FIRST EDITION

ADVANCED NUTRITION AND REGULATION OF METABOLISM

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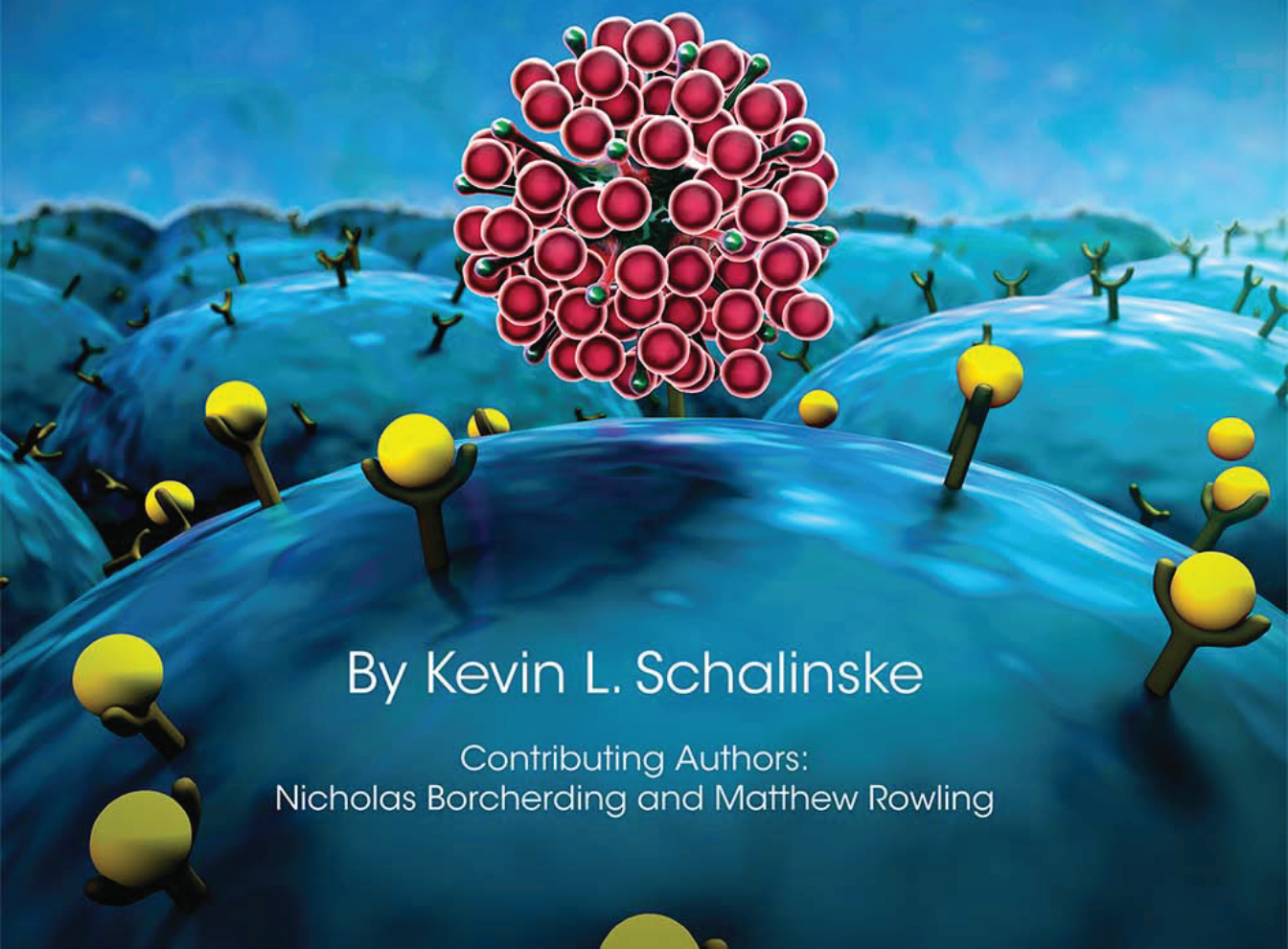

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Advanced Nutrition and Regulation of Metabolism

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CHAPTER 1

Cell Biology and Basic Biological Concepts

Learning Objectives

- Understand the concepts of homeostasis and turnover
- Understand different components of the cell and their function
- Understand the process of gene expression
- Understand how proteins are regulated
- Understand the role of hormones, bond formation, and oxidation-reduction reactions

Learning Outcomes

- Apply what is known about cell structure to how nutrients function
- Understand how protein regulation meets the environmental needs of the cell
- Understand how enzyme kinetics can be utilized to regulate substrate utilization
- Recognize the various levels of gene expression and how it is regulated
- Apply what is known about homeostasis and turnover to health, nutrition, and disease

INTRODUCTION

The body, and metabolism in particular, is a very dynamic process that has to respond appropriately to an ever-changing environment, including the supply of nutrients. The vast majority of cells in the body are quite similar with respect to structure; however, not all cells respond the same to nutrients and hormones or produce the same proteins to carry out specific metabolic processes and other protein-driven functions. But before we can discuss nutrition, the first goal is to have a basic understanding of cell biology, as well as some additional biological concepts.

Activity

Proteins are dynamic and regulated molecules that perform a diverse array of functions. Develop a "real-life" analogy to describe how proteins function and how they are regulated. For example, a transport protein like transferrin, which transports iron in the circulation to cells in the body, is similar to a car using roads to transport people from one place to another.

Enzyme kinetics can be difficult to understand—we will have an in-class activity where you, the student, will function as an enzyme as a method to illustrate the concept of maximal velocity, affinity, and how maximal velocity can be altered.

Reflection/Discussion Questions

Discuss in small groups what happens when a protein is dysfunctional? What could make a protein dysfunctional? Relate this back to the analogy you developed initially. For the car example, if the car cannot accommodate the people, then they cannot get where they need to go; if transferrin cannot bind iron, other cells in the body can become deficient in iron.

HOMEOSTASIS AND TURNOVER

Nutrition and the regulation of metabolism are key components to optimize health and prevent disease. This requires the adequate supply of essential nutrients, regulation of specific hormones, and appropriate regulation of gene expression in response to changing environmental conditions. For adults, the goal is **homeostasis**—being in **balance** with respect to meeting the energy and biosynthetic needs of cells and tissues.

