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Overview of Molecular Biology

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After much deliberation, the authors have decided to include a preliminary chapter concerned with molecular biology and the immune system. This decision was based on the fact that the book was written for engineers and applied scientists who may not have a background in biology. Why the inclusion? The authors felt that these topics, for those interested, could provide the readers with a better understanding of how cells function under normal circumstances, and thus better comprehend how viruses take over and use these mechanisms against the body.

Biology, as the science of life, involves the general study of living forms. *Molecular* biology, which includes *biophysics* and *biochemistry*, has made fundamental contributions to modern biology. Thus, more information is now available about the structure and function of nucleic acids – the base of DNA and proteins, and the key molecules of all living matter. *Cellular* biology is closely related to molecular biology (the title of this chapter). It primarily deals with the functions of the cell – the basic structural unit of life – which studies its components and their

A Guide to Virology for Engineers and Applied Scientists: Epidemiology, Emergency Management, and Optimization, First Edition. Megan M. Reynolds and Louis Theodore.

interactions. The life functions of multicellular organisms are governed by the activities and interactions of their cellular components. The study of organisms includes not only their growth and development but also how they function.

When a virus infects a host, it utilizes the genetic code of the invaded cell to hijack the normal replication process in order to replicate numerous copies of itself. Thus, it is helpful to have some understanding as to how genetic coding works under normal circumstances, in order to fully comprehend the complex mechanism with which the virus commandeers a cell for its own purposes. While viruses are not themselves cellular, they do contain the same basic genetic materials as cells, either DNA or RNA.

This chapter attempts to provide the reader with some of the key terms that have become integral to the study of molecular biology. The chapter also endeavors to offer a framework of normal cell functions critical to the understanding of virology. Chapter 2: Basics of Virology, the next chapter, utilizes this framework to depict how viruses invade and hijack standard cell function. Hopefully, the importance of the definitions and explanations in this earlier section will become clear. In the same vein, those already familiar with molecular biology may wish to skip this chapter in favor of Chapter 2.

1.1 CELL BASICS

This section describes the basic concepts of *eukaryotic* cells, which compose all multicellular organisms, such as humans, animals, and plants. Alternatively, single-celled microorganisms such as bacteria are referred to as *prokaryotes*. Different viruses infect different types of cells. As discussed above, Chapter 2 further examines how viruses invade and infect human cells.

Within each eukaryotic cell is a highly complex system of *organelles* – the tiny cellular structures that perform specific functions within the cell. These structures keep the cell running, much like organs do in the human body. Several key organelles are shown below in Figure 1.1.

(Louten 2016)

The following subsections below highlight a few organelles that play a crucial role during virus invasion leading to infection. These include the cytoplasm, ribosomes, and nucleus.

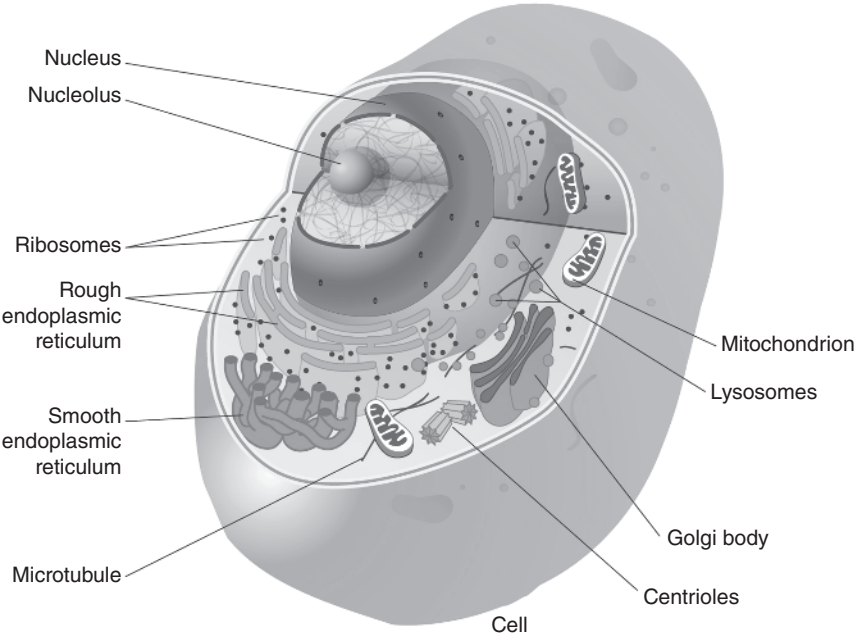

Figure 1.1 Illustration of organelles within a cell. *Source*: National Cancer Institute/U.S. Department of Health and Human Services/Public Domain.

