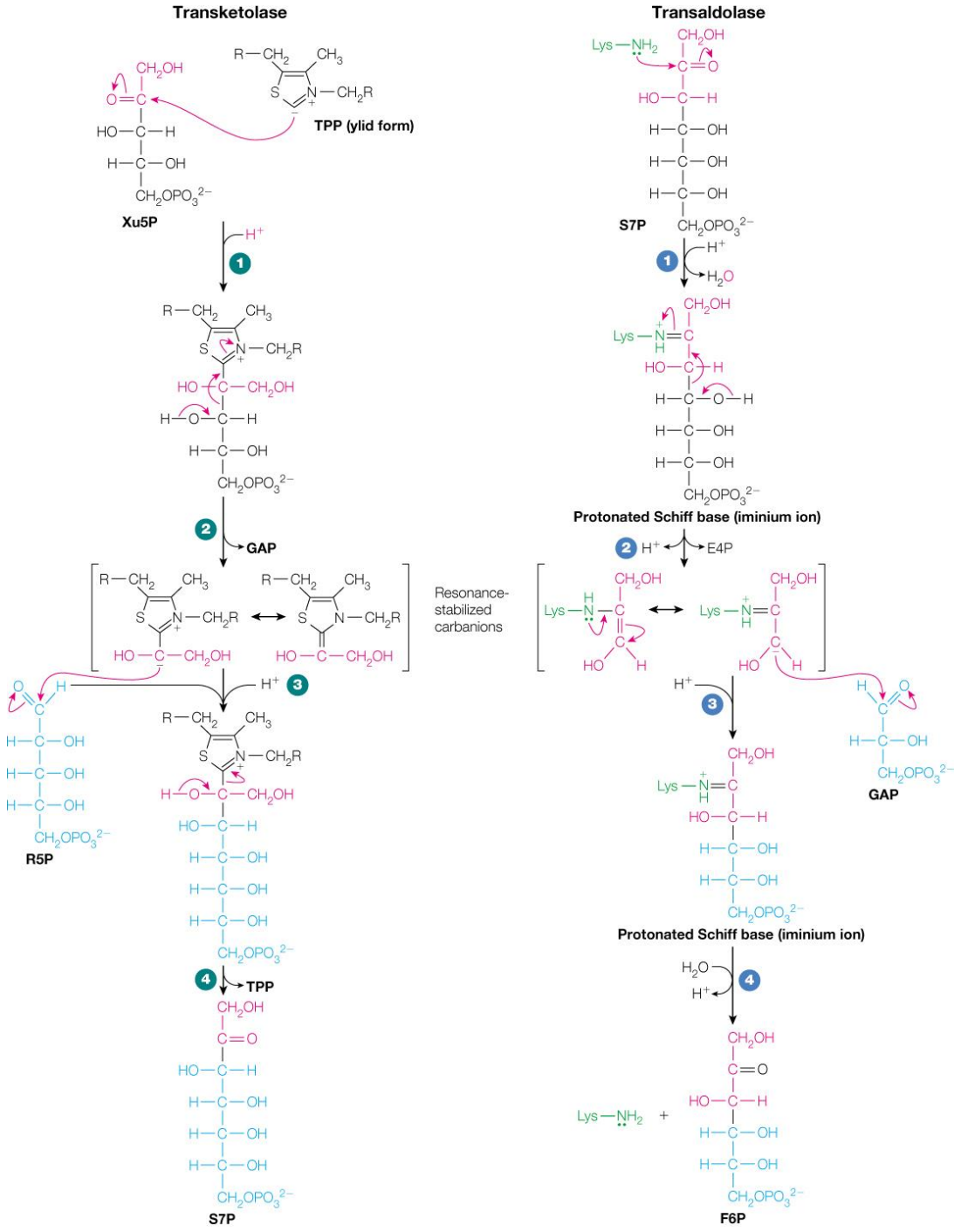


Ribulose 5-phosphate can also be converted to the pentose sugar xylulose 5-phosphate (X5P) by ribulose 5-phosphate epimerase.

The Nonoxidative Phase of PPP (Stages 3 and 4)

Transketolase and transaldolase reactions

- R5P (a C5 sugar) and X5P (C5) are converted to glyceraldehyde 3-phosphate (GAP; C3) and sedoheptulose 7-phosphate (S7P; C7) by transketolase (cofactor, thiamine pyrophosphate: TPP; Vit. B1) , which are further metabolized to fructose 6-phosphate (F6P; C6) and erythrose 4-phosphate (E4P; a C4 sugar) by transaldolase
  - ➢2*5C = 3C + 7C
  - ➢3C + 7C = 6C + 4C
- Moreover, E4P and X5P can be altered to GAP and F6P in a second transketolase reaction
  - ➢5C + 4C = 6C + 3C



Relationship Between Glycolysis & Pentose Phosphate Pathway

Oxidative phase:

3 Glucose-6-phosphate + $6NADP^+ + 3H_2O \rightarrow$

3 pentose-5-phosphate + $6NADPH + 6H^+ + 3CO_2$

Non-oxidative phase:

2 Xylulose-5-phosphate + ribose-5-phosphate →

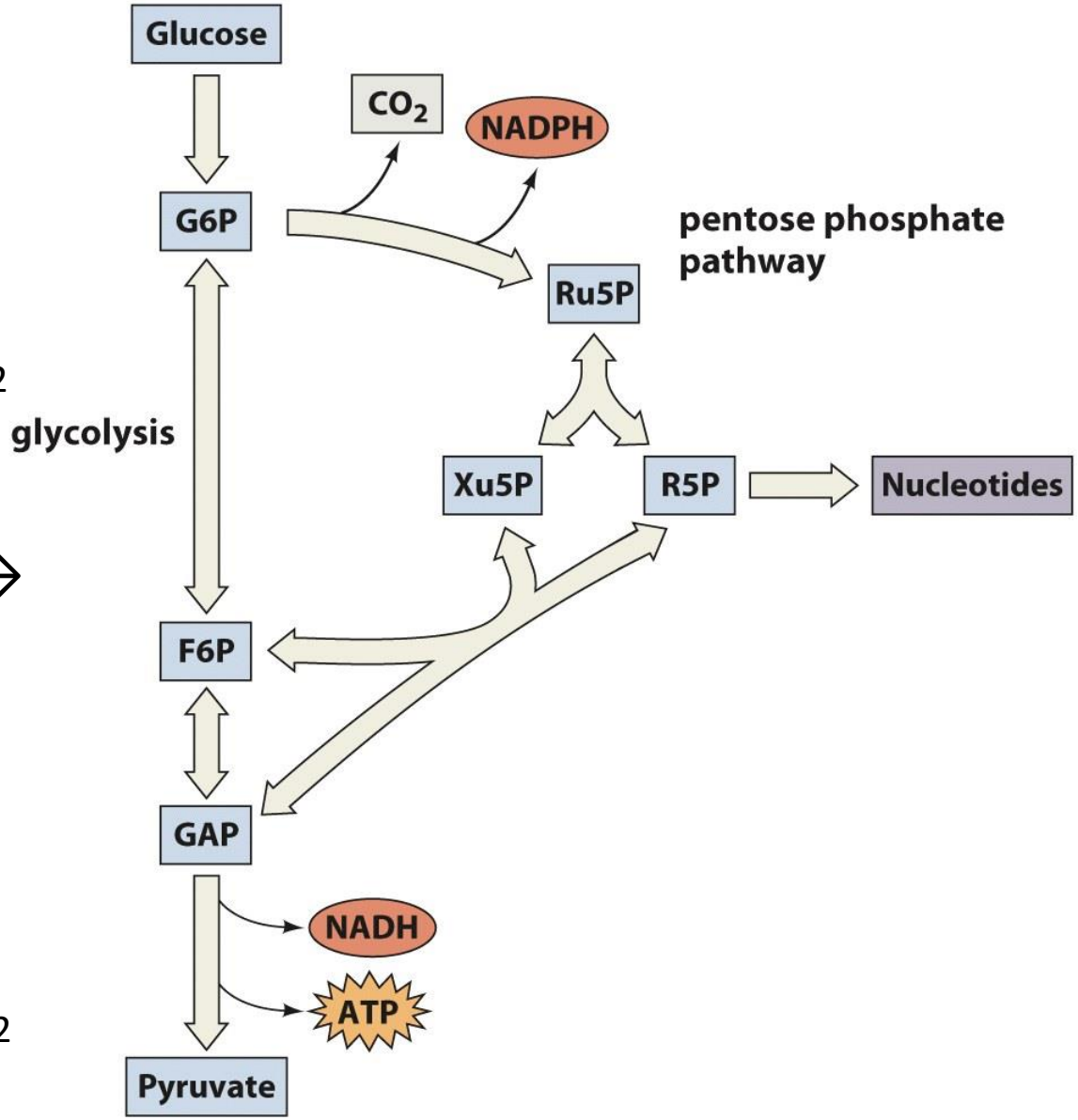
2 fructose-6-phosphate + glyceraldehyde-3-phosphate