Homeostasis is not a static process but is very dynamic—all components of the body turn over and must be replaced such that balance is maintained, except in the situation of a growing organism. Everything in the body experiences **turnover**, from whole cells to mRNA and proteins. Some body components have a very short "life span," and for others it is much longer. For example, mucosal cells that line the gastrointestinal tract have a **life span** of 3 to 5 days, whereas for red blood cells it is 120 days. Quantitatively, the rate of turnover can be referred to as the **half-life**; that is, the time it takes for one-half of the abundance of a specific substance or organelle to be degraded or lost. In either case, at some point all cells and their contents must be replaced with new cells, of which some material is recycled; other components must come from the diet, along with the necessary cofactors. All proteins are synthesized according to a given gene sequence that provides the recipe to make a given protein. Some proteins are made based on need, and the blueprint to make them, DNA, must be regulated. Like entire cells, these proteins, along with the mRNA they are translated from, have a finite life span, after which they are degraded. This ability to turn on the synthesis of specific proteins is based on changes in our environment, particularly our intake of essential nutrients. Throughout this book, we will focus on **adaptive** genes and their protein products, meaning these are the genes that can be turned on in response to a particular need to result in increased abundance of the corresponding protein. It is also important to recognize that there are other genes and their respective proteins that are **constitutive**, meaning they are produced at a fairly constant rate regardless of environmental changes. The DNA that codes for these proteins is referred to as "**housekeeping genes**." Finally, after we synthesize a protein, there are typically a number of modifications that need to be made before it is fully functional. We can refer to an inactive protein as an **apoprotein**, whereas once it is fully functional, it is considered a **holoprotein**. For example, the production of hemoglobin requires the synthesis of four globin subunits, followed by the insertion of heme, which consists of protoporphyrin IX and iron.

CELL COMPONENTS AND BIOLOGY

The human body contains many structures, tissues, and fluids—but everything is composed of specific cell types. Related to the concept of homeostasis and turnover, it should be kept in mind that to achieve turnover, cells must undergo **proliferation**—that is, divide to produce new cells. New cells must then undergo **differentiation** to achieve full maturity and carry out their specific functions. After a specified life span, be it 3 to 5 days for enterocytes or 120 days for red blood cells, cells must undergo **apoptosis**, which is defined as **programmed cell death**. Following apoptosis, the degraded older cells are replaced by the production of new cells. This process of proliferation, differentiation, and apoptosis can be viewed as one version of the **cell cycle**—having a normal cell cycle is essential for health, and when it is disrupted

or not properly regulated, it can contribute to disease. Carcinogenesis—that is, the development of cancer—is an example where cells continue to proliferate, are poorly differentiated, and fail to undergo apoptosis. One of the roles of nutrition in carcinogenesis is readily explored by determining if a nutrient, a derivative of a nutrient, or any other dietary bioactive compound has the ability to inhibit proliferation and promote differentiation and apoptosis. Compounds that can accomplish this are considered to be potential anticarcinogenic agents.

It is important to understand the basic components and general functions that reflect nearly all cells in the body. The primary cellular structures to discuss are the (1) **plasma membrane**, (2) **cytosol**, (3) **mitochondria**, (4) **nucleus**, and (5) **rough endoplasmic reticulum**. There are certainly many other important structures that are important in cell biology; however, our discussion will be focused on these five.

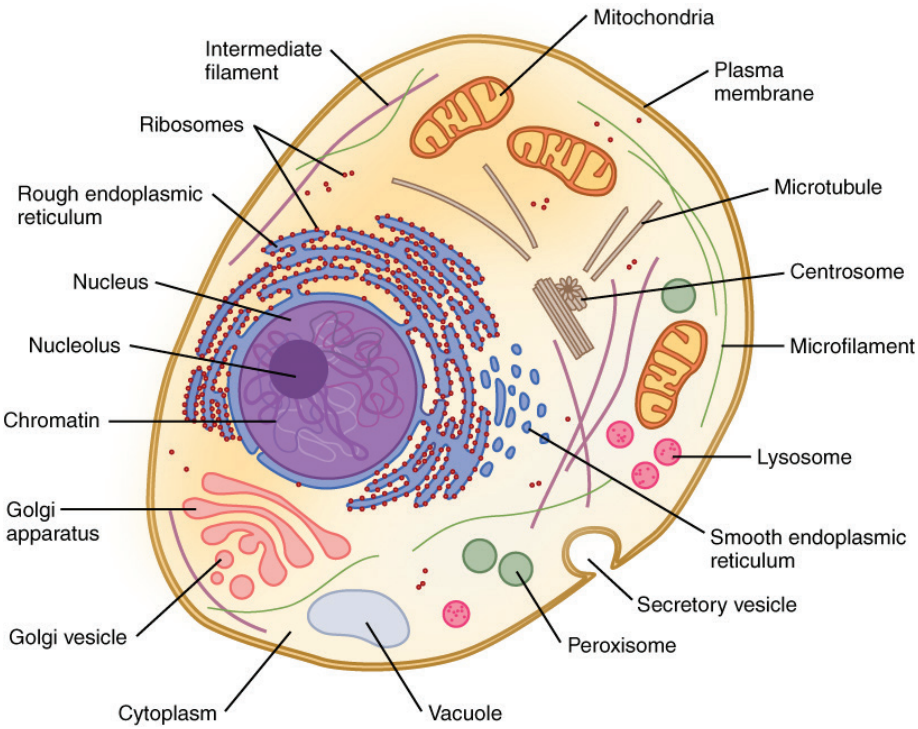

FIGURE 1-1 Cell Structure

Plasma Membrane

The plasma membrane represents a barrier between the inner contents of the cell and the plasma. It is important to keep in mind that essentially all cells are "bathed" by the circulatory