1.1.1 Cytoplasm

The cytoplasm is one of the most important organelles within the cell membrane since it holds the other organelles together in its gel-like composition and allows for numerous processes to occur within the cell through the suspension of organelles and cellular molecules. Cytoplasm also allows for the occurrence of biochemical reactions within the cell, such as the replication of RNA viruses and protein synthesis (Denison 2008). The replication of RNA viruses occurs here in the cytoplasm as a majority of the enzymes used to replicate RNA are virally encoded.

1.1.2 Ribosomes

Ribosomes found in the cytosol play an important role in the manufacture of proteins within the cell. These ribosomes are located not only attached to the *rough*

endoplasmic reticulum (rER), but also floating within the cytosol. The ribosomes attached to the endoplasmic reticulum have the ability to create proteins. Once transferred to the lumen, proteins are modified to be utilized by the remaining organelles throughout the cell. This is all possible due to the binding of the ribosomes to the messenger RNA (mRNA) prior to the production of proteins (Louten 2016). Viruses have the ability to overtake the production of these proteins by the ribosomes for their own use.

1.1.3 Nucleus

The nucleus within eukaryotic cells contains organelles necessary for the regulation of cellular activities as well as the structures that contain the cell's DNA and other hereditary information. These structures inside the nucleus are comprised of chromosomes, the nuclear matrix, nucleoli, the nucleoplasm, the outer and inner nuclear membranes as well as the nuclear pores (Louten 2016).

The nucleus also allows for the replication of DNA which is then transcribed into messenger RNA to be used throughout the cell. Because of this, viruses must be able to have access to the cell's nucleus in order to replicate their DNA and attack other cells (Geer and Messersmith 2002).

1.2 CELL REPLICATION

Cell replication is a detailed process involving the copying of DNA to make new cells. DNA contains the genetic code that is present in every cell in the human body. DNA and RNA are both made up of nucleic acids, which are described below. They are critical to the process of replication and survival, not only for the cell but also for the invading virus (Denison 2008).

1.2.1 Nucleic Acids

This subsection will review the structure and function of DNA and RNA, which, as previously mentioned, are both made up of various nucleotides. Nucleotides are the basic building blocks for all living organisms, and are a crucial component in all cells. Nucleotides are comprised of three basic components:

- A five-carbon sugar molecule (deoxyribose for DNA or ribose for RNA)
- A phosphate group containing phosphorus and oxygen
- A *nitrogenous base*, a ringed molecule of nitrogen, oxygen, and hydrogen

There are four variations of nitrogenous bases, and together they form the basic building blocks for all living organisms: adenine (A), guanine (G), cytosine (C), and thymine (T). (Note: Uracil (U) replaces thymine in RNA) Together, these four different nucleotides combine to form polynucleotide base pairs. In DNA, adenine

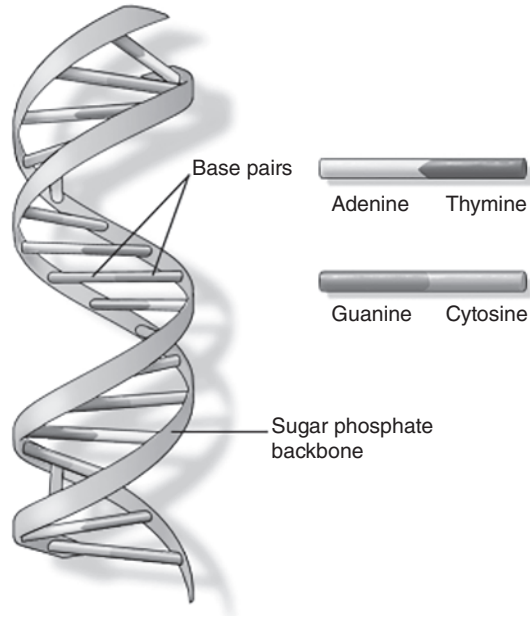

Figure 1.2 DNA polynucleotide base pairs with sugar--phosphate backbone https://medlineplus.gov/genetics/understanding/basics/dna/

