Microbial Metabolism

Introduction

- Throughout earth's history, microbial metabolism has been a driving force behind the development and maintenance of the planet's biosphere.
- Eukaryotic organisms such as plants and animals typically depend on organic molecules for energy, growth, and reproduction.

•Prokaryotes, on the other hand, can metabolize a wide range of organic as well as inorganic matter, from complex organic molecules like cellulose to inorganic molecules and ions such as atmospheric nitrogen (N_2), molecular hydrogen (H_2), sulfide (S^{2-}), manganese (II) ions (Mn^{2+}), ferrous iron (Fe^{2+}), and ferric iron (Fe^{3+}), to name a few.

•By metabolizing such substances, microbes chemically convert them to other forms.

•In some cases, microbial metabolism produces chemicals that can be harmful to other organisms; in others, it produces substances that are essential to the metabolism and survival of other life form.

Energy, Matter, and Enzymes

- The term used to describe all of the chemical reactions inside a cell is **metabolism**.
- Cellular processes such as the building or breaking down of complex molecules occur through series of stepwise, interconnected chemical reactions called **metabolic pathways**.
- Reactions that are spontaneous and release energy are exergonic reactions, whereas endergonic reactions require energy to proceed.
- The term anabolism refers to those endergonic metabolic pathways involved in biosynthesis, converting simple molecular building blocks into more complex molecules, and fueled by the use of cellular energy.

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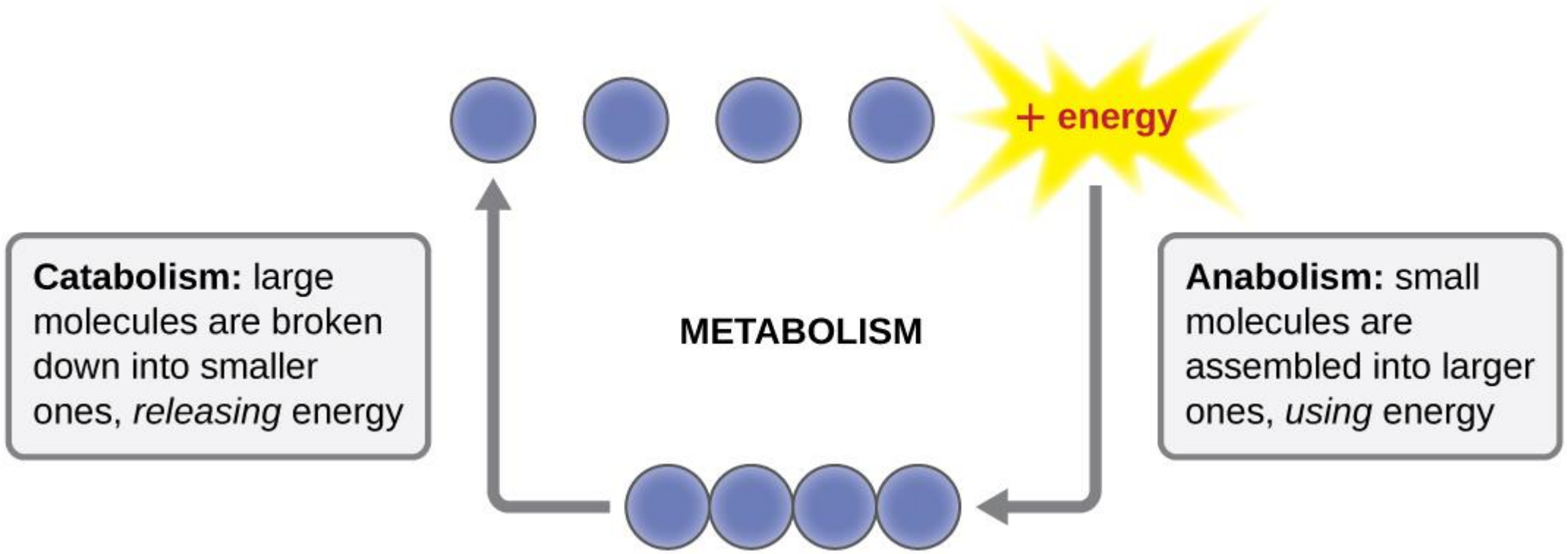
- Conversely, the term catabolism refers to exergonic pathways that break down complex molecules into simpler ones.
- Molecular energy stored in the bonds of complex molecules is released in catabolic pathways and harvested in such a way that it can be used to produce high-energy molecules, which are used to drive anabolic pathways.
- Thus, in terms of energy and molecules, cells are continually balancing catabolism with anabolism.

Metabolism includes catabolism and anabolism. Anabolic pathways require energy to synthesize larger molecules. Catabolic pathways generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.



Classification by Carbon and Energy Source

- Organisms can be identified according to the source of carbon they use for metabolism as well as their energy source.
- The prefixes auto- (“self”) and hetero- (“other”) refer to the origins of the carbon sources various organisms can use.
- Organisms that convert inorganic carbon dioxide (CO2) into organic carbon compounds are autotrophs.
- Plants and cyanobacteria are well-known examples of autotrophs. Conversely, heterotrophs rely on more complex organic carbon compounds as nutrients; these are provided to them initially by autotrophs.
- Many organisms, ranging from humans to many prokaryotes, including the well-studied Escherichia coli, are heterotrophic.

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- Organisms can also be identified by the energy source they use.
- All energy is derived from the transfer of electrons, but the source of electrons differs between various types of organisms.
- The prefixes photo- (“light”) and chemo- (“chemical”) refer to the energy sources that various organisms use.
- Those that get their energy for electron transfer from light are phototrophs, whereas chemotrophs obtain energy for electron transfer by breaking chemical bonds.
- There are two types of chemotrophs: **organotrophs and lithotrophs**.
- **Organotrophs,** including humans, fungi, and many prokaryotes, are chemotrophs that obtain energy from organic compounds.
- **Lithotrophs** (“litho” means “rock”) are chemotrophs that get energy from inorganic compounds, including hydrogen sulfide (H2S) and reduced iron.
- Lithotrophy is unique to the microbial world.

- The strategies used to obtain both carbon and energy can be combined for the classification of organisms according to nutritional type.
- Most organisms are chemoheterotrophs because they use organic molecules as both their electron and carbon sources.

Classifications		Energy Source	Carbon Source	Examples
Chemotrophs	Chemoautotrophs	Chemical	Inorganic	Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria
	Chemoheterotrophs	Chemical	Organic compounds	All animals, most fungi, protozoa, and bacteria
Phototrophs	Photoautotrophs	Light	Inorganic	All plants, algae, cyanobacteria, and green and purple sulfur bacteria
	Photoheterotrophs	Light	Organic compounds	Green and purple nonsulfur bacteria, heliobacteria

Oxidation and Reduction in Metabolism

- The transfer of electrons between molecules is important because most of the energy stored in atoms and used to fuel cell functions is in the form of high-energy electrons.
- The transfer of energy in the form of electrons allows the cell to transfer and use energy incrementally; that is, in small packages rather than a single, destructive burst.
- Reactions that remove electrons from donor molecules, leaving them oxidized, are oxidation reactions; those that add electrons to acceptor molecules, leaving them reduced, are reduction reactions.
- Because electrons can move from one molecule to another, oxidation and reduction occur in tandem.
- These pairs of reactions are called oxidation-reduction reactions, or redox reactions.

Energy Carriers: NAD+ NADP+ FAD, and ATP

- The energy released from the breakdown of the chemical bonds within nutrients can be stored either through the reduction of electron carriers or in the bonds of adenosine triphosphate (ATP).
- In living systems, a small class of compounds functions as mobile electron carriers, molecules that bind to and shuttle high-energy electrons between compounds in pathways.
- The principal electron carriers consider originate from the B vitamin group and are derivatives of nucleotides; they are nicotinamide adenine dinucleotide, nicotine adenine dinucleotide phosphate, and flavin adenine dinucleotide.

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- These compounds can be easily reduced or oxidized. Nicotinamide adenine dinucleotide (NAD+ /NADH) is the most common mobile electron carrier used in catabolism.