system—this is critical to provide the essential nutrients, hormones, and other regulatory compounds that function to control cell health, and ultimately the heath of the entire body. The plasma membrane is composed of a **phospholipid bilayer**—this important characteristic has a significant impact on the ability of compounds, including nutrients, to enter the cell. The structure of the phospholipids that are organized into this bilayer consists of a glycerol (three-carbon molecule) backbone, two fatty acids attached to two of the carbons, and a polar head group attached to the third carbon atom. Related to the structure of a phospholipid, an important concept in nutrition that will have vast implications throughout this book is solubility—that is, whether a compound or parts of a compound are **water soluble** versus **fat soluble**. Many nutrients, particularly vitamins, can be classified as being either water soluble or fat soluble, and this characteristic has significant implications for digestion, absorption, transport, toxicity, and even daily requirements. There are other terms related to this concept of water versus fat soluble that we can introduce. For water soluble, this is the same as stating something is **hydrophilic**, **aqueous**, or **polar**. For fat or lipid soluble, this is the same as stating something is **hydrophobic**, **nonaqueous**, or **nonpolar**. In referring back to our discussion of the phospholipid bilayer, barrier is important in providing a cell definition, but even more so to control the transport of molecules across the membrane, such as nutrients, and also provide a point for other molecules to bind and transmit signals to the rest of the cell. The polar component of the phospholipids are orientated toward the plasma and interior of the cell, whereas the nonpolar, hydrophobic fatty acid tails face each other, thereby creating a lipid barrier that must be navigated for a substance to enter the cell. The plasma membrane also contains specific transport proteins, which are also referred to as **integral** proteins since they span the cellular membrane bilayer, as well as proteins that reside on the plasma membrane surface, referred to as **peripheral** proteins. Because the plasma membrane is a lipid environment, many molecules require a transport protein to enter the cell. This includes nutrients that are water soluble, such as glucose, amino acids, and water-soluble vitamins. This is an essential requirement, because a compound that is water soluble cannot cross a lipid bilayer without the assistance of a protein transporter. Peripheral proteins, such as receptors, represent a "docking station" for substances to bind to and subsequently elicit a response or initiate a signal to the cell, a process called **signal transduction**. An example of this type of interaction that we will discuss at length is the binding of the hormone insulin to the insulin receptor on the plasma membrane of a cell, thereby promoting specific cellular processes, such as the increased activity and/or abundance of key metabolic enzymes, or increased glucose uptake from the plasma. Peripheral receptors also represent a means by which a compound, following binding to its receptor, may be engulfed by the cell. Transport mechanisms will be discussed at more length in chapter 2. There are other membranes that we will consider in addition to the plasma membrane, such as the mitochondrial membrane—in all cases the membrane serves as a control point to separate different processes and to regulate what occurs on either side of a given membrane. It also means that mechanisms must exist to move important compounds across the membrane when they are needed.

Cytoplasm

The interior of a cell is referred to as the cytosol or **cytoplasm**. Many processes occur exclusively in the cytosol (e.g., glycolysis), and consequently they must be linked to subsequent processes in other places within the cell, such as the mitochondria (e.g., TCA cycle). The cytosol is not simply a "swimming pool" where the interior components of the cell are freely "floating around." Many components within the cytosol are "held in place" by the microtrabecular matrix, essentially a lattice-like framework of cytoskeletal components that ensures organelles and related processes are in close proximity to each other to make metabolism an efficient process. It is important to note that although the cytosolic structure provides a framework for where things are located, the ability to move things within cells through **trafficking** is also very important. Quite often various intracellular molecules are made or originate in one location but must be trafficked to other locations to exert their function.

Mitochondria

The mitochondrion is an organelle within the cell that serves as the primary location for the production of energy, which in biological terms is the production of **adenosine triphosphate**, or **ATP**. ATP is the "currency" within the body to fuel processes, such as transport across membranes, movement, metabolism, and many essential functions that otherwise would not occur. The primary basis for the consumption of carbohydrates is to serve as a substrate for the production of ATP. Although some ATP is generated by the oxidation of carbohydrates in the cytosol via glycolysis, the vast majority of ATP is subsequently produced within the mitochondria through the action of the **tricarboxylic acid (TCA)** cycle, also known as the **citric acid cy**cle and the **Krebs cycle**. Glycolysis is an anaerobic process, whereas the TCA cycle is dependent on oxygen to produce ATP (i.e., aerobic), which also results in the production of metabolic water. In addition to carbohydrates, fatty acids can also serve as a substrate within the mitochondria for the generation of ATP via the TCA cycle and a process called **β-oxidation**. A topic that will be discussed in chapter 6 is the mitochondrial density of a cell, and in chapter 8 the consequences of inefficient mitochondria that produce harmful by-products, particularly as we age.

Nucleus

The nucleus in the cell is where **deoxyribonucleic acid (DNA)** resides—DNA is essentially the "recipe book" for how we make a given protein—and DNA is what needs to be copied or **replicated** when a cell divides to create new cells. DNA is composed of four bases—adenine,

guanine, cytosine, and thymine (A, G, C, T)—and it is these four bases that serve as a "code" for instructing the cell what amino acids need to be put together in the proposer sequence to create a functional protein. That is why when there is a **mutation** in the DNA sequence—whether it is something that is inherited or something that arises from an environmental exposure—it can result in a "mistake" in the code or recipe that leads to the wrong amino acid being used in the synthesis of a given protein. Sometimes this results in a protein that doesn't work at all, and other times it is a protein that is functional but not to the same degree as a normal protein, and sometimes it has no impact on the function of a protein at all. Our discussion will go much deeper into the process of DNA leading to synthesis of proteins—what we will refer to as **gene expression**—later in this chapter. Gene expression will be a principle framework of discussion throughout this textbook as we talk about nutrition, because nutrition and consuming nutrients can have a major impact on gene expression by ensuring that we have the proper proteins being synthesized at the proper time, thus allowing us to adapt to the many environmental changes (e.g., being in a fed state versus a fasted state) that occur throughout the life span.

Rough Endoplasmic Reticulum

The rough endoplasmic reticulum (RER) is the site within the cytosol where the actual synthesis of proteins occurs. **Ribosomes** or **polysomes**, strings of ribosomes actively translating a single mRNA, exist on the RER—and hence the basis for the term *rough*—with the primary function of putting together the correct amino acids in the right sequence, based ultimately on the DNA sequence, to create a peptide or protein. As stated earlier, the protein that is produced often will undergo further modifications to eventually result in a completely functional protein. Some of these modifications will occur in the **Golgi apparatus**, where proteins are subsequently packaged into vesicles to be transported to where they are needed in the cell or for secretion into the bloodstream.