Overall:

3 Glucose-6-phosphate + $6NADP^+ + 3H_2O \rightarrow$

2 fructose-6-phosphate + $6NADPH + 6H^+ + 3CO_2$

glyceraldehyde-3-phosphate.



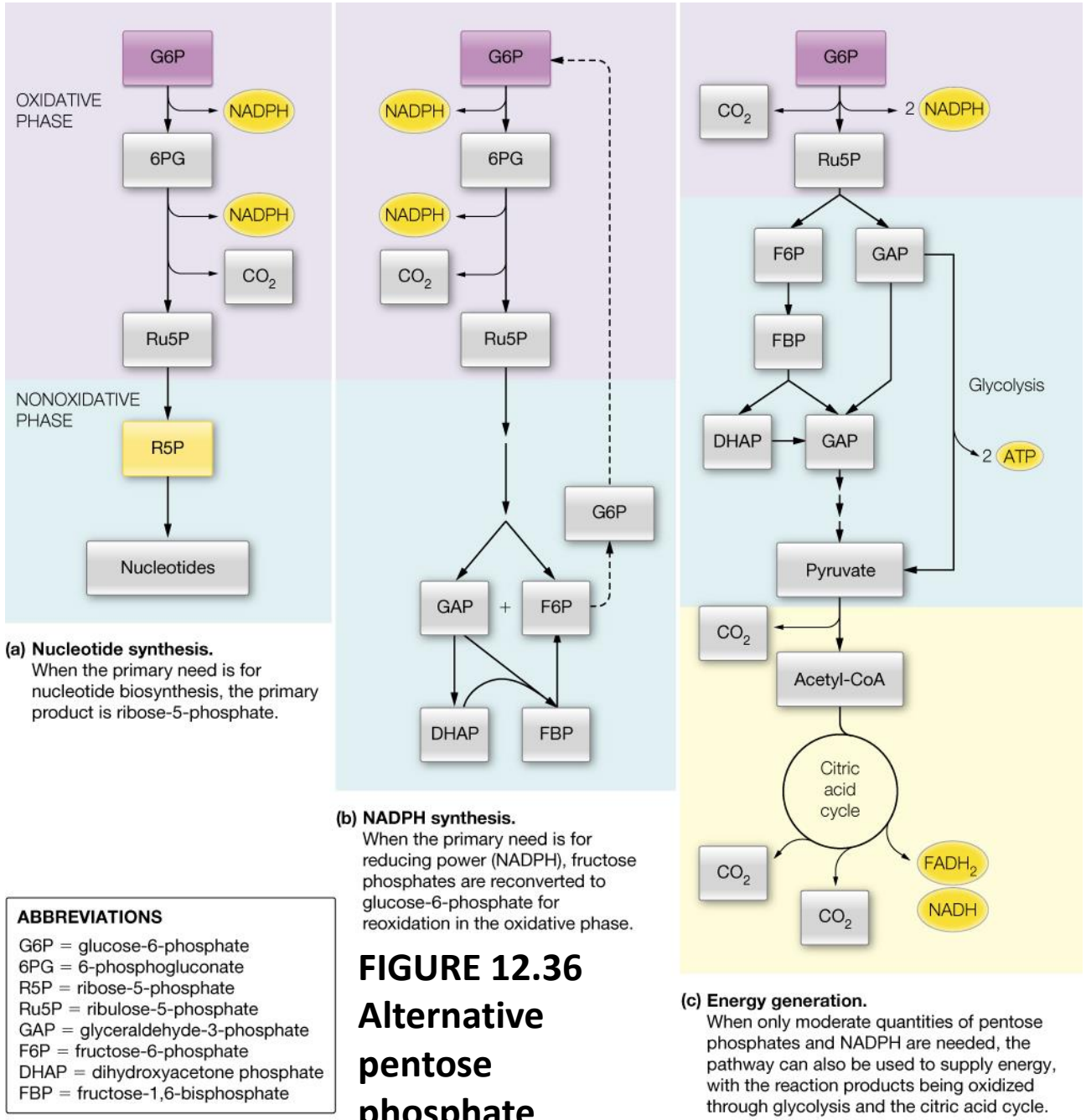
Modes of PPP

The pentose phosphate pathway is not primarily an energy-generating pathway. The actual fate of the sugar phosphates depends on the metabolic needs of the cell in which the pathway is occurring.

(a) For nucleotide and nucleic acid synthesis: the major product is ribose-5-phosphate, and most of the rearrangements do not occur.

(b) For the generation of NADPH (for fatty acid or steroid synthesis): the nonoxidative phase generates compounds that can easily be reconverted to glucose-6-phosphate, for subsequent passage through the oxidative phase. In this mode, repeated turns of the cycle result ultimately in the complete oxidation of glucose-6-phosphate to CO_2 and water, while generating the maximum number of reducing equivalents.

(c) In a cell with **moderate needs for both NADPH and pentose phosphates**, the fructose-6-phosphate and glyceraldehyde-3-phosphate produced in the nonoxidative phase can be further catabolized by glycolysis and the citric acid cycle.



(a) **Nucleotide synthesis.** When the primary need is for nucleotide biosynthesis, the primary product is ribose-5-phosphate.

(b) **NADPH synthesis.** When the primary need is for reducing power (NADPH), fructose phosphates are reconverted to glucose-6-phosphate for reoxidation in the oxidative phase.

(c) **Energy generation.** When only moderate quantities of pentose phosphates and NADPH are needed, the pathway can also be used to supply energy, with the reaction products being oxidized through glycolysis and the citric acid cycle.

FIGURE 12.36 Alternative pentose phosphate pathway modes.

Regulation of PPP

- PPP competes with glycolysis for glucose-6-phosphate.
- Whereas glycolysis is regulated primarily by energy charge and fuel availability, flux through the pentose phosphate pathway is sensitive to the $NADP^+$/NADPH ratio of the cell.
- The first enzyme of the pathway, **glucose-6-phosphate dehydrogenase**, represents the committed step, and its activity controls flux through the entire pentose phosphate pathway.
- Glucose-6-phosphate dehydrogenase is regulated by the availability of $NADP^+$.
  - If the $NADP^+$/NADPH ratio is low, indicating that the cell has plenty of reducing power, glucose-6-phosphate dehydrogenase activity will be low and the pathway will not divert glucose-6-phosphate from glycolysis.
  - If the cell needs more reducing equivalents, however, the high $NADP^+$/NADPH ratio will stimulate flux through glucose-6-phosphate dehydrogenase, regenerating the necessary NADPH.

Pathway Map

- Glycolysis: ATP + NADH + Pyruvate
- Glycogen metabolism: uses glycogen as a storage polysaccharide
- PPP: makes ribose for nucleotides
- Gluconeogenesis: Glucose

- The 3C pyruvate can provide more energy by generating 3 CO_2
- In the Citric Acid Cycle!