•NAD+ is the oxidized form of the molecule; NADH is the reduced form of the molecule. Nicotine adenine dinucleotide phosphate (NADP+), the oxidized form of an NAD+ variant that contains an extra phosphate group, is another important electron carrier; it forms NADPH when reduced.

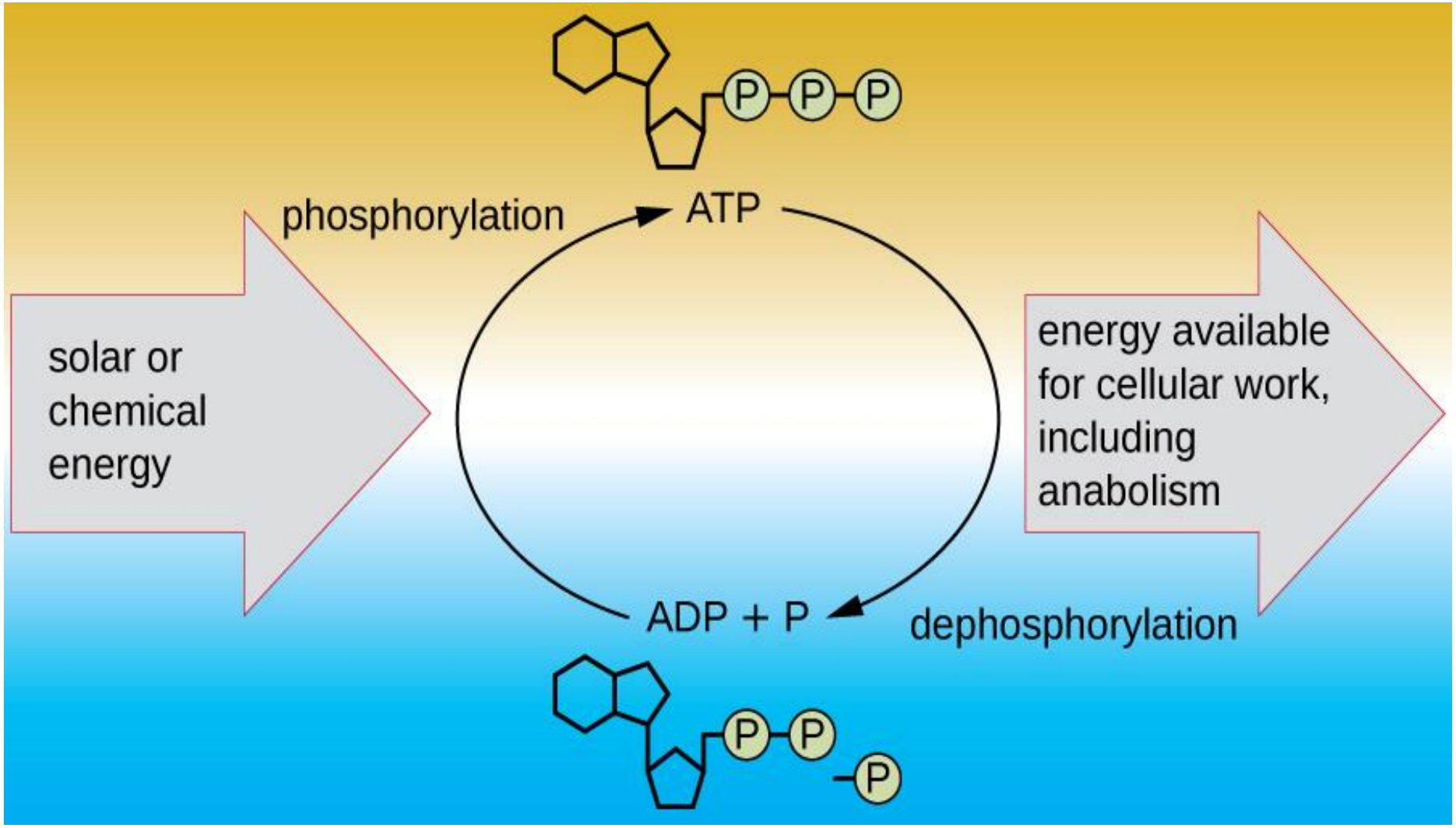
•The oxidized form of flavin adenine dinucleotide is FAD, and its reduced form is FADH2. Both NAD+/NADH and FAD/FADH2 are extensively used in energy extraction from sugarsduring catabolism in chemoheterotrophs, whereas NADP+ /NADPH plays an important role in anabolic reactions andphotosynthesis.

•Collectively, FADH2, NADH, and NADPH are often referred to as having reducing power due to their ability to donate electrons to various chemical reactions.

•A living cell must be able to handle the energy released during catabolism in a way that enables the cell to store energy safely and release it for use only as needed.

•Living cells accomplish this by using the compound adenosine triphosphate (ATP). ATP is often called the “energy currency” of the cell, and, like currency, this versatile compound can be used to fill any energy need of the cell.

The energy released from dephosphorylation of ATP is used to drive cellular work, including anabolic pathways. ATP is regenerated through phosphorylation, harnessing the energy found in chemicals or from sunlight.



Enzyme Structure and Function

- A substance that helps speed up a chemical reaction is a catalyst.
- Catalysts are not used or changed during chemical reactions and, therefore, are reusable.
- Whereas inorganic molecules may serve as catalysts for a wide range of chemical reactions, proteins called enzymes serve as catalysts for biochemical reactions inside cells.
- Enzymes thus play an important role in controlling cellular metabolism.
- An enzyme functions by lowering the activation energy of a chemical reaction inside the cell.
- Activation energy isthe energy needed to form or break chemical bonds and convert reactants to products.
- Enzymes lower the activation energy by binding to the reactant molecules and holding them in such a way as to speed up the reaction.

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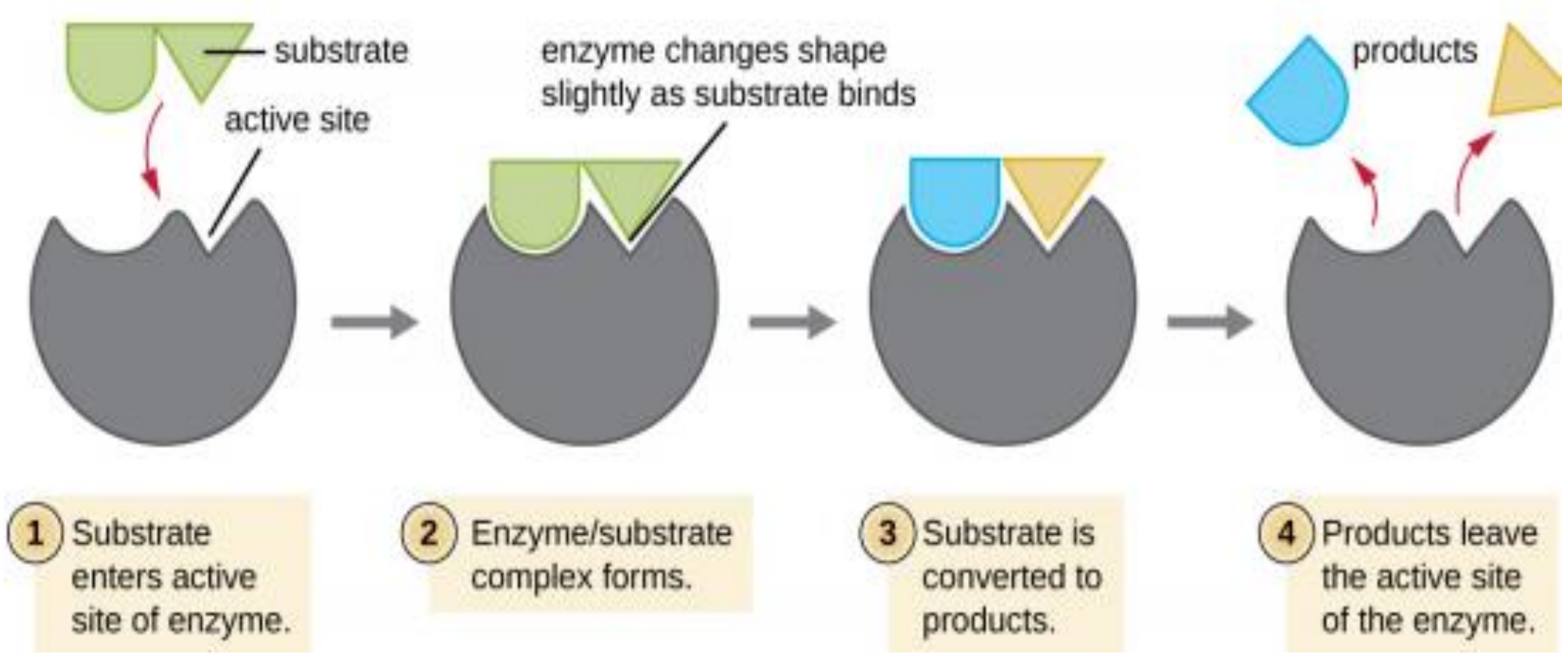
- The chemical reactants to which an enzyme binds are called substrates, and the location within the enzyme where the substrate binds is called the enzyme's active site.
- The characteristics of the amino acids near the active site create a very specific chemical environment within the active site that induces suitability to binding, albeit briefly, to a specific substrate (or substrates).
- Due to this jigsaw puzzle-like match between an enzyme and its substrates, enzymes are known for their specificity.
- In fact, as an enzyme binds to its substrate(s), the enzyme structure changes slightly to find the best fit between the transition state (a structural intermediate between the substrate and product) and the ctive site, just as a rubber glove molds to a hand inserted into it.
- This active-site modification in the presence of substrate, along with the simultaneous formation of the transition state, is called induced fit .
- Overall, there is a specifically matched enzyme for each substrate and, thus, for each chemical reaction; however, there is some flexibility as well.
- Some enzymes have the ability to act on several different structurally related substrates.