GENE EXPRESSION

A fundamental requirement of humans is to have the ability to produce the appropriate proteins, in response to specific environmental factors or other stimuli, that are required for cell function and overall health. For example, when we are in the fed state, we need to ensure that we have adequate levels of specific enzymatic proteins to metabolize our food by the process of glycolysis and the TCA cycle so that we can produce ATP. Moreover, these enzymatic proteins need to be fully active and not in an inactive form. For some proteins, this also means that specific signals that arise in the fed state need to communicate with the DNA to increase the synthesis and ultimately the abundance of these proteins. It is important to recognize that

all cells contain the same DNA, but not all of the DNA is expressed in every cell type. A great example of this is the protein hormone insulin—the DNA sequence is present in all cells, but only specific singular cells in the pancreas have the ability to actually express the DNA sequence to result in the production of insulin; thus, gene expression is very tissue- and cell type–specific. For our discussion, the term *gene expression* refers to the entire process, from DNA sequence to the production of mRNA to the synthesis of new proteins. Any signal that can stimulate this process at any point will be referred to as an increase in gene expression.

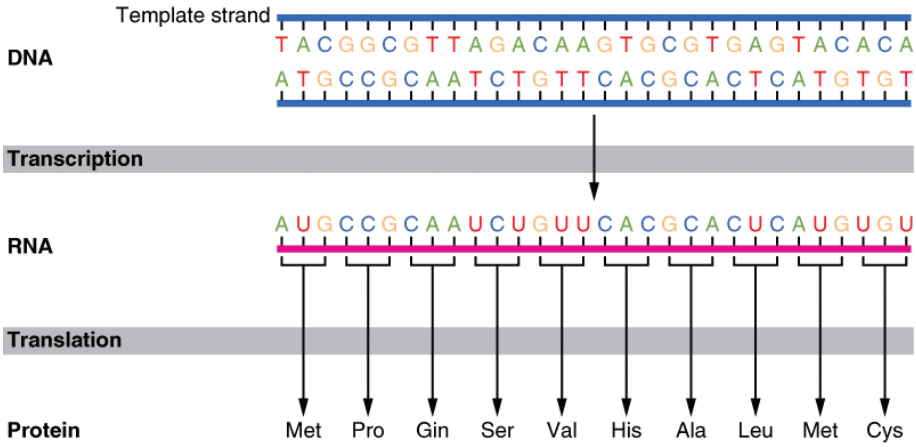

FIGURE 1-2 Gene Expression

There are a number of stages to gene expression that need to be discussed, including (**1**) **transcription**, (**2**) **translation**, and (**3**) **posttranslational modification**. As stated earlier, DNA consists of four bases—**adenine**, **guanine**, **cytosine**, **thymine** (A, G, C, T)—linked sequentially and arranged in a double-helix formation, meaning that there are two strands of base sequences that are intertwined around each other. This double-helix formation of DNA is held together by the hydrogen bonding that occurs between the bases A and T (two hydrogen bonds) and the bases G and C (three hydrogen bonds). The process of transcription occurs in the nucleus and involves specific enzymes reading or transcribing the base sequence of one DNA strand to create a "copy" that is called **messenger ribonucleic acid (mRNA)** molecule. Although mRNA is essentially a copy of a specific DNA sequence, there are two key differences: (1) mRNA is single stranded, and (2) the thymine bases in DNA are replaced by a fifth base, **uracil**. This will become important when we discuss the B vitamins involved in the conversion of uracil to thymine. Thus, mRNA is a copy of a specific sequence of DNA, with the differences noted above, that can leave the nucleus and travel to the RER to serve as a template in the production of a specific protein. As its name "messenger" implies,

mRNA is carrying the recipe, originally from the gene, to the ribosomes to make a particular protein in response to a given signal, such as a nutrient or a hormone.

Following the translocation of nuclear mRNA to the cytosol, the base sequence of the mRNA is read by the polysomes and serves as the instruction for the construction of a specific protein by putting the correct amino acids together in the proper order. The mRNA is simply the instructions, or codes, for the polysomes to produce a protein. The polysomes read the mRNA three bases at a time, and each sequence of three bases, referred to as a **codon**, corresponds to a specific amino acid that will be used to build the correct amino acid sequence. Amino acids are delivered to the polysome-mRNA complex by **transfer RNA (tRNA)**. The process of transcription and translation is why a single change in a DNA base results in the wrong base in the mRNA, thereby changing the codon, and ultimately the wrong amino acid is inserted into the amino acid sequence, potentially resulting in a protein that may not function properly. RNA also can undergo **splicing** prior to being utilized for ribosomal translation. This process removes specific regions of the RNA, thus resulting in specific differences in the amino acid sequence that is produced and the resultant protein. RNA splicing is a mechanism by which a single mRNA, following **differential splicing**, can result in different proteins being produced. This process can be thought of similar to translating a sentence in a book from one language to another—if the entire sentence is translated as such, the result is one specific sentence, but if pieces of the sentence (i.e., words) are

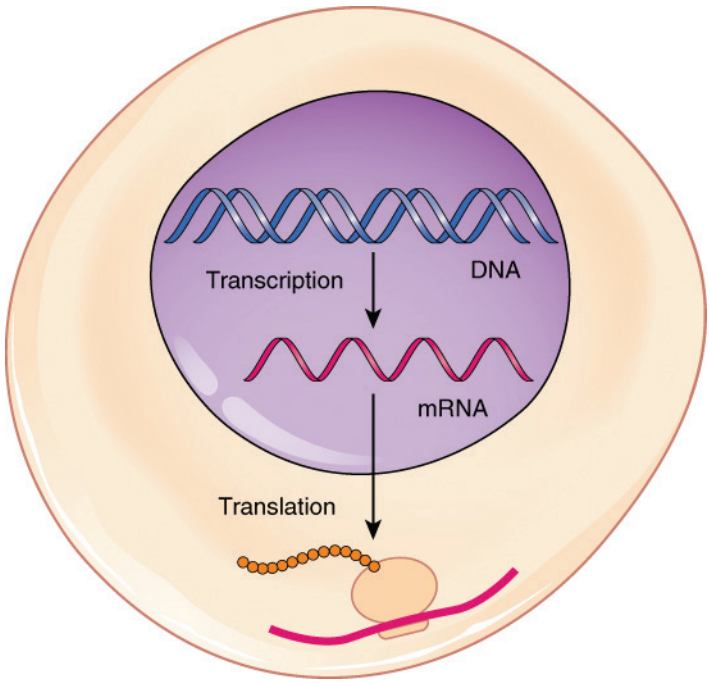

FIGURE 1-3 Transcription and Translation

removed, the result can be a very different in its meaning. Moreover, different tissues may undergo different mRNA splicing, resulting in different proteins being expressed from the same gene in a process that is tissue specific or cell type specific. Many proteins have isomeric forms. **Isomers** are proteins that carry out the same function but have different characteristics, such as substrate affinity if it is an enzyme, or differ in how they are allosterically regulated or how they respond to a hormone. Isomers can each arise from their own distinct genes, or they can be the product of one gene, owing to differential splicing to create different protein products. We will discuss examples of these two scenarios in chapter 3.