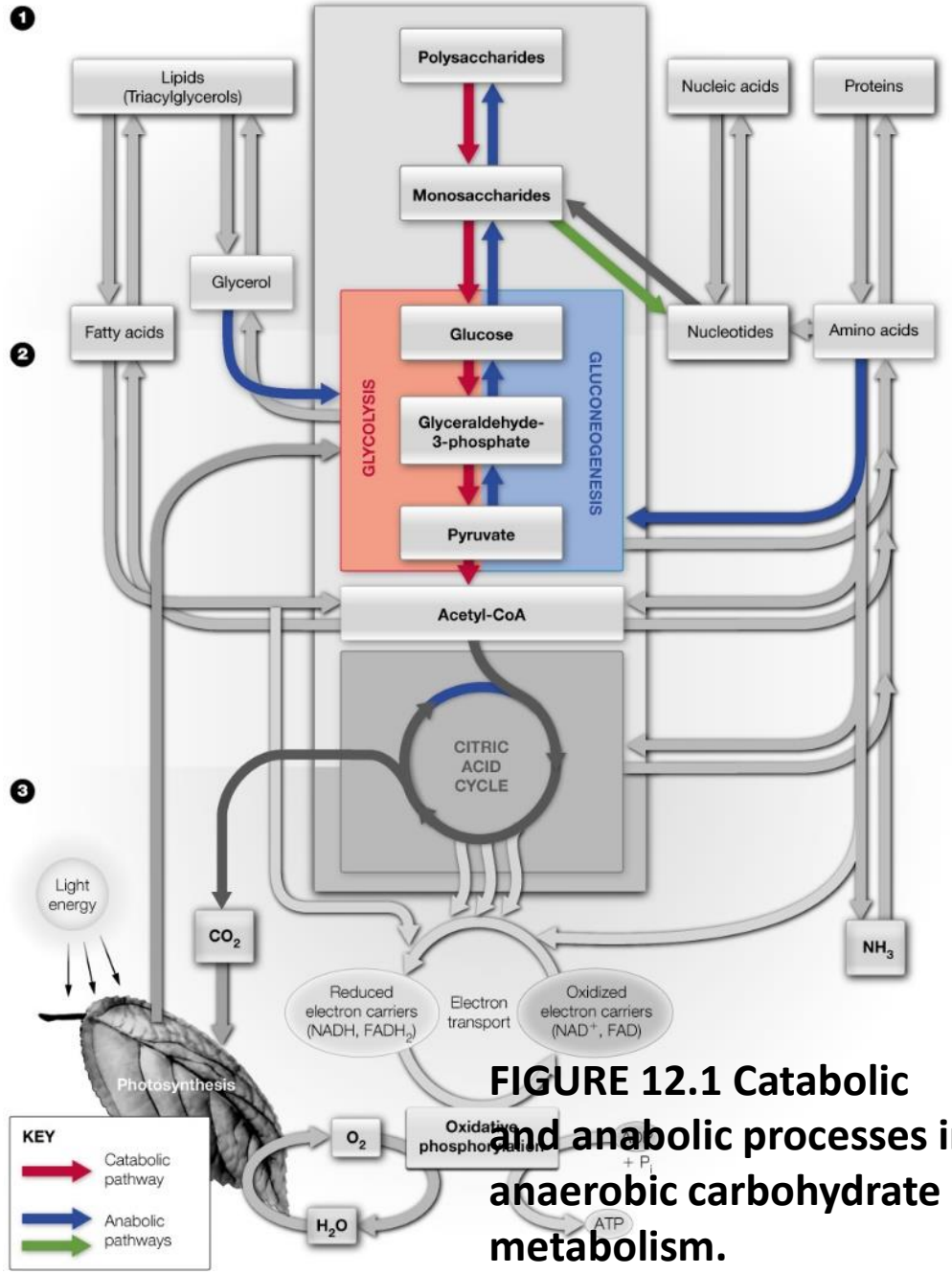
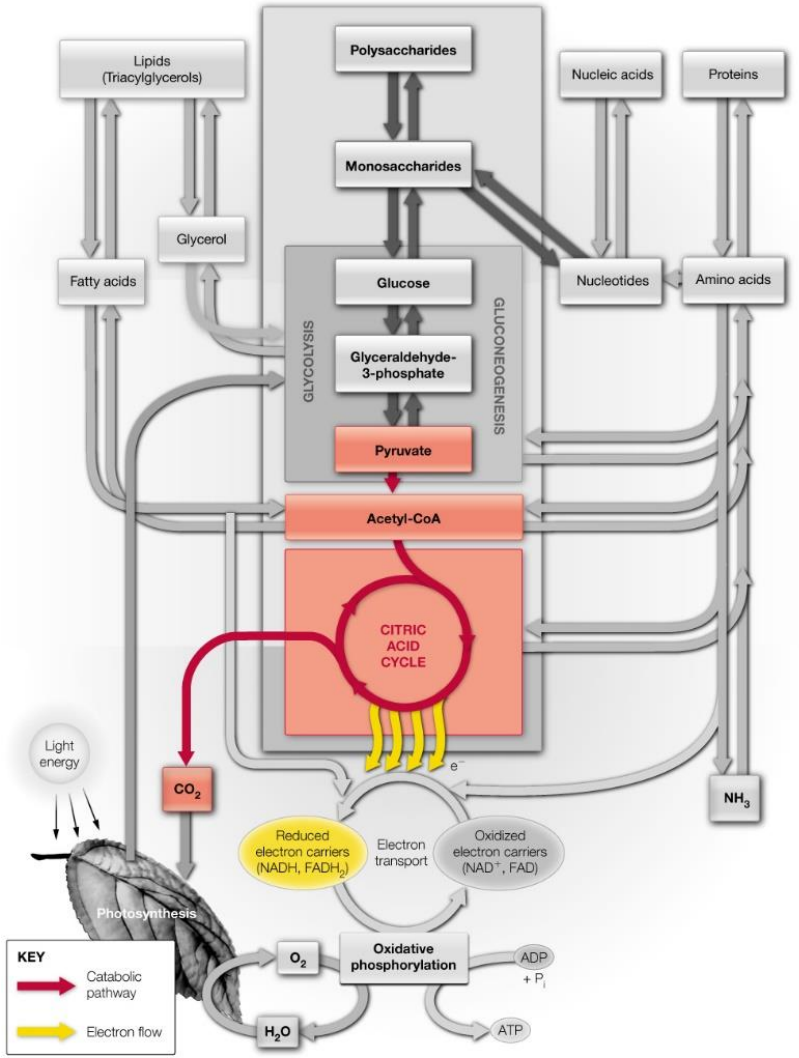


FIGURE 12.1 Catabolic and anabolic processes in anaerobic carbohydrate metabolism.

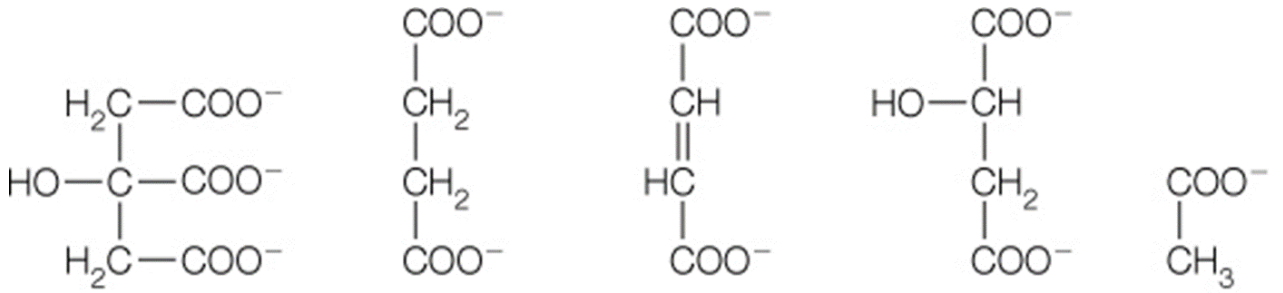
Oxidative Processes in the Generation of Metabolic Energy

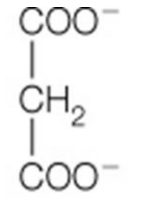
- The **citric acid cycle** is the central pathway for oxidizing all metabolic fuels
- Most of the energy yield from substrate oxidation in the citric acid cycle is stored in reduced electron carriers such as NADH
- The citric acid cycle is an anaerobic pathway.