According to the induced-fit model, the active site of the enzyme undergoes conformational change upon binding with the substrate.



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- Enzymes are subject to influences by local environmental conditions such as pH, substrate concentration, and temperature.
- Although increasing the environmental temperature generally increases reaction rates, enzyme catalyzed or otherwise, increasing or decreasing the temperature outside of an optimal range can affect chemical bonds within the active site, making them less well suited to bind substrates.
- High temperatures will eventually cause enzymes, like other biological molecules, to denature, losing their three-dimensional structure and function.
- Enzymes are also suited to function best within a certain pH range, and, as with temperature, extreme environmental pH values (acidic or basic) can cause enzymes to denature.
- Active-site amino-acid side chains have their own acidic or basic properties that are optimal for catalysis and, therefore, are sensitive to changes in pH.
- Another factor that influences enzyme activity is **substrate concentration**: Enzyme activity is increased at higher concentrations of substrate until it reaches a saturation point at which the enzyme can bind no additional substrate.
- Overall, enzymes are optimized to work best under the environmental conditions in which the organisms that produce them live.

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- For example, while microbes that inhabit hot springs have enzymes that work best at high temperatures, human pathogens have enzymes that work best at 37°C.
- Similarly, while enzymes produced by most organisms work best at a neutral pH, microbes growing in acidic environments make enzymes optimized to low pH conditions, allowing for their growth at those conditions.
- Many enzymes do not work optimally, or even at all, unless bound to other specific nonprotein helper molecules, either temporarily through ionic or hydrogen bonds or permanently through stronger covalent bonds.
- Binding to these molecules promotes optimal conformation and function for their respective enzymes.
- Two types of helper molecules are cofactors and coenzymes.
- **Cofactors** are inorganic ions such as iron (Fe2+) and magnesium (Mg2+) that help stabilize enzyme conformation and function.
- One example of an enzyme that requires a metal ion as a cofactor is the enzyme that builds DNA molecules, DNA polymerase, which requires a bound zinc ion (Zn2+) to function.

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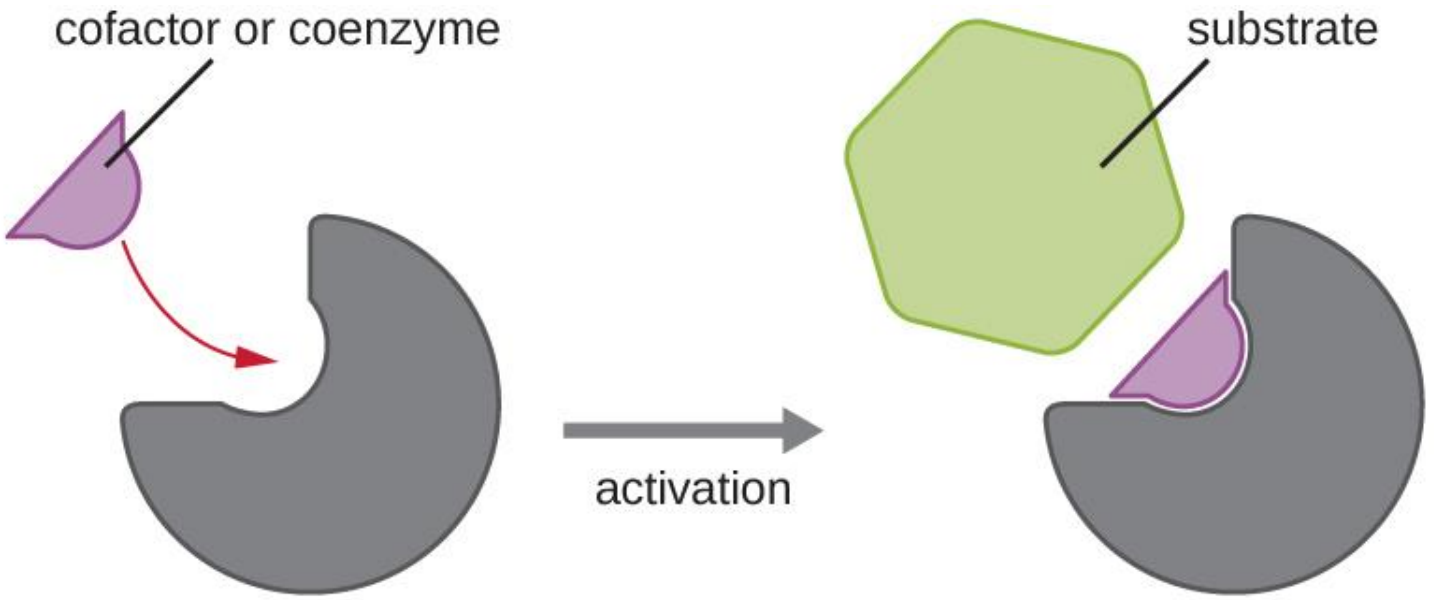
- Coenzymes are organic helper molecules that are required for enzyme action.
- Like enzymes, they are not consumed and, hence, are reusable.
- The most common sources of coenzymes are dietary vitamins.
- Some vitamins are precursors to coenzymes and others act directly as coenzymes.
- Some cofactors and coenzymes, like coenzyme A (CoA), often bind to the enzyme's active site, aiding in the chemistry of the transition of a substrate to a product
- In such cases, an enzyme lacking a necessary cofactor or coenzyme is called an **apoenzyme** and is inactive.
- Conversely, an enzyme with the necessary associated cofactor or coenzyme is called a **holoenzyme** and is active.
- NADH and ATP are also both examples of commonly used coenzymes that provide high-energy electrons or phosphate groups, respectively, which bind to enzymes, thereby activating them.

The binding of a coenzyme or cofactor to an apoenzyme is often required to form an active holoenzyme.



Catabolism of Carbohydrates

- Extensive enzyme pathways exist for breaking down carbohydrates to capture energy in ATP bonds.
- In addition,many catabolic pathways produce intermediate molecules that are also used as building blocks for anabolism.

Understanding these processes is important for several reasons.

First,

Because the main metabolic processes involved are common to a wide range of chemoheterotrophic organisms, we can learn a great deal about human metabolism by studying metabolism in more easily manipulated bacteria like E. coli.

Second,

Because animal and human pathogens are also chemoheterotrophs, learning about the details of metabolism in these bacteria, including possible differences between bacterial and human pathways, is useful for the diagnosis of pathogens as well as for the discovery of antimicrobial therapies targeting specific pathogens.