Once a specific protein or polypeptide is synthesized, typically there are a number of alterations that are made to produce the final functional protein. Because this occurs after the process of translation, this is referred to as **covalent** or posttranslational modification. Posttranslational modification can occur as a normal process of producing a given protein, or it can be stimulated to alter a protein in response to a specific signal as a means to make it active or inactive. The key for discussing posttranslational modification is that the substrate is a protein and the modification involves the production or removal of specific covalent bonds. It should be noted that this is often a reversible process—for example, a protein can be phosphorylated (i.e., adding a phosphate group) to make it active, and it can be dephosphorylated to make it inactive. The process of adding or removal covalent bonds typically requires an enzyme to carry out the process. In addition to posttranslational modification, we will discuss proteins that require a cofactor to be inserted into their structure for them to be functional or need multiple subunits to combine to form the final protein. A great singular example of this is the oxygen transporter in the bloodstream, hemoglobin. The final hemoglobin molecule consists of four globulin subunits that are brought together, where each globulin subunit has a heme molecule inserted into its structure. That is also why each molecule of hemoglobin can bind to and carry four molecules of oxygen within a red blood cell.

In summary, stimulation of gene expression or the "turning on" of a gene results in the production of a protein and an increase in its abundance. The specific proteins we produce are in response to our physiological state and the environment. It is important to recognize that only 1 percent to 2 percent of the total DNA within a cell codes for protein—the other regions can be regulatory, such as promoters, can result in noncoding RNAs, or are simply regions where the function is not well understood. We will discuss many examples of these possibilities throughout the remainder of this book.

NUTRIENTS AND GENE EXPRESSION

Now that we have a basic understanding of gene expression and why it is important, we must consider the role of nutrients in this process. Nutrients can directly and indirectly alter gene expression, and this can be accomplished at both the transcriptional and translational level.

An example of directly altering gene expression is the action of all-trans-retinoic acid, one of the most active forms of vitamin A. All-trans-retinoic acid can enter a cell and traffic to the nucleus, where it binds to specific transcriptional proteins to ultimately turn on a gene to increase the abundance of the protein that is coded for by the gene. An example of indirect regulation would be carbohydrates, such as glucose. High concentrations of glucose in the circulation, such as in a fed state, results in the release of the hormone insulin by the pancreas. Insulin is the signal that can bind to the insulin receptor on the plasma membrane of a specific cell and via signal transduction turn on specific genes, also resulting in the increased abundance of a particular protein. Lastly, a nutrient such as iron directly alters the synthesis and abundance of specific proteins, but rather than turning on a gene at the transcriptional level, it alters the translation of existing mRNA. Nutrients can also play a role in the posttranslational modification of a protein to make it active or inactive, but we will not consider that part of the gene expression process—posttranslational modification occurs after the protein is made and is often carried out on existing proteins in response to a signal, rather than newly synthesized proteins.

REGULATION OF PROTEIN FUNCTION

Proteins are arguably the most versatile and dynamic type of molecule in the body. Proteins function as enzymes, plasma membrane transporters, circulatory transporters, antibodies, molecules important in water and acid-base balance, hormones, and transcription factors, and of course, they serve a structural role. Thus, the ability to turn the production of proteins on and off, at the appropriate time, is critical—and nutrients, as well as the physiologic state of the body, play a key role in that process. Initially, we will consider three mechanisms by which proteins and their function are regulated. The three mechanisms are (**1**) **induction**, (**2**) **posttranslational modification**, and (**3**) **allosteric** regulation. Both nutrients and hormones can utilize induction and posttranslational modification to regulate protein function. Allosteric regulation only applies to proteins that are enzymes, whereas the other two mechanisms can function in the regulation of a variety of proteins beyond just enzymes, such as transport proteins. A key feature of **induction** is an increase in the **abundance** of a particular protein, thereby resulting in a higher capacity to carry out the function of a particular protein. Typically, this is achieved via stimulation of transcription for that protein, but as stated earlier, it can also be the result of increased translation of existing mRNA. Thus, a better definition for induction is *an increase in the abundance of a protein via increasing some aspect of gene expression, which includes both transcription and translation.*

As discussed earlier, **posttranslational modification**, or **PTM**, involves the "making or breaking" of covalent bonds—it does *not* involve a change in the abundance of a protein. By producing or removing a covalent bond, PTM can either activate or inactivate the function of

a protein, or "tag it" to be degraded. Types of PTM include phosphorylation, carboxylation, glycosylation, methylation, acetylation, and hydroxylation, to name just a few. As mentioned earlier, all of these processes are reversible and require an enzyme to carry out the modification. For example, we have a specific enzyme that can phosphorylate a specific protein, as well as a specific enzyme that can dephosphorylate the same protein. Thus, having the appropriate signals to stimulate or inhibit the enzymes that are involved in PTM is a highly significant point of regulation. PTM can also be achieved by the simple cleavage of a peptide bond, thereby removing a particular amino acid sequence, to turn an inactive protein into an active protein—we will see this when we discuss digestive enzymes and hormones, like insulin. In contrast to induction, PTM is a rapid process to activate or inactivate proteins as a means to ensure we have a proper and timely response to a given signal. For example, if we consume food, we need the already present enzymes to digest our meal to be rapidly activated via PTM, rather than being dependent on stimulating gene expression to synthesize the required digestive enzymes.