The Citric Acid Cycle

- From 1932, Krebs tested oxidation of various organic acids from the rate of oxygen consumption in liver and kidney slices. He found that citrate, succinate, fumarate, malate, and acetate were readily oxidized in these tissues.

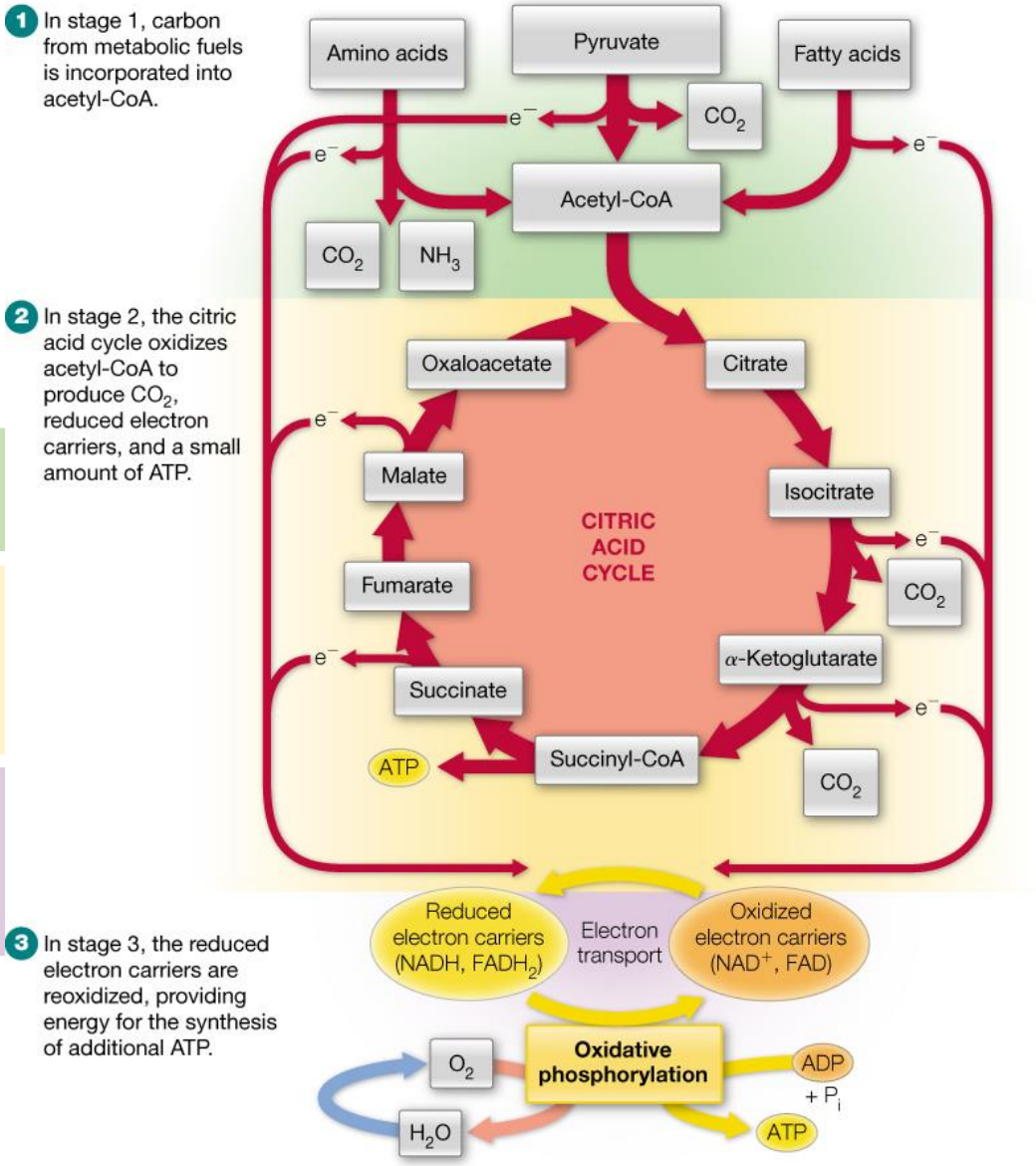




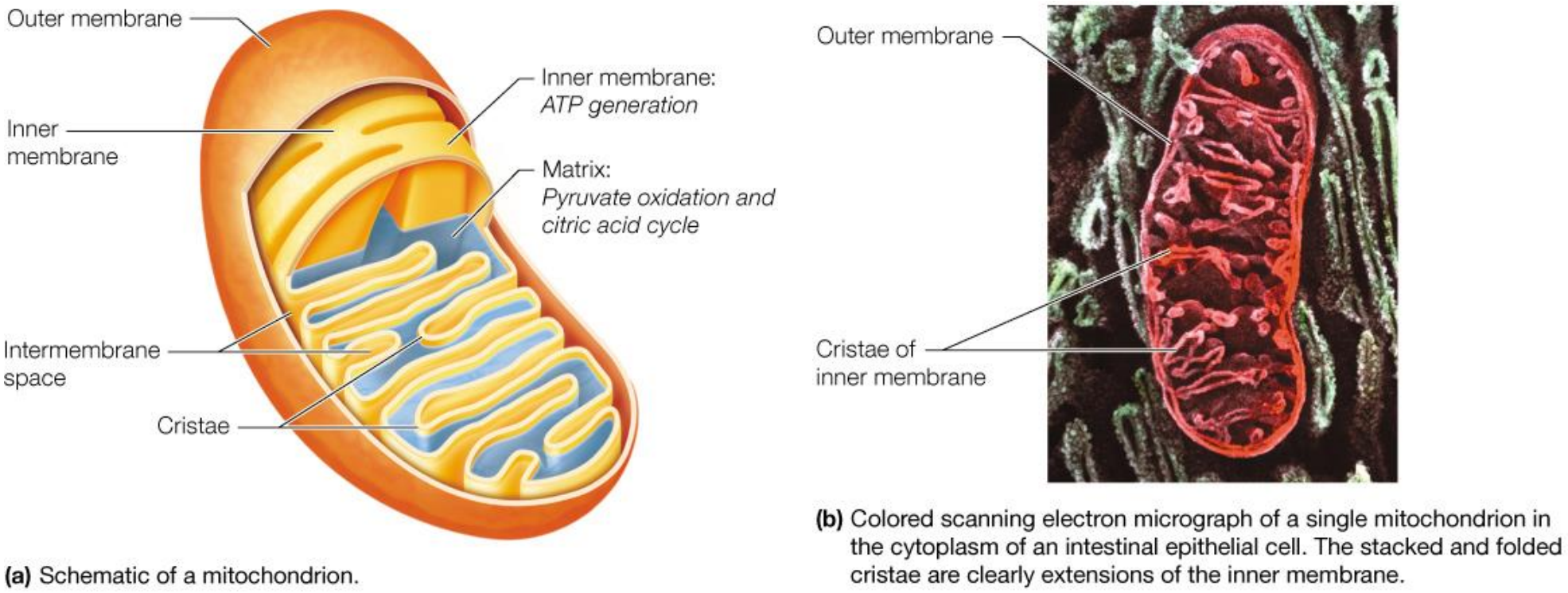
- In 1935, Albert Szent-Györgyi found that oxygen consumption of pigeon breast muscle was enhanced by small amounts of added dicarboxylic acids succinate, fumarate, or malate.
- In 1937, Carl Martius and Franz Knoop discovered that citrate is converted to α-ketoglutarate, which was already known to undergo conversion to succinate.
- In 1937 by Hans Krebs proposed that organic fuels are oxidized via a cyclic pathway - he later shared a Nobel Prize for this with Fritz Lipmann (the discoverer of coenzyme A).
- He also noted that malonate, an analog of succinate and a known inhibitor of succinate dehydrogenase, blocked the oxidation of pyruvate.