The typical example used to introduce concepts of metabolism to is carbohydrate catabolism.

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- For chemoheterotrophs, our examples of metabolism start with the catabolism of polysaccharides such as glycogen, starch, or cellulose.
- Enzymes such as amylase, which breaks down glycogen or starch, and cellulases, which break down cellulose, can cause the hydrolysis of glycosidic bonds between the glucose monomers in these polymers, releasing glucose for further catabolism.
- **Glycolysis:**
- For bacteria, eukaryotes, and most archaea, glycolysis is the most common pathway for the catabolism of glucose; it produces energy, reduced electron carriers, and precursor molecules for cellular metabolism.
- Every living organism carries out some form of glycolysis, suggesting this mechanism is an ancient universal metabolic process.
- The process itself does not use oxygen; however, glycolysis can be coupled with additional metabolic processes that are either aerobic or anaerobic.

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- Glycolysis takes place in the cytoplasm of prokaryotic and eukaryotic cells.
- It begins with a single six-carbon glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate.
- Pyruvate may be broken down further after glycolysis to harness more energy through aerobic or anaerobic respiration,
- But many organisms, including many microbes, may be unable to respire; for these organisms, glycolysis may be their only source of generating ATP.
- The type of glycolysis found in animals and that is most common in microbes is the Embden-Meyerhof-Parnas (EMP) pathway, named after Gustav Embden (1874–1933), Otto Meyerhof (1884–1951), and Jakub Parnas (1884–1949).
- Glycolysis using the EMP pathway consists of **two** distinct phases

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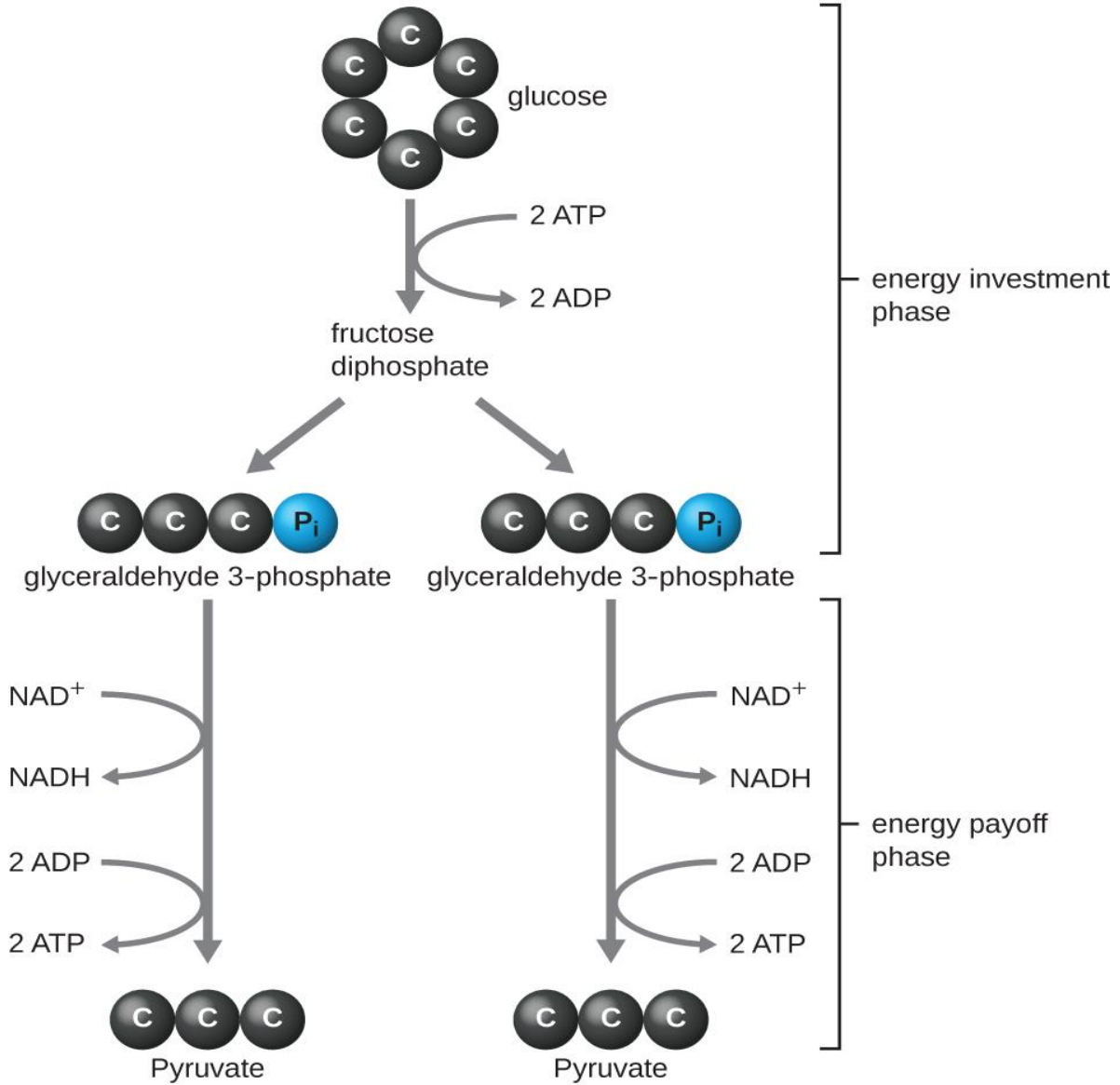
- The first part of the pathway, called the energy investment phase, uses energy from two ATP molecules to modify a glucose molecule so that the six-carbon sugar molecule can be split evenly into two phosphorylated three-carbon molecules called glyceraldehyde 3-phosphate (G3P).
- The second part of the pathway, called the energy payoff phase, extracts energy by oxidizing G3P to pyruvate, producing four ATP molecules and reducing two molecules of NAD+ to two molecules of NADH, using electrons that originated from glucose.
- The ATP molecules produced during the energy payoff phase of glycolysis are formed by substrate-level hosphorylation, one of two mechanisms for producing ATP.
- In substrate-level phosphorylation, a phosphate group is removed from an organic molecule and is directly transferred to an available ADP molecule, producing ATP.
- During glycolysis, high-energy phosphate groups from the intermediate molecules are added to ADP to make ATP.
- Overall, in this process of glycolysis, the net gain from the breakdown of a single glucose molecule is:
- two ATP molecules
- two NADH molecule, and
- two pyruvate molecules.

The energy investment phase of the Embden-Meyerhof-Parnas glycolysis pathway uses two ATP molecules to phosphorylate glucose, forming two glyceraldehyde 3-phosphate (G3P) molecules. The energy payoff phase harnesses the energy in the G3P molecules, producing four ATP molecules, two NADH molecules, and two pyruvates.



Other Glycolytic Pathways

- When we refer to glycolysis, unless otherwise indicated, we are referring to the EMP pathway used by animals
- and many bacteria. However, some prokaryotes use alternative glycolytic pathways. One important alternative is the Entner-Doudoroff (ED) pathway, named after its discoverers Nathan Entner and Michael Doudoroff (1911–1975).
- Although some bacteria, including the opportunistic gram-negative pathogen Pseudomonas aeruginosa, contain only the ED pathway for glycolysis, other bacteria, like E. coli, have the ability to use either the ED pathway or the EMP pathway.
- A third type of glycolytic pathway that occurs in all cells, which is quite different from the previous two pathways, is the pentose phosphate pathway (PPP) also called the phosphogluconate pathway or the hexose monophosphate shunt.
- Evidence suggests that the PPP may be the most ancient universal glycolytic pathway.
- The intermediates from the PPP are used for the biosynthesis of nucleotides and amino acids. Therefore, this glycolytic pathway may be favored when the cell has need for nucleic acid and/or protein synthesis, respective.