Allosteric regulation involves the ability to stimulate or inhibit enzymes—like PTM, a rapid and important process in the regulation of metabolism. The term *allosteric* translates as "other site," meaning a site on the enzyme that is not the active site where an enzyme carries out catalysis. Enzymes are proteins that function to catalyze a given chemical reaction—that is, converting a substrate into a product, a reaction that would be highly unfavorable without the enzyme. Enzymes have a specific protein conformation that allows the substrate to bind to the active site, and then the product is released. In viewing a metabolic pathway, keep in mind that typically every step requires an enzyme to carry out the conversion of substrate to an intermediate and ultimately to a product. For allosteric regulation, other molecules—such as the substrate, intermediates, or products in a given pathway—can bind to the allosteric or "other site," resulting in a conformational change in the protein that can result in it being more active or inhibited. Like PTM, allosteric regulation does *not* involve any change in the abundance of the enzyme, but rather regulating the activity of existing protein. Often, substrates in a pathway function to stimulate key enzymes downstream—essentially turn on an enzyme when there is an abundance of substrate. Conversely, products often inhibit an enzyme—**feedback inhibition** is a common mechanism to reduce the activity of an enzyme when enough product has been made. It is also important to recognize that a given protein can be regulated by all three of these mechanisms: induction, PTM, and if it is an enzyme, allosterically. We will discuss many examples of all three mechanisms throughout the subsequent chapters in this book. There will also be some discussion of additional mechanisms that can result in a protein being "active," or having "increased abundance," that do not fit into these three categories. For example, we can increase the "abundance" of a plasma membrane transport protein, not by increasing its synthesis, but simply by moving existing protein to the proper location on the membrane—this is called **translocation**. In essence, the transport protein's abundance, relative to where it is functional (i.e., on the membrane) has increased, even though synthesizing more of the protein was not part of the

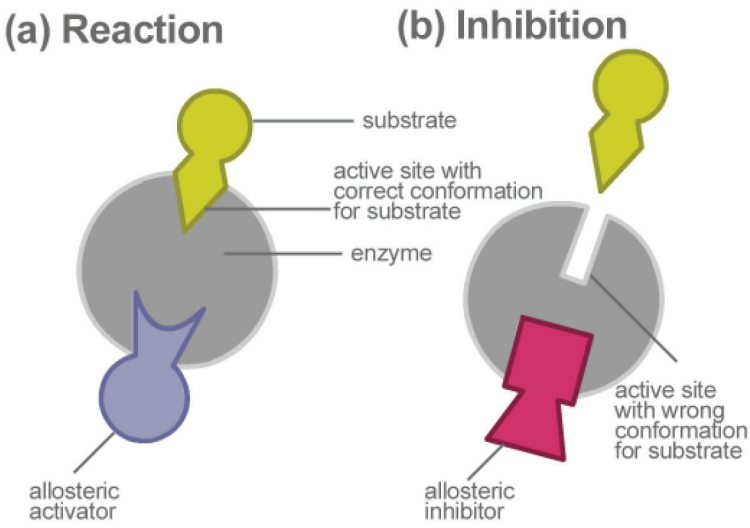

FIGURE 1-4 Allosteric Regulation of Proteins

process. A membrane transporter that is in the cytosol and not on the membrane can be considered nonfunctional—it cannot do its job if it is not in the right location. Increasing abundance by induction assumes the resultant protein is in the proper location to carry out its function; if this is not the case, then nothing has been achieved.

ENZYME KINETICS

Related to allosteric regulation, it is important to understand enzyme kinetics, because this is another point of metabolic regulation within a cell. All proteins that function as enzymes have specific characteristics, namely how efficiently they can convert a substrate into a product. Two important metrics to describe an enzyme's ability to convert a substrate into a product are its **maximum velocity** ($\mathbf{V_{max}}$) and its **Michaelis-Menten constant** ($\mathbf{K_m}$). As substrate concentrations increase, there is a relatively linear increase in velocity rate with respect to the enzyme converting the substrate into product per unit time. However, at some point with increasing substrate concentration, the enzyme will be functioning at its maximal velocity—it simply cannot operate faster, regardless of how much the substrate concentration increases. It is this rate that is referred to as V_{max}. A good analogy would be if a bunch of people were in a room and their role was to pick up a piece of candy off of the floor and place it in a bowl. The candy moving to the bowl is not going to happen without the participation of the people, or enzymes. When there are only a few pieces of candy scattered about the room (i.e., low

substrate concentration), it requires some time to locate the piece of candy and place it where it needs to go. As more and more candy is added to the room, the "enzyme" becomes more efficient at finding a piece of candy and doing its job, because it is easier to locate a piece of candy. But if we fast-forward to the point where the room is two feet deep in candy, the only limitation to the rate or speed of placing candy in the bowel is the actual process of picking it up and placing it in the bowl—there is no lag time required to accomplish this, because the substrate concentration is so high—and that is V_{max}.

The second component related to V_{max} is K_m—if we know the maximal velocity and then determine half of the maximal velocity, we can find the substrate concentration wherein the enzyme is functioning at one-half of its maximum velocity, and that substrate concentration is referred to as the K_m. The reason it is useful to know the K_m of an enzyme is that it is a measure of an enzyme's **affinity** for a substrate. A low K_m indicates high affinity—the enzyme does a great job of converting a low amount of substrate concentration into product. An enzyme with a high K_m means it will take a greater substrate concentration for the enzyme to reach maximal velocity; thus, it has a lower affinity. The best way to think of this is to picture two enzymes competing for the same substrate, and there are many biological examples of this that we will discuss. If a given substrate could be metabolized down to different pathways, more of it will go down the pathway that is regulated by the enzyme with the low K_m and high affinity when substrate concentrations are low. But as the substrate concentration increases, the low K_m enzyme will reach V_{max} and allow the enzyme with the higher K_m to metabolize more of the substrate. The substrate is still being metabolized by the first enzyme. But because the substrate concentration is high, more of it will be metabolized by the enzyme with lower affinity. It is important to recognize that this entire discussion is also true for transport proteins and the rate at which they transport something across a plasma membrane. We will use transport proteins in our discussion below on how to alter the V_{max} of a protein, thereby increasing how much a substance is transported across a membrane as a function of time.