Stages of Cellular Respiration

- Metabolic oxidation of organic substrates (cellular respiration) occurs in three stages:
  - In stage 1, carbon from metabolic fuels is incorporated into acetyl-CoA
  - In stage 2, the citric acid cycle oxidizes acetyl-CoA to produce CO_2, reduced electron carriers, and a small amount of ATP
  - In stage 3, the reduced electron carriers are reoxidized, providing energy for the synthesis of additional ATP
- In eukaryotic organisms, **<u>these three stages are located in the mitochondria</u>**.



Structure of Mitochondria



(a) Schematic of a mitochondrion.

(b) Colored scanning electron micrograph of a single mitochondrion in the cytoplasm of an intestinal epithelial cell. The stacked and folded cristae are clearly extensions of the inner membrane.

FIGURE 13.3 Structure of the mitochondrion.

The reactions of stages 1 and 2 of respiration occur in the mitochondrial matrix. Reactions of stage 3 are catalyzed by membrane-bound enzymes in the inner mitochondrial membrane

Mitochondrial membrane has two lipid bilayers!

- Outer membrane: 52% proteins
  - ➢ Like plasma membrane
- Inner membrane: 76% proteins!
  - ❖ Lots of important pathways are based here!
  - ❖ Important for **electron transport chain** and **oxidative phosphorylation**!

TABLE 10.5 Protein, lipid, and carbohydrate content of some membranes

	Percent by Weight		
Membrane	Protein	Lipid	Carbohydrate
Myelin	18	79	3
Human erythrocyte (plasma membrane)	49	43	8
Mitochondria (outer membrane)	52	48	0
Sarcoplasmic reticulum (muscle cells)	67	33	0
Chloroplast lamellae	70	30	0
Gram-positive bacteria	75	25	0
Mitochondria (inner membrane)	76	24	0

Adapted from *Annual Review of Biochemistry* 41:731, G. Guidotti, Membrane proteins.

Pyruvate Oxidation : A Major Entry Route for Carbon into the Citric Acid Cycle

- Pyruvate, from glycolysis, is transported into mitochondria, where it is converted to acetyl-CoA by the pyruvate dehydrogenase (PDH) multienzyme complex, an example of a compartmentation in a multisubunit complex
- **3 enzymes, requiring 5 coenzymes, make up the PDH complex**:
    1. Pyruvate dehydrogenase (E1)
    2. Dihydrolipoamide transacetylase (E2)
    3. Dihydrolipoamide dehydrogenase (E3)

TABLE 13.1 Coenzymes of the pyruvate dehydrogenase reaction

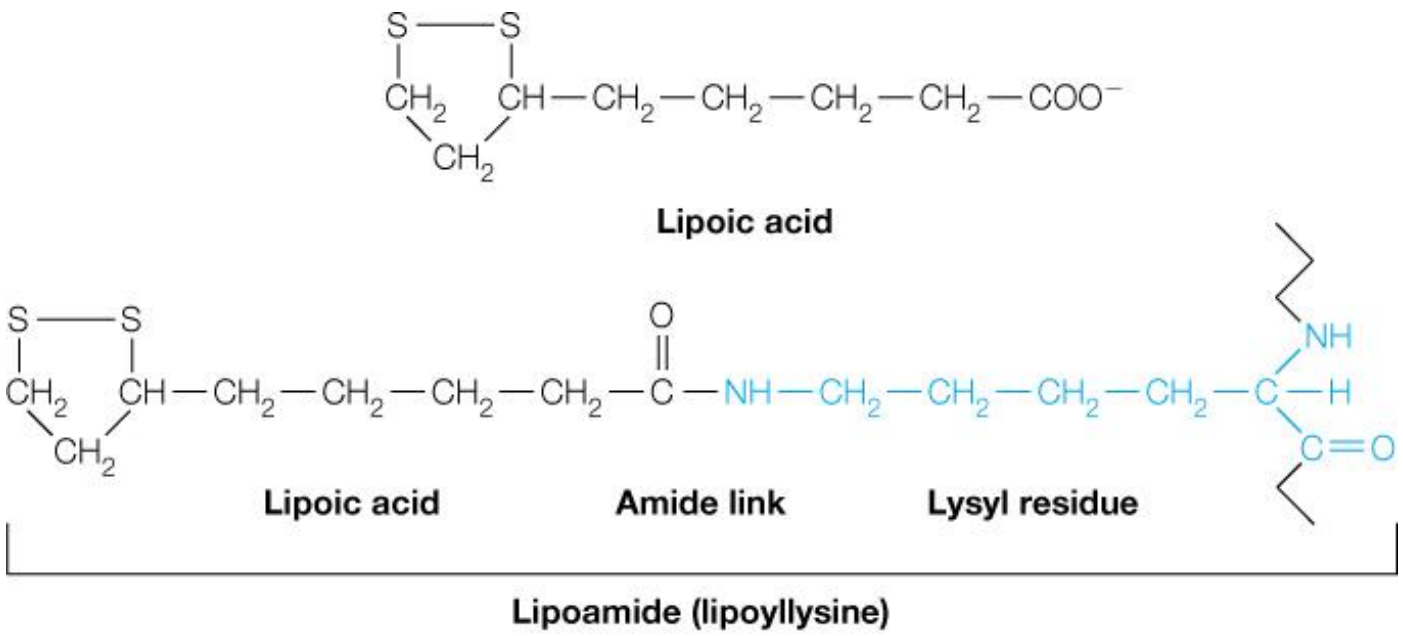
Cofactor	Location	Function
Thiamine pyrophosphate (TPP)	Tightly bound to E_1	Decarboxylates pyruvate, yielding hydroxyethyl-TPP
Lipoic acid (lipoamide)	Covalently bound to E_2 via lysine ("swinging arm")	Accepts hydroxyethyl carbanion from TPP as acetyl group
Coenzyme A (CoA)	Dissociable substrate for E_2	Accepts acetyl group from lipoamide
Flavin adenine dinucleotide (FAD)	Tightly bound to E_3	Accepts pair of electrons from reduced lipoamide
Nicotinamide adenine dinucleotide (NAD^+)	Dissociable substrate for E_3	Accepts pair of electrons from reduced $FADH_2$

Thiamine Pyrophosphate (TPP), a Cofactor of E1

Thiamine pyrophosphate (TPP)