Transition Reaction, Coenzyme A, and the Krebs Cycle

- Glycolysis produces pyruvate, which can be further oxidized to capture more energy.
- For pyruvate to enter the next oxidative pathway, it must first be decarboxylated by the enzyme complex pyruvate dehydrogenase to a two-carbon acetyl group in the transition reaction, also called the bridge reaction.
- In the transition reaction, electrons are also transferred to NAD+ to form NADH.
- To proceed to the next phase of this metabolic process, the comparatively tiny two-carbon acetyl must be attached to a very large carrier compound calledcoenzyme A (CoA).
- The transition reaction occurs in the mitochondrial matrix of eukaryotes; in prokaryotes, it occurs in the cytoplasm because prokaryotes lack membrane-enclosed organelles.

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- The Krebs cycle transfers remaining electrons from the acetyl group produced during the transition reaction to electron carrier molecules, thus reducing them.
- The Krebs cycle also occurs in the cytoplasm of prokaryotes along with glycolysis and the transition reaction, but it takes place in the mitochondrial matrix of eukaryotic cells where the transition reaction also occurs.
- The Krebs cycle is named after its discoverer, British scientist Hans Adolf Krebs (1900–1981) and is also called the citric acid cycle, or the tricarboxylic acid cycle (TCA) because citric acid has three carboxyl groups in its structure.
- Unlike glycolysis, the Krebs cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of chemical reactions that capture the two-carbon acetyl group (the CoA carrier does not enter the Krebs cycle) from the transition reaction, which is added to a four-carbon intermediate in the Krebs cycle, producing the six-carbon intermediate citric acid (giving the alternate name for this cycle).
- As one turn of the cycle returns to the starting point of the four carbon intermediate, the cycle produces two CO_2 molecules, one ATP molecule (or an equivalent, such as guanosine triphosphate [GTP]) produced by substrate-level phosphorylation, and three molecules of NADH and one of $FADH_2$.

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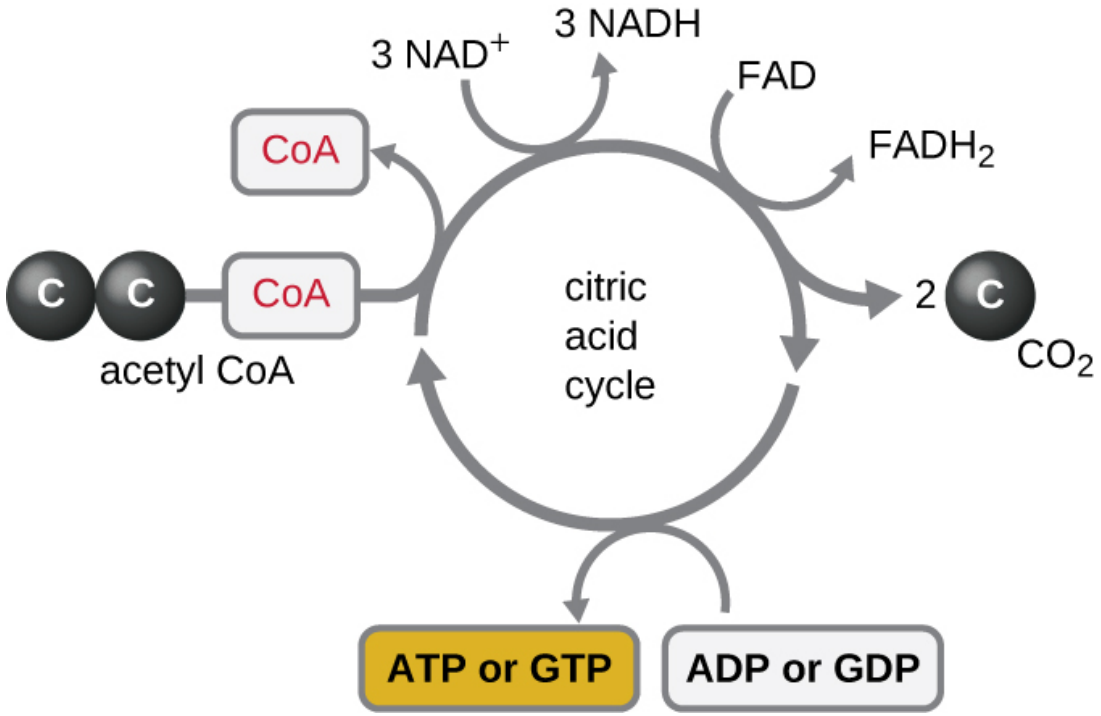
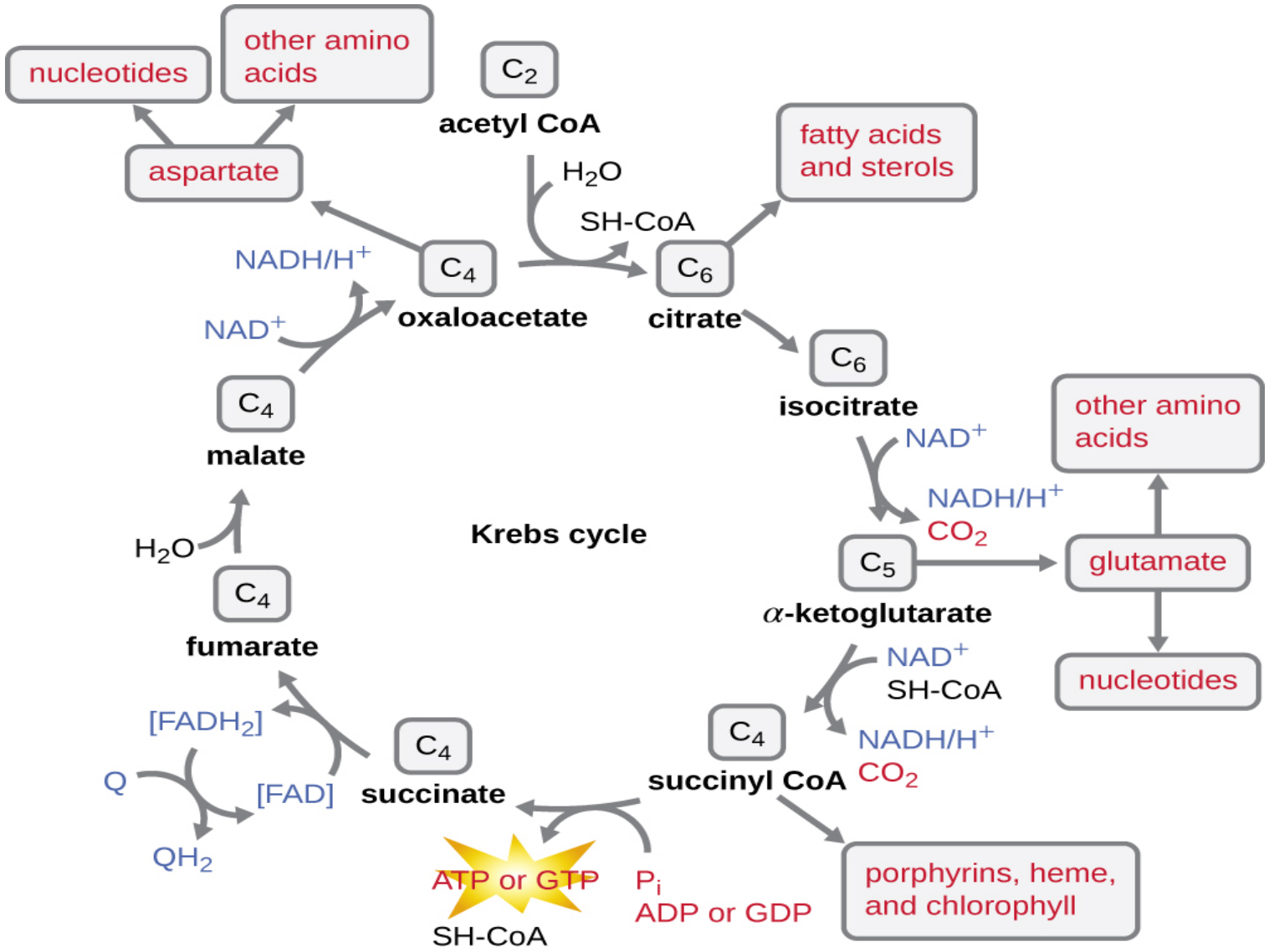
- Although many organisms use the Krebs cycle as described as part of glucose metabolism, several of the intermediate compounds in the Krebs cycle can be used in synthesizing a wide variety of important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides; therefore, the cycle is both anabolic and catabolic.



Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.



Cellular Respiration

- Most ATP, however, is generated during a separate process called oxidative phosphorylation, which occurs during cellular respiration.
- Cellular respiration begins when electrons are transferred from NADH and FADH2—made in glycolysis, the transition reaction, and the Krebs cycle—through a series of chemical reactions to a final inorganic electron acceptor (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration).
- These electron transfers take place on the inner part of the cell membrane of prokaryotic cells or in specialized protein complexes in the inner membrane of the mitochondria of eukaryotic cells.
- The energy of the electrons is harvested to generate an electrochemical gradient across the membrane, which is used to make ATP by oxidative phosphorylation.

Electron Transport System

- The electron transport system (ETS) is the last component involved in the process of cellular respiration
- It comprises a series of membrane-associated protein complexes and associated mobile accessory electron carriers

• Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons from NADH and FADH2 are passed rapidly from one ETS electron carrier to the next.

• These carriers can pass electrons along in the ETS because of their redox potential.

•For a protein or chemical to accept electrons, it must have a more positive redox potential than the electron donor.

• Therefore, electrons move from electron carriers with more negative redox potential to those with more positive redox potential.

•The four major classes of electron carriers involved in both eukaryotic and prokaryotic electron transport systems are the cytochromes, flavoproteins, iron-sulfur proteins, and the quinones.

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- In aerobic respiration, the final electron acceptor (i.e., the one having the most positive redox potential) at the end of the ETS is an oxygen molecule (O_2) that becomes reduced to water (H_2O) by the final ETS carrier.
- This electron carrier, cytochrome oxidase, differs between bacterial types and can be used to differentiate closely related bacteria or diagnoses.
- For example, the gram-negative opportunist Pseudomonas aeruginosa and the gram-negative cholera causing Vibrio cholerae use cytochrome c oxidase, which can be detected by the oxidase test, whereas other gram negative Enterobacteriaceae, like E. coli, are negative for this test because they produce different cytochrome oxidase types.

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- There are many circumstances under which aerobic respiration is not possible, including any one or more of the following:
- The cell lacks genes encoding an appropriate cytochrome oxidase for transferring electrons to oxygen at the end of the electron transport system.
- The cell lacks genes encoding enzymes to minimize the severely damaging effects of dangerous oxygen radicals produced during aerobic respiration, such as hydrogen peroxide (H_2O_2) or superoxide
- The cell lacks a sufficient amount of oxygen to carry out aerobic respiration.
- One possible alternative to aerobic respiration is anaerobic respiration, using an inorganic molecule other than oxygen as a final electron acceptor.
- There are many types of anaerobic respiration found in bacteria and archaea.

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- Denitrifiers are important soil bacteria that use nitrate (NO_3) and nitrite (NO_2) as final electron acceptors, producing nitrogen gas (N_2).
- Many aerobically respiring bacteria, including E. coli, switch to using nitrate as a final electron acceptor and producing nitrite when oxygen levels have been depleted.
- Microbes using anaerobic respiration commonly have an intact Krebs cycle, so these organisms can access the energy of the NADH and $FADH_2$ molecules formed.
- However, anaerobic respirers use altered ETS carriers encoded by their genomes, including distinct complexes for electron transfer to their final electron acceptors.
- Smaller electrochemical gradients are generated from these electron transfer systems, so less ATP is formed through anaerobic respiration.

Chemiosmosis, Proton Motive Force, and Oxidative Phosphorylation

- In each transfer of an electron through the ETS, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions (H+) across a membrane.
- In prokaryotic cells, H+ is pumped to the outside of the cytoplasmic membrane (called the periplasmic space in gram-negative and gram-positive bacteria), and in eukaryotic cells, they are pumped from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space.
- There is an uneven distribution of H+ across the membrane that establishes an electrochemical gradient because H+ ions are positively charged (electrical) and there is a higher concentration (chemical) on one side of the membrane.
- This electrochemical gradient formed by the accumulation of H+ (also known as a proton) on one side of the membrane compared with the other is referred to as the proton motive force (PMF).
- Because the ions involved are H+ a pH gradient is also established, with the side of the membrane having the higher concentration of H+ being more acidic.
- Beyond the use of the PMF to make ATP, the PMF can also be used to drive other energetically unfavorable processes, including nutrient transport and flagella

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- The potential energy of this electrochemical gradient generated by the ETS causes the H+ to diffuse across a membrane (the plasma membrane in prokaryotic cells and the inner membrane in mitochondria in eukaryotic cells).
- This flow of hydrogen ions across the membrane, called chemiosmosis, must occur through a channel in the membrane via a membrane-bound enzyme complex called ATP synthase.
- The tendency for movement in this way is much like water accumulated on one side of a dam, moving through the dam when opened.
- ATP synthase (like a combination of the intake and generator of a hydroelectric dam) is a complex protein that acts as a tiny generator, turning by the force of the H+ diffusing through the enzyme, down their electrochemical gradient from where there are many mutually repelling H+ to where there are fewer H+ .
- In prokaryotic cells, H+ flows from the outside of the cytoplasmic membrane into the cytoplasm, whereas in eukaryotic mitochondria, H+ flows from the intermembrane space to the mitochondrial matrix.

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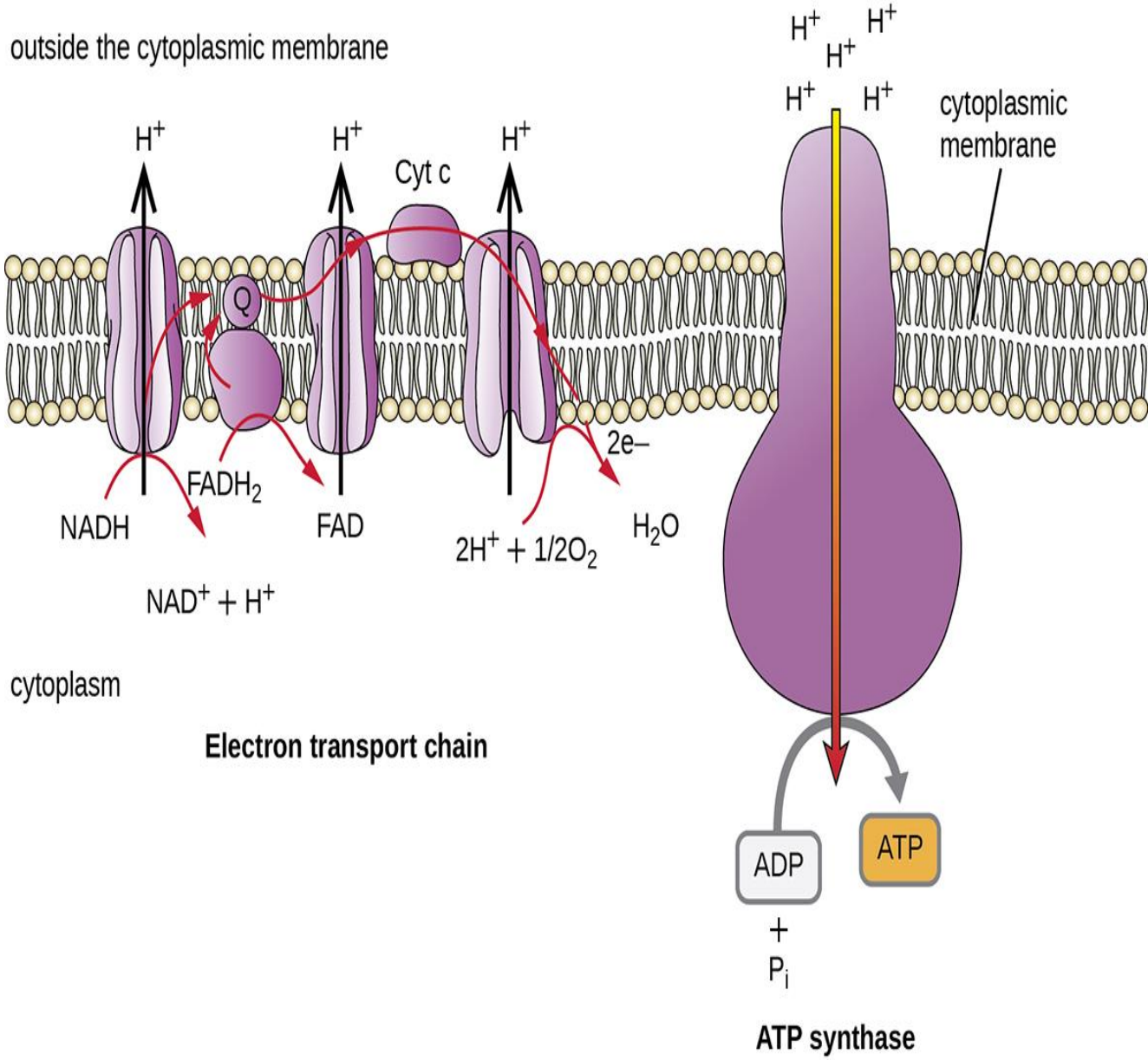
- The turning of the parts of this molecular machine regenerates ATP from ADP and inorganic phosphate (Pi) by oxidative phosphorylation, a second mechanism for making ATP that harvests the potential energy stored within an electrochemical gradient.