A significant question is whether we can change the V_{max} of a protein, be it an enzyme or a transporter. If we can increase the V_{max} of an enzyme, we can instruct more substrate to be metabolized down a given pathway, or we can transport more of a given substance across a membrane as a function of time. *How can we increase Vmax?* There are two ways that we can increase V_{max}, and both are based on the concept of increasing the "functional abundance" of the enzyme or transport protein. First, if we induce a gene and increase the abundance of an enzyme or transport protein, the ability to convert more substrate per unit time into product or move a compound across a membrane per unit time will increase V_{max}. A good analogy relative to a membrane transport protein are "turnstiles" to enter a football game—when the substrate concentration is low (i.e., few people entering the stadium), the amount of people coming into the stadium per unit time is low. As the substrate concentration increases, the rate will also increase—but when there are thousands of people *waiting* to get in, you reach maximal velocity, because only one person can enter the stadium at a

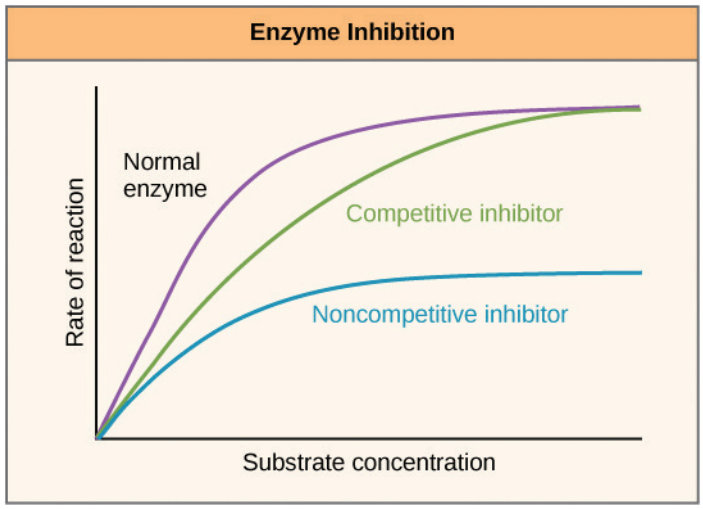

FIGURE 1-5 Enzymatic Kinetics

time. So how do we increase that rate? Simply have more turnstiles available—with respect to the entire stadium, more total people can enter per unit time if there are more turnstiles for them to use. An excellent example of this that we will discuss often is how the active form of vitamin D increases calcium absorption in the small intestine. When blood calcium levels are low, the active form of vitamin D travels via the bloodstream to enterocytes, diffuses across the plasma membrane, enters the nucleus, and induces the expression of the protein that transports calcium from the gastrointestinal lumen to inside the enterocyte—more transporters on the enterocyte membrane facing the lumen results in more "turnstiles" for calcium to enter the cell over time.

Besides synthesis of more transporters via induction, we could also use translocation to get more transporters in the right location without having to synthesize more protein. A good example of this that we will discuss in chapter 3 is with respect to glucose transporters. When blood glucose levels are high, the pancreas releases insulin, which can bind to its receptor on the plasma membrane of certain cells and send a signal to the glucose transporters that are "resting" in the cytosol to translocate to the membrane and increase glucose uptake. For either example, whether it is induction or translocation, the abundance of the transporter on the plasma membrane has increased; thus, V_{max} has been increased. For enzymes, this can also be accomplished by increasing the abundance of an enzyme via induction, or by the PTM of existing enzymes to convert them from an inactive state to an active state. From a functional point of view, this is equivalent to increasing the abundance via increased gene expression—PTM simply "turned on" existing enzymes, which is the equivalent of increasing the abundance of the active enzymatic proteins. In the end, one has more **functional protein** to carry out the role of that particular protein—for enzymes, this translates into

more substrate going down a given pathway, or for transporters, more of a given compound entering a cell. Therefore, it should be clear that *regulating* this process of increasing the V_{max} of a particular protein is critical in both metabolism and transport.

OTHER BIOLOGICAL CONCEPTS

Hormones

A simple definition of a hormone is a compound that is synthesized at one site, travels through the circulation, and exerts its action elsewhere. That said, a number of hormones are made and function in the same tissue or location. Hormones are powerful compounds that can influence many biological processes in the body, particularly metabolism. Structurally, there are two types of hormones—protein or polypeptide hormones, and steroid hormones. Our focus will be on the protein hormones that are critical for nutrition and metabolism, namely insulin and glucagon. Insulin and glucagon are considered polypeptide hormones since their entire structure consists of less than one hundred amino acids—the term *protein* is reserved for those structures that exceed one hundred amino acids. As will be evident in the next chapter, the response to pancreatic insulin and glucagon is essential in how our body responds to the consumption of food or when there is a lack of food over time.

Types of Bonds and Reactions

There are a number of basic biological concepts that are critical in understanding advanced nutrition and metabolism. The first is the type of bonds that can be formed within or between molecules. Bond formation is essential for the proper function of molecules, whether they be proteins, nucleic acids, triglycerides, or saccharides. We will see that often bonds have to be broken and made in order for proper nutrition and metabolism to occur. Covalent bonds are the most common bonds between all the elements of a molecule. This includes the bonds to form glucose from six carbon, twelve hydrogen, and six oxygen molecules, or the peptide bond between the amino acids that constitute a protein. They are also the bonds involved in the PTM of a protein to make it active or inactive, or the bonds that link a fatty acid to glycerol to form triglycerides and phospholipids. The making or breaking of covalent bonds is not easy and often requires an enzyme to carry out the process. Ionic bonds are the loose associations between elements, such as sodium (Na^+) and chloride (Cl^-) to form NaCl (i.e., salt), or iron (Fe^{2+}) and sulfate ($SO2^{2-}$) to form ferrous sulfate, a commonly prescribed supplement to treat anemia. As discussed earlier in this chapter, nucleic acids can also form

hydrogen bonds between the different bases—for DNA, this is the pairing of A with T, and G with C—resulting in the double-helix formation. It can also occur in mRNA and lead to secondary structure that is important in the regulation of mRNA to be translated. Different types of bond formations are also highly critical in the structure and function of proteins and will be discussed in more detail in chapter 5.

There are many types of reactions that occur in the discussion of nutrients and metabolism. Some of the most important are **oxidation** and **reduction** reactions, commonly referred to as **redox reactions**. Oxidation and reduction reactions are the cornerstone of **catabolic** and **anabolic** pathways. Catabolic pathways typically involved the "breaking down" of compounds for a purpose, such as energy production via the catabolism of macronutrients, an oxidative process. Anabolic pathways are associated with the synthesis or construction of new compounds and are considered a reduction process. Many micronutrients are essential in both catabolic (i.e., oxidative) and anabolic (i.e., reductive) processes. A working definition of oxidation is the loss of electrons—typically, in biological processes this means the loss of hydrogen and/or the gain of oxygen. For reduction, this would be the gain of hydrogen and/or the loss of oxygen. Another rule of thumb we will see frequently is that to achieve balance and recycle molecules, essential in nutrition, when one compound is oxidized it is coupled to another compound that is reduced, and vice versa. When it comes to nutrients, recycling is critically important for metabolism to be efficient and reduce the amount of a given nutrient that we need to consume. This is true for many vitamins, proteins/amino acids, and even enzymes following the performance of their function.