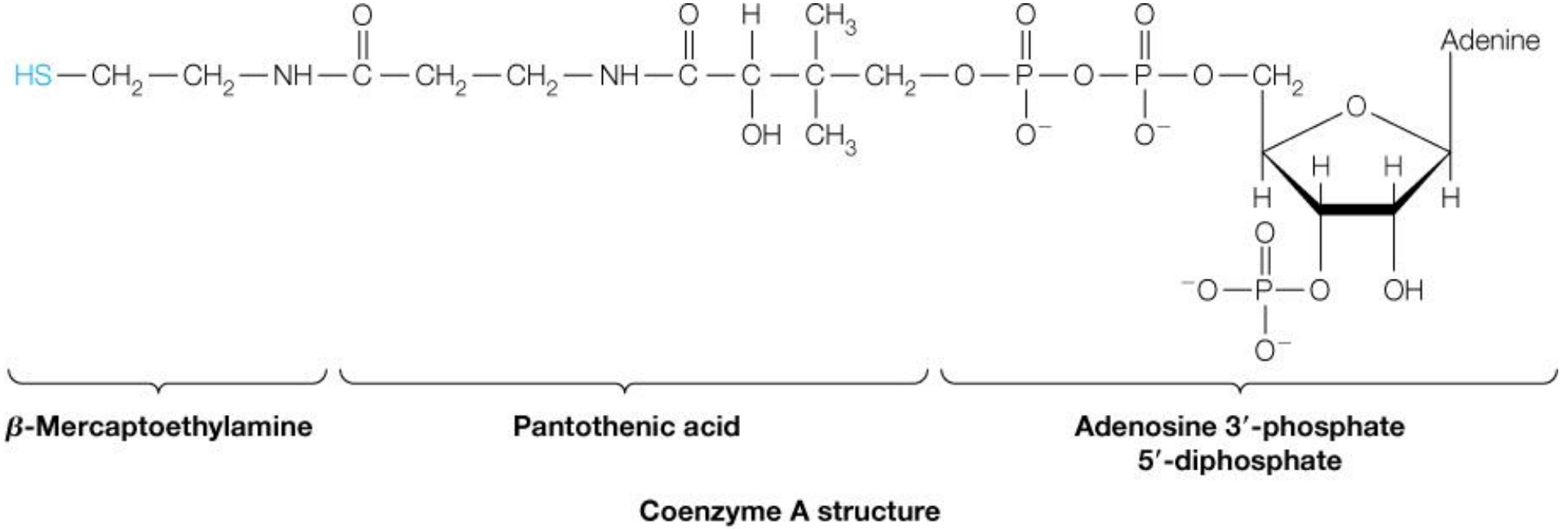
- Unlike β-keto acids, α-keto acids develop an unstable carbanion intermediate during decarboxylation
- TPP (vitamin B_1) catalyzes the cleavage of C-C bonds by stabilizing this carbanion intermediate

Lipoamide, a Cofactor of E2



- Lipoamide is a coenzyme located on the E_2 subunit that participates in the transfer of acyl groups
- The 14 Å long carbon chain acts as a swinging arm to interact with the E_1 and E_3 subunits

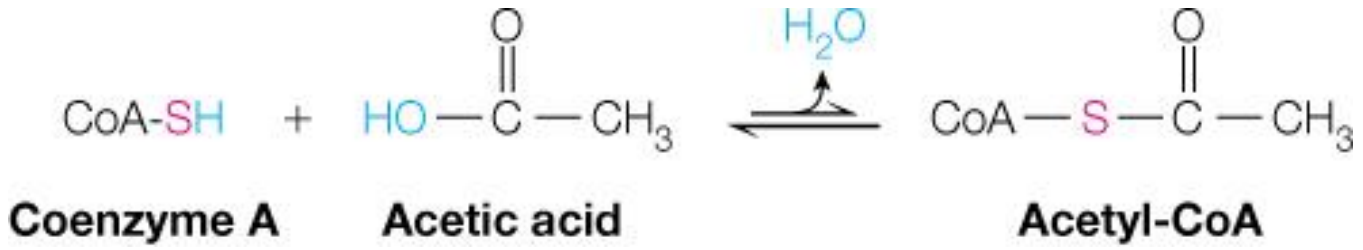
Coenzyme A, a Cofactor of E2

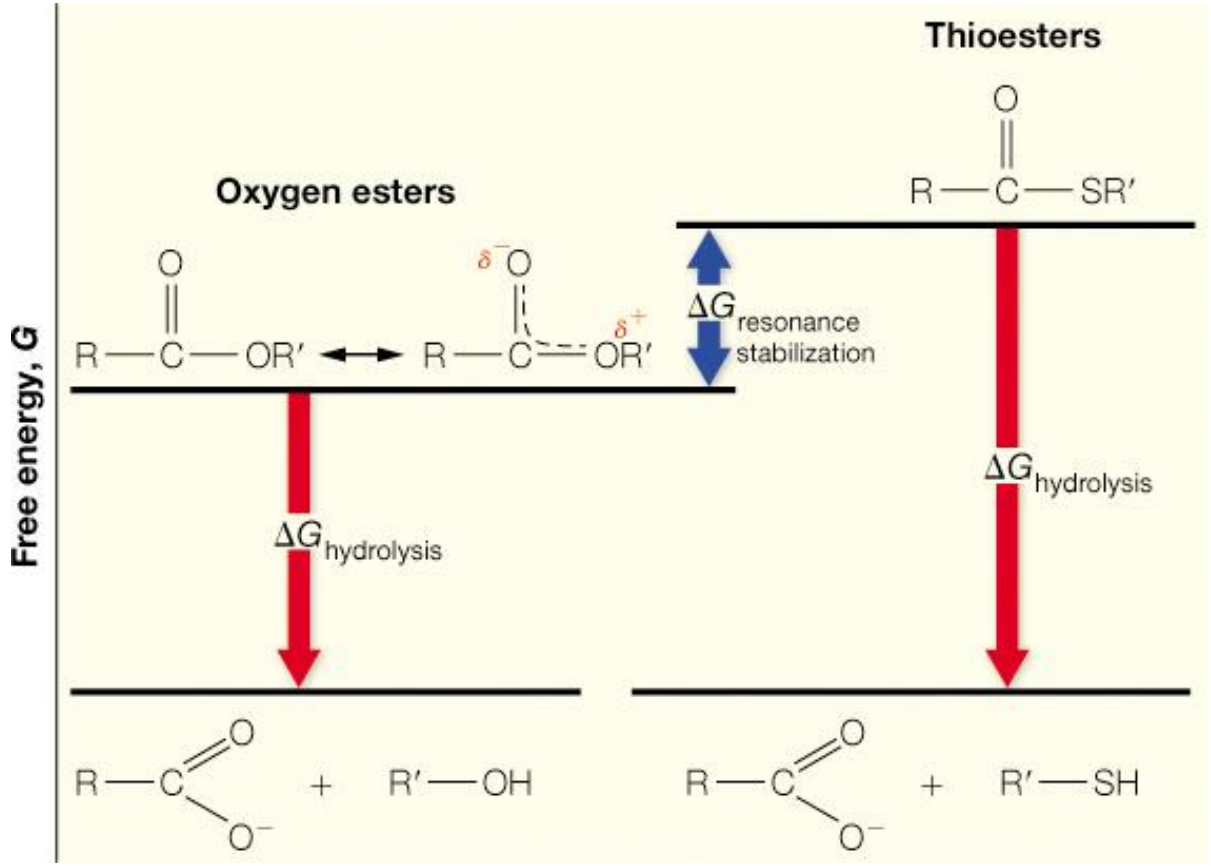


Coenzyme A structure

- The free thiol of β-mercaptoethylamine reacts with an acyl group to form an thioester, such as acetyl-CoA
- Pantothenic acid is vitamin B_5
- A thioester is an energy-rich compound

Coenzyme A, a Cofactor of E2





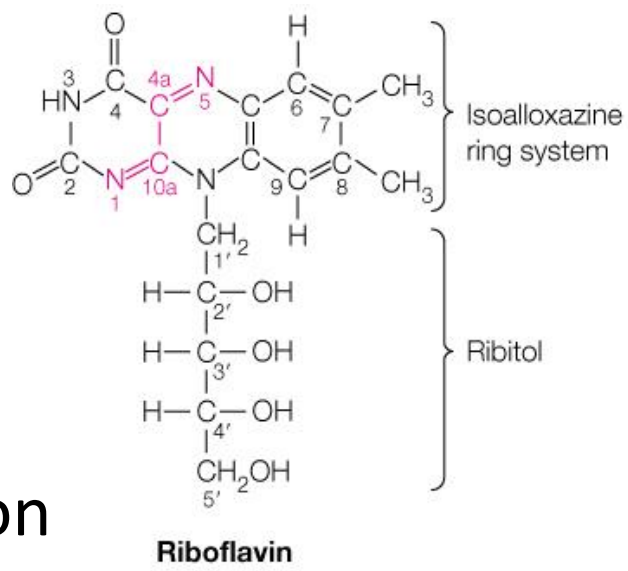
The larger atomic size of sulfur compared with the ester oxygen results in poor resonance stabilization.

Therefore, **a thioester** has a **higher potential energy** for acyl group transfer than an ester.