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- The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport system complexes can pump through the membrane varies between different speciesof organisms.
- In aerobic respiration in mitochondria, the passage of electrons from one molecule of NADH generates enough proton motive force to make three ATP molecules by oxidative phosphorylation, whereas the passage of electrons from one molecule of FADH2 generates enough proton motive force to make only two ATP molecules.
- Thus, the 10 NADH molecules made per glucose during glycolysis, the transition reaction, and the Krebs cycle carry enough energy to make 30 ATP molecules, whereas the two FADH2 molecules made per glucose during these processesprovide enough energy to make four ATP molecules.
- Overall, the theoretical maximum yield of ATP made during the complete aerobic respiration of glucose is 38 molecules, with four being made by substrate-level phosphorylation and 34 being made by oxidative phosphorylation.
- In reality, the total ATP yield is usually less, ranging from one to 34 ATP molecules, depending on whether the cell is using aerobic respiration or anaerobic respiration; in eukaryotic cells, some energy is expended to transport intermediates from the cytoplasm into the mitochondria, affecting ATP yield.

Source	Carbon Flow	Molecules of Reduced Coenzymes Produced	Net ATP Molecules Made by Substrate-Level Phosphory-lation	Net ATP Molecules Made by Oxidative Phosphory-lation	Theoretical Maximum Yield of ATP Molecules
Glycolysis (EMP)	Glucose (6C) $\longrightarrow$ 2 pyruvates (3C)	2 NADH	2 ATP	6 ATP from 2 NADH	8
Transition reaction	2 pyruvates (3C) $\longrightarrow$ 2 acetyl (2C) + 2 CO_2	2 NADH		6 ATP from 2 NADH	6
Krebs cycle	2 acetyl (2C) $\longrightarrow$ 4 CO_2	6 NADH 2 $FADH_2$	2 ATP	18 ATP from 6 NADH 4 ATP from 2 $FADH_2$	24
Total:	glucose (6C) $\longrightarrow$ 6 CO_2	10 NADH 2 $FADH_2$	4 ATP	34 ATP	38 ATP

Fermentation

- Many cells are unable to carry out respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any appropriate, inorganic, final electron acceptor to carry out cellular respiration.

2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system.

3. The cell lacks genes to make one or more enzymes in the Krebs cycle.

Whereas lack of an appropriate inorganic final electron acceptor is environmentally dependent, the other two conditions are genetically determined.

Thus, many prokaryotes, including members of the clinically important genus Streptococcus, are permanently incapable of respiration, even in the presence of oxygen.

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- Conversely, many prokaryotes are facultative, meaning that, should the environmental conditions change to provide an appropriate inorganic final electron acceptor for respiration, organisms containing all the genes required to do so will switch to cellular respiration for glucose metabolism because respiration allows for much greater ATP production per glucose molecule.
- If respiration does not occur, NADH must be reoxidized to NAD+ for reuse as an electron carrier for glycolysis, the cell's only mechanism for producing any ATP, to continue.
- Some living systems use an organic molecule (commonly pyruvate) as a final electron acceptor through a process called **fermentation.**

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- Fermentation does not involve an electron transport system and does not directly produce any additional ATP beyond that produced during glycolysisby substrate-level phosphorylation.
- Organisms carrying out fermentation, called fermenters, produce a maximum of two ATP molecules per glucose during glycolysis.

Comparison of Respiration Versus Fermentation

Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules
Aerobic respiration	*Pseudomonas aeruginosa*	O_2	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 34
			Total	**38**
Anaerobic respiration	*Paracoccus denitrificans*	NO_3^-, SO_4^{-2}, Fe^{+3}, CO_2, other inorganics	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 1–32
			Total	**5–36**
Fermentation	*Candida albicans*	Organics (usually pyruvate)	EMP glycolysis (SLP) Fermentation	2 0
			Total	**2**

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- Microbial fermentation processes have been manipulated by humans and are used extensively in the production of various foods and other commercial products, including pharmaceuticals.
- Microbial fermentation can also be useful for identifying microbes for diagnostic purposes.
- Fermentation by some bacteria, like those in yogurt and other soured food products, and by animals in muscles during oxygen depletion, is lactic acid fermentation.

The chemical reaction of lactic acid fermentation is as follows:

- Pyruvate + NADH $\leftrightarrow$ lactic acid + NAD+

- Bacteria of several gram-positive genera, including Lactobacillus, Leuconostoc, and Streptococcus, are collectively known as the lactic acid bacteria (LAB), and various strains are important in food production.
- During yogurt andcheese production, the highly acidic environment generated by lactic acid fermentation denatures proteins containedin milk, causing it to solidify.
- When lactic acid is the only fermentation product, the process is said to be homolactic fermentation; such is the case for Lactobacillus delbrueckii and S. thermophiles used in yogurt production.
- However, many bacteria perform heterolactic fermentation, prCO2 as a result, because of their use of the branched pentose phosphate pathway instead of the EMP pathway for glycolysis
- One important heterolactic fermenter is Leuconostoc mesenteroides, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.oducing a mixture of lactic acid, ethanol and/or acetic acid, and CO2 as a result, because of their use of the branched pentose phosphate pathway instead of the EMP pathway for glycolysis.
- One important heterolactic fermenter is Leuconostoc mesenteroides, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.

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- Lactic acid bacteria are also important medically.
- The production of low pH environments within the body inhibits the establishment and growth of pathogens in these areas.
- For example, the vaginal microbiota is composed largely of lactic acid bacteria, but when these bacteria are reduced, yeast can proliferate, causing a yeast infection.
- Additionally, lactic acid bacteria are important in maintaining the health of the gastrointestinal tract and, as such, are the primary component of probiotics.