CONCEPTS IN NUTRITION RESEARCH

This is a good point to introduce a framework for nutrition-related research and the questions that are addressed. There are a number of experimental approaches to conducting research that addresses a question of nutritional relevance. **Clinical nutrition** is the most obvious, because it typically uses human subjects to explore nutrition and health—that is a clear strength. A limitation in this approach is that it is typically noninvasive and thereby limits the type of data that can be gathered; compliance by the subjects to adhere to the protocol of the experimental design can be an issue; and the inherent variability from human to human can introduce a lot of variation in the results. Nutrition with human subjects can include randomized clinical trials, epidemiological studies, or observational studies. Often the data generated seeks to establish associations between the treatment groups or populations in the study.

Using **animal models** for nutrition research allows the types of analysis to be performed to be highly invasive, since most studies are terminal, thereby allowing the researcher to collect any tissue or biological fluid that is relevant to the goals of the study. Subsequently, the analysis is often at the biochemical or molecular level in the samples that are collected,

thereby allowing the research to focus on understanding the research question at a mechanistic level. This is important, because if the specific mechanism is understood, the potential to intervene with therapeutic approaches is possible. In contrast to clinical nutrition with humans, animals are highly compliant with the study protocol, and a significant degree of genetic homogeneity can be achieved, thereby reducing variability. Another strength is that a researcher can generate a specific genetic alteration in the animal by removing or adding specific genes, referred to as **knockout** and **knockin** models, respectively. This approach is a very powerful method to precisely identify the specific genes/proteins that are involved in a given process. Although there are genetic or chemically induced animal models for a large number of disease states, there is often a question as to how precisely such models reflect the same disease in humans. Moreover, there are differences in metabolism and physiology that need to be considered when using an animal model to draw conclusions with respect to humans. Rats and mice are the most common animal used in nutrition research, but many other species are also utilized for various reasons.

A complement to both human- and animal-based approaches is the use of **cell culture**. Cell culture involves the maintenance of a specific cell type under conditions that allow the cell to continuously replicate generation after generation. This characteristic is because the cells that are used in cell culture are **transformed** cells, which essentially means they are cancer cells, often derived from a human or manipulated to be carcinogenic and continuously replicate. The clear advantage in using cell culture is that researchers have a single cell type of their choosing and can specifically manipulate the media in terms of its composition and what compounds are added to the media at specific concentrations. One can think of the media as the "blood" that is bathing a specific cell type or "tissue." Humans and animals are in vivo models; therefore, a given treatment to address a nutrition question results in many changes in the molecules and hormones that are in the bloodstream, as well as how different cells or tissues interact with each other. Nearly all of these variables are eliminated using cell culture. However, it is critical to remember that these cells are transformed and may not always reflect what is occurring in normal cells. Another form of cell culture that circumvents using transformed cells is **primary cell culture**. These are cells that are isolated from a given tissue or the bloodstream and then cultured in media. The humans or animals that these cells come from may have been subject to the treatment arm of the study, or the cells after isolation are then subject to treatment. A limitation in this approach is that these cells do not propagate, and there is a finite amount of time that a researcher can work with primary cells—essentially, they are going to die after a given period of time under cell culture conditions.

Community nutrition involves a close interaction between the researcher and a specific population of people, usually to teach them subject matter or practices that reflect a healthy lifestyle with respect to nutrition. Research involved in community nutrition, like clinical, can result in finding associations between nutrition practices and people, as well as result in developing information to provide to communities to enable them to make healthy choices.

Comprehension Questions and Study Guide

Prior to working on these study questions, it would be best to first review your notes along with the relevant sections of the book (i.e., treat this like the exam itself). Likewise, don't just read a question and think "I know that" and move on to the next question. Take the time to write out your answers completely to all of the questions—this will ensure that you know the material, and it is also good practice to work on writing concise answers.

1. Name the various parts of the cell and write a brief (one to three sentences) definition for each.
2. How do we *regulate* proteins? Name the three mechanisms we discussed and describe, in detail, how they work.
3. What is the difference between adaptive and constitutive expression of genes? What is meant by apoprotein versus holoprotein? What is the importance of knowing these definitions?
4. Describe enzyme kinetics and any relevant terms we introduced. How are the inherent kinetic characteristics of an enzyme related to regulation of a metabolic pathway/process?
5. You should know the following concepts/terms and their interrelationship with each other: homeostasis, turnover, and half-life.
6. You should know the following concepts/terms and their interrelationship with each other: proliferation, differentiation, and apoptosis.

CONCLUSION

The body undergoes constant changes with respect to environment, particularly nutrient supply, which is presented sporadically to cells. The body is also trying to maintain homeostasis even though nearly all components of the body, from cells to proteins, undergo turnover throughout the life span. Thus, it is important that cells can respond to hormones and nutrition to provide the proper proteins, energy sources, and biosynthetic processes to maintain balance, a key to health and disease prevention. Key concepts from this chapter include:

- Homeostasis is the concept of maintaining balance in the presence of constant turnover, including cellular turnover through the process of apoptosis.
- Cells are "mini cities" in that they take in nutrients, respond to hormones, provide energy in the form of ATP, and carry out biosynthetic processes over their life span.

- Proteins are the dynamic molecules of the body, and as such, must be regulated at the appropriate time to maintain homeostasis.
- Regulation of gene expression is a tissue- and cell type–specific process that allows adaptive production of proteins in response to environmental changes, including nutrition.
- Proteins can be regulated by induction, posttranslational modification, and allosteric mechanisms in response to a specific signal.
- Gene expression can occur at the transcriptional and/or translational level.
- Enzyme kinetics are also a key control point for regulating metabolism and other cellular processes.
- Bond formation and its regulation is a key aspect of protein regulation, nutrient procurement, and nutrient distribution.
- Redox reactions are key steps in catabolic and anabolic metabolic processes.

Application Activity

A number of diseases are the result of a genetic mutation—describe this with respect to protein synthesis and function. How does a genetic mutation result in a physiologic or phenotypic alteration? Use PubMed to find an example of a genetic mutation that results in a dysfunctional protein that is associated with a specific disease or abnormality.

Taking It Outside the Classroom

Google the top ten causes of death in humans and identify which ones have a nutrition component. Reflect on the role of nutrition in these causes of death in the context of what was discussed in this chapter. How is this different in other parts of the world where nutrition and sources of nutrients can be vastly different than the United States?

IMAGE CREDITS

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