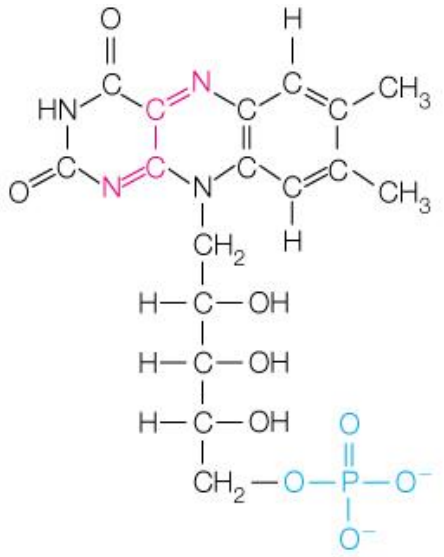
Acetyl-CoA is a classic example of a high energy S bond.

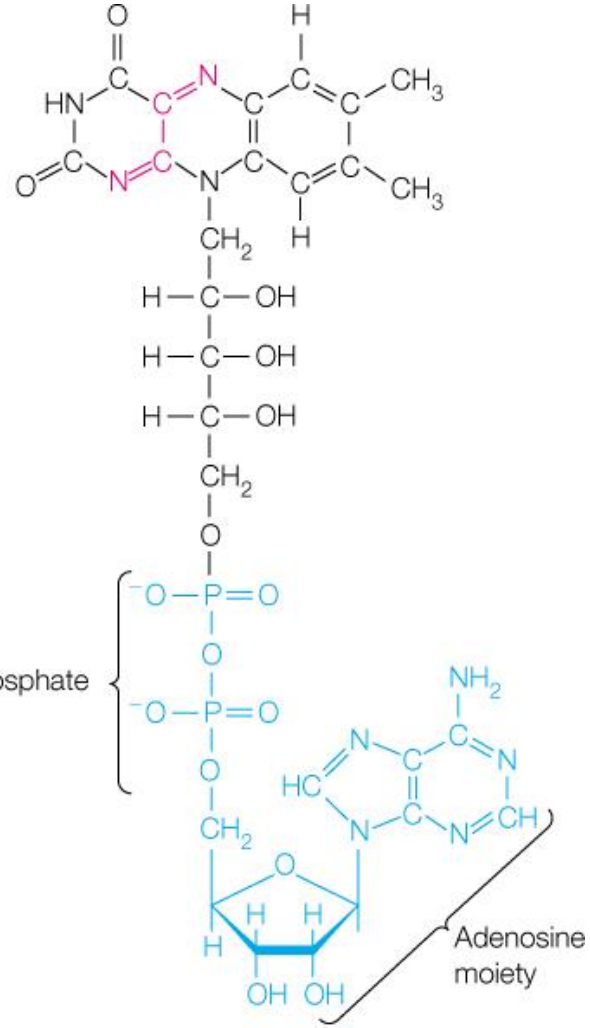
Flavine Adenine Dinucleotide (FAD), a Cofactor of E3

- Flavin coenzymes (riboflavin or Vitamin B_2, FMN, or FAD) participate in two-electron oxidoreduction reactions that proceed **one electron at a time**
- Flavin reduction proceeds through a stable semiquinone free radical species
- In the PDH complex, FAD accepts a pair of electrons from reduced lipoamide



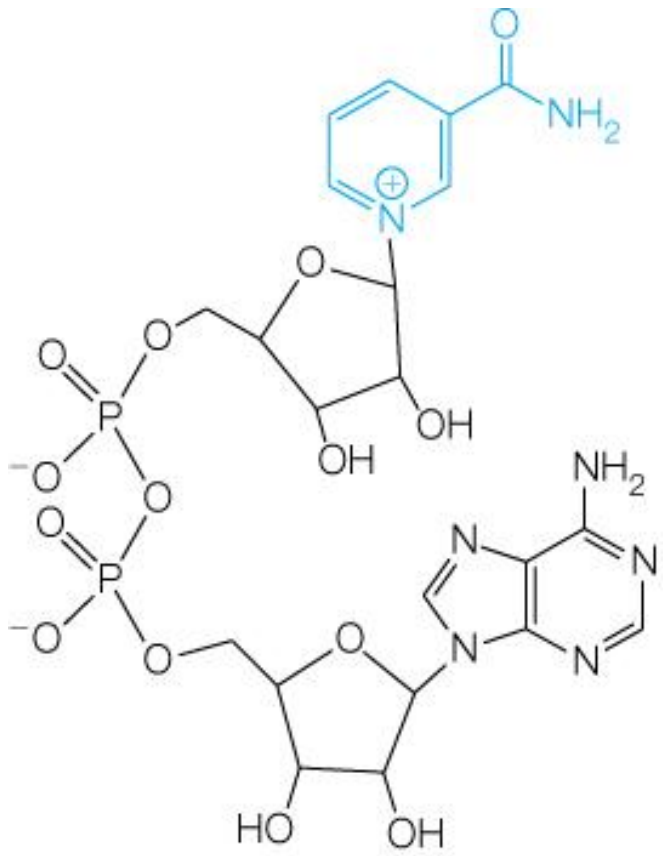
Riboflavin


Flavin mononucleotide (FMN)
(also called riboflavin phosphate)

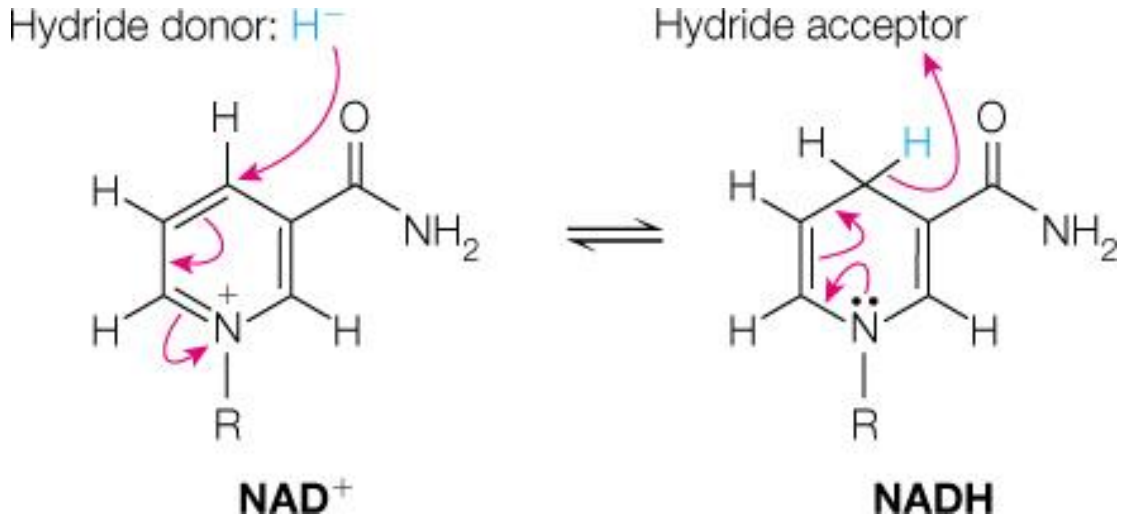


Flavin adenine dinucleotide (FAD)

Nicotinamide Adenine Dinucleotide (NAD^+), a Cofactor of E3



NAD^+



NAD^+ **NADH**

- Derived from niacin, vitamin B_3
- The nicotinamide portion of **NAD^+** can accept **two electrons** in the form of a hydride (H^-), yielding the reduced species, **NADH**
- In the PDH complex, electrons are transferred from reduced FAD to NAD^+

Structure of the Eukaryotic PDH Complex

- Pyruvate dehydrogenase (E_1): about 30 tetramers (roughly 160 kDa each tetramer)
- Dihydrolipoamide transacetylase (E_2): 60 monomers (roughly 58 kDa each monomer)
- Dihydrolipoamide dehydrogenase (E_3): 12 homodimers (roughly 112 kDa each homodimer)
- The overall mass of the PDH complex is about 10,000,000 Da (10 MDa; larger than the ribosome)