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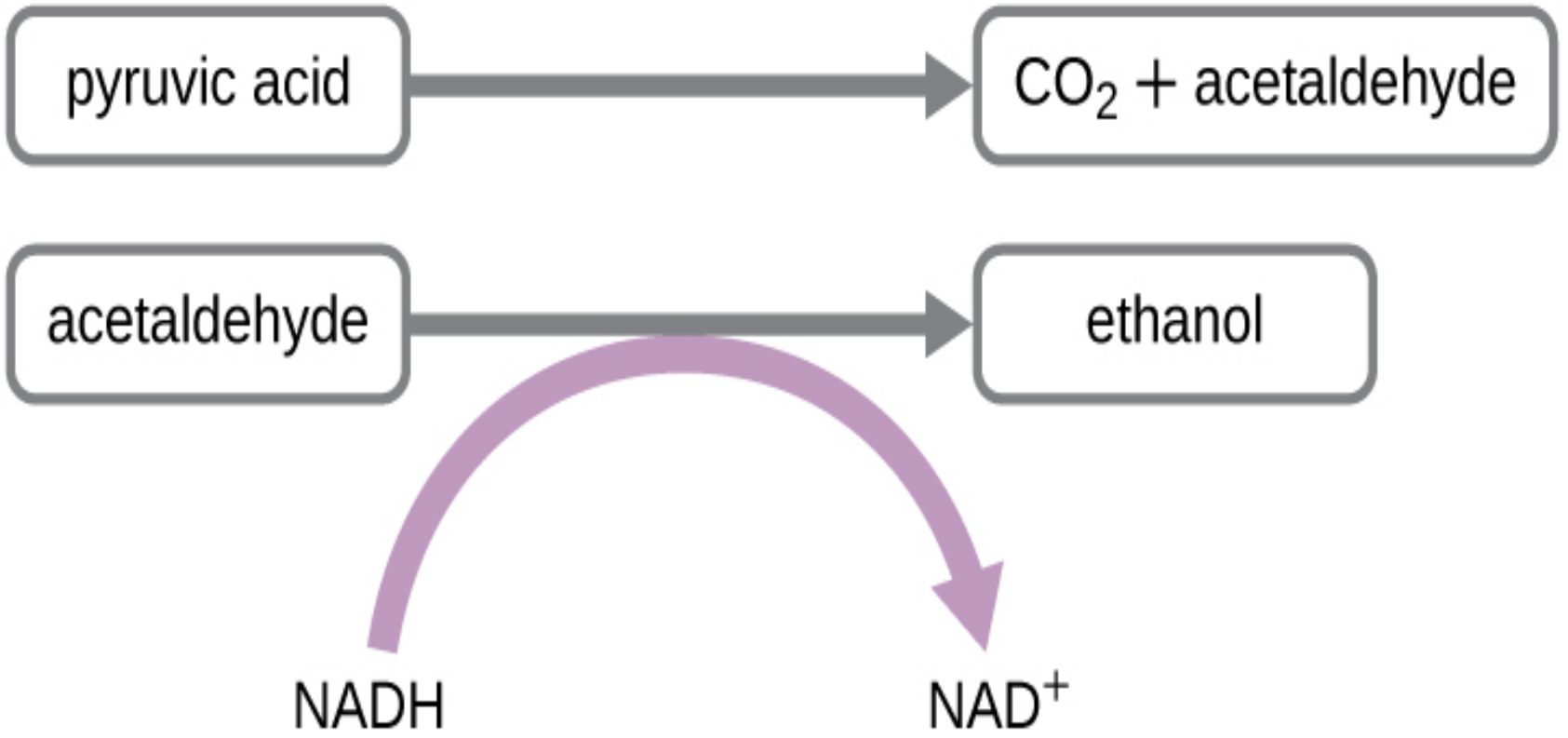
- Another familiar fermentation process is alcohol fermentation, which produces ethanol.
- In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing CO2 gas while producing the two-carbon molecule acetaldehyde.
- The second reaction, catalyzed by the enzyme alcohol dehydrogenase, transfers an electron from NADH to acetaldehyde, producing ethanol and NAD+.
- The ethanol fermentation of pyruvate by the yeast Saccharomyces cerevisiae is used in the production of alcoholic beverages and also makes bread products rise due to CO2 production.
- Outside of the food industry, ethanol fermentation of plant products is important in biofuel production.

The chemical reactions of alcohol fermentation are shown here. Ethanol fermentation is important in the production of alcoholic beverages and bread.



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- Microbes can also be differentiated according to the substrates they can ferment. For example, E. coli can ferment lactose, forming gas, whereas some of its close gram-negative relatives cannot.
- The ability to ferment the sugar alcohol sorbitol is used to identify the pathogenic enterohemorrhagic O157:H7 strain of E. coli because, unlike other E. coli strains, it is unable to ferment sorbitol.
- Last, mannitol fermentation differentiates the mannitol-fermenting Staphylococcus aureus from other non–mannitol-fermenting staphylococci.

Common Fermentation Pathways

Pathway	End Products	Example Microbes	Commercial Products
Acetone-butanol-ethanol	Acetone, butanol, ethanol, CO_2	*Clostridium acetobutylicum*	Commercial solvents, gasoline alternative
Alcohol	Ethanol, CO_2	*Candida*, *Saccharomyces*	Beer, bread
Butanediol	Formic and lactic acid; ethanol; acetoin; 2,3 butanediol; CO_2; hydrogen gas	*Klebsiella*, *Enterobacter*	Chardonnay wine
Butyric acid	Butyric acid, CO_2, hydrogen gas	*Clostridium butyricum*	Butter
Lactic acid	Lactic acid	*Streptococcus*, *Lactobacillus*	Sauerkraut, yogurt, cheese
Mixed acid	Acetic, formic, lactic, and succinic acids; ethanol, CO_2, hydrogen gas	*Escherichia*, *Shigella*	Vinegar, cosmetics, pharmaceuticals
Propionic acid	Acetic acid, propionic acid, CO_2	*Propionibacterium*, *Bifidobacterium*	Swiss cheese

Table 8.3

Lipid Catabolism

- Triglycerides are a form of long-term energy storage in animals. They are made of glycerol and three fatty acids.
- Phospholipids compose the cell and organelle membranes of all organisms except the archaea.
- Phospholipid structure is similar to triglycerides except that one of the fatty acids is replaced by a phosphorylated head group.
- Triglycerides and phospholipids are broken down first by releasing fatty acid chains (and/or the phosphorylated head group, in the case of phospholipids) from the three-carbon glycerol backbone.
- The reactions breaking down triglycerides are catalyzed by lipases and those involving phospholipids are catalyzed by phospholipases.
- These enzymes contribute to the virulence of certain microbes, such as the bacterium Staphylococcus aureus and the fungus Cryptococcus neoformans.
- These microbes use phospholipases to destroy lipids and phospholipids in host cells and then use the catabolic products for energy

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- The resulting products of lipid catabolism, glycerol and fatty acids, can be further degraded.
- Glycerol can be phosphorylated to glycerol-3-phosphate and easily converted to glyceraldehyde 3-phosphate, which continues through glycolysis.
- The released fatty acids are catabolized in a process called β-oxidation, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains, reducing NAD+ and FAD to produce NADH and FADH2, respectively, whose electrons can be used to make ATP by oxidative phosphorylation.
- The acetyl groups produced during β-oxidation are carried by coenzyme A to the Krebs cycle, and their movement through this cycle results in their degradation to CO2, producing ATP by substrate-level phosphorylation and additional NADH and FADH2 molecules.

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- Other types of lipids can also be degraded by certain microbes. For example, the ability of certain pathogens, like Mycobacterium tuberculosis, to degrade cholesterol contributes to their virulence.
- The side chains of cholesterolcan be easily removed enzymatically, but degradation of the remaining fused rings is more problematic.
- The four fused rings are sequentially broken in a multistep process facilitated by specific enzymes, and the resulting products, including pyruvate, can be further catabolized in the Krebs cycle.

Protein Catabolism

- Proteins are degraded through the concerted action of a variety of microbial protease enzymes.
- Extracellular proteases cut proteins internally at specific amino acid sequences, breaking them down into smaller peptides that can then be taken up by cells.
- Some clinically important pathogens can be identified by their ability to produce a specific type of extracellular protease. For example, the production of the extracellular protease gelatinase by members of the genera Proteus and Serratia can be used to distinguish them from other gram-negative enteric bacteria.
- Following inoculation and growth of microbes in gelatin broth, degradation of the gelatin protein due to gelatinase production prevents solidification of gelatin when refrigerated.
- Other pathogens can be distinguished by their ability to degrade casein, the main protein found in milk.

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- When grown on skim milk agar, production of the extracellular protease caseinase causes degradation of casein, which appears as a zone of clearing around the microbial growth.
- Caseinase production by the opportunist pathogen Pseudomonas aeruginosa can be used to distinguish it from other related gram-negative bacteria.
- After extracellular protease degradation and uptake of peptides in the cell, the peptides can then be broken down further into individual amino acids by additional intracellular proteases, and each amino acid can be enzymatically deaminated to remove the amino group.
- The remaining molecules can then enter the transition reaction or the Krebs cycle.