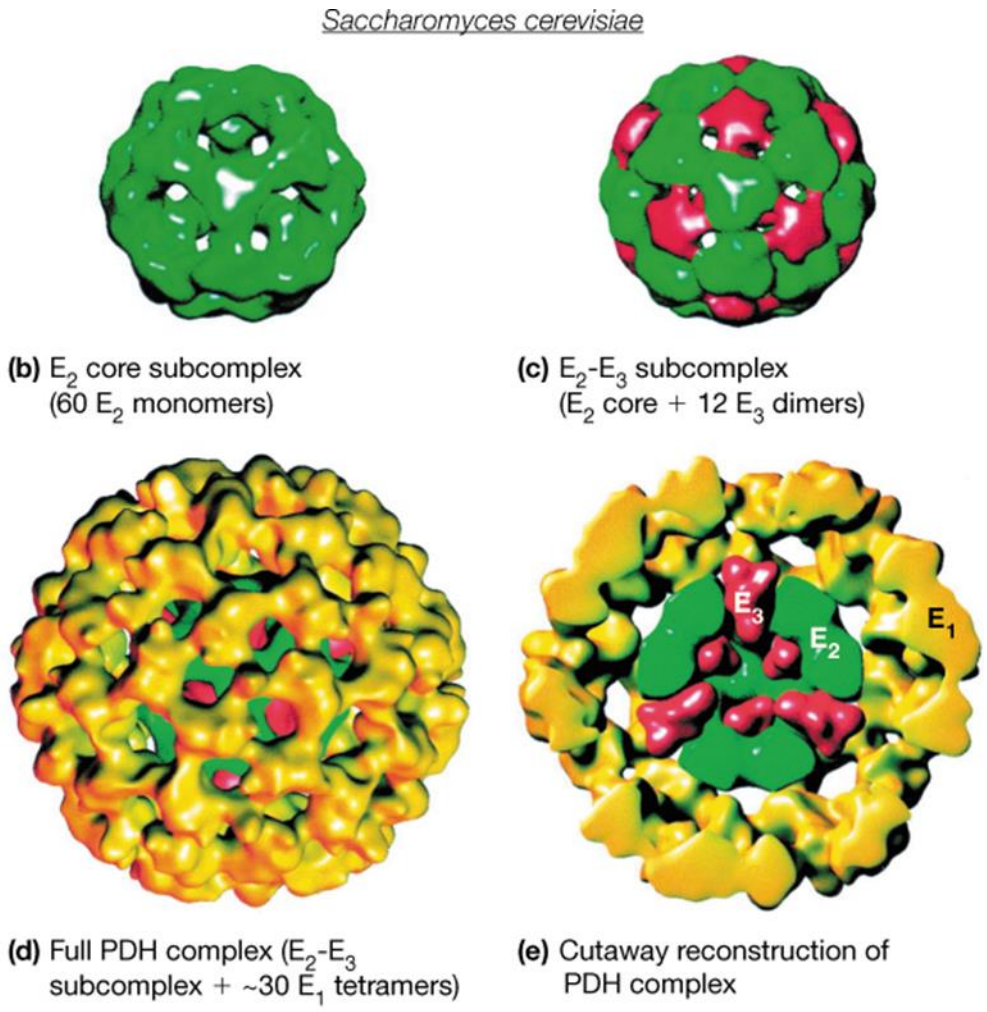
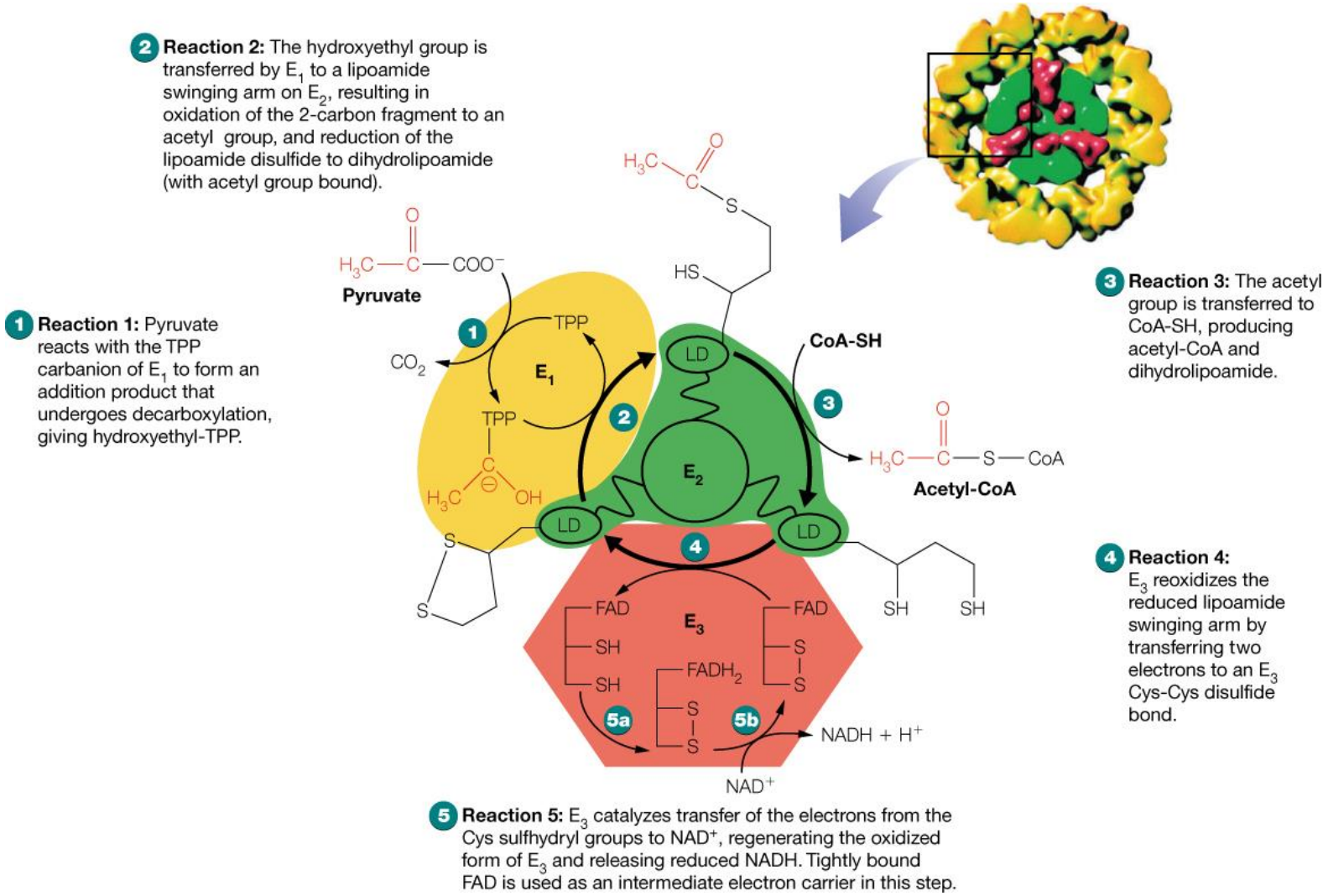


FIGURE 13.5 Structure of the pyruvate dehydrogenase complex.

Concerted function of PDH



Stage 2: the CAC

- Acetyl-CoA entering the citric acid cycle is highlighted (in blue) to show the fate of its two carbons through reaction 4.
- After reaction 5 , the carbon atoms contributed by the acetyl-CoA acetyl group from this turn of the cycle are no longer highlighted because succinate and fumarate are symmetrical molecules.
  - Thus, C1 and C2 become indistinguishable from C3 and C4 beyond this point in the cycle.
- Carboxyl groups that leave the cycle as CO_2 in reactions 3 and 4 are highlighted in green.
- These departing CO_2 groups derive from the two oxaloacetate carboxyl groups that were incorporated as acetyl-CoA in earlier turns of the citric acid cycle.