always pairs with thymine, while cytosine pairs with guanine. The pairs are bound together by hydrogen bonds. These base pairs form the coding sequences within the DNA double helix, as shown in Figure 1.2. (Seladi-Schulman 2019; NIH 2010).

The double helix of DNA is structured as two complementary polynucleotide strands, with the leading strand running from the 5′ to 3′ carbon and the lagging strand running from the 3′ to 5′ carbon, as shown in the middle of Figure 1.3, below. The base pairs are located within the resulting double helix. The code, which is the order of nucleotides, determines which amino acids will be produced, and therefore, which proteins. Each amino acid is encoded by the order of three nucleotides (Geer and Messersmith 2002).

1.2.2 DNA Replication

The cell cycle consists of four stages including gap 1(G_1), synthesis (S Phase), gap 2(G_2), and mitosis. Within these four stages, each cell has the ability to grow and divide while also replicating its DNA. The process of DNA replication occurs when the cell creates a direct copy of its chromosomes either during synthesis or during the s-phase of the cell cycle.

As depicted in Figure 1.3, the DNA molecule is untwined during replication, and the two DNA strands are separated from one another through the presence of *cellular enzymes* within the cell. *DNA polymerase* is one of the main

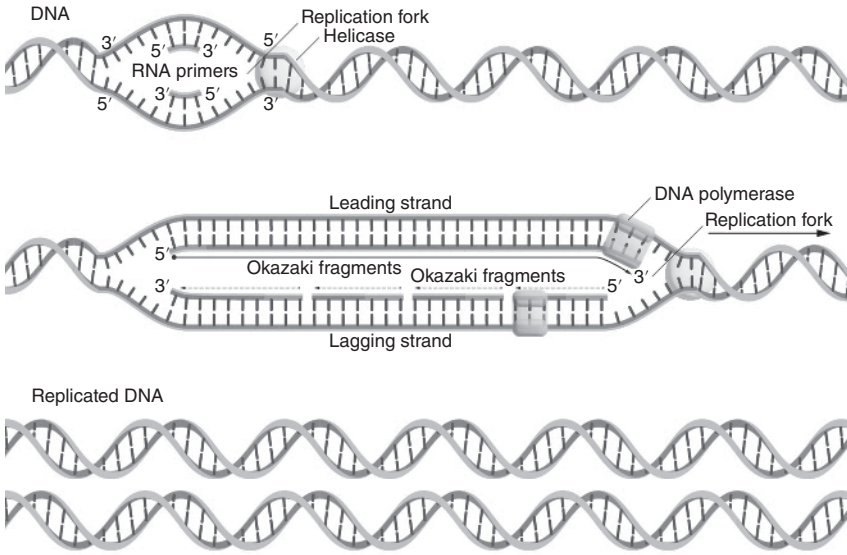

Figure 1.3 DNA Replication https://www.genome.gov/genetics-glossary/DNA-Replication

enzymes utilized in DNA replication due to its ability to place the complementary nucleotides of the new DNA strand in the 5′ to 3′ direction. DNA polymerase also adds in nucleotides based upon the complementary base pair rules as discussed in the previous section and is highly accurate, so there is a very low rate of misplaced nucleotides. This is shown in Figure 1.3. (Geer and Messersmith 2002).

Working alongside DNA polymerase is an enzyme known as *RNA polymerase* that synthesizes RNA. Similar to DNA polymerase, this enzyme uses a DNA template to produce a section of RNA that adds nucleotides in a 5′ to 3′ direction while also using the complementary base pair rule. RNA polymerase is known to have a lower rate of fidelity as compared to DNA polymerase (Louten 2016). This fact is highly relevant to virus replication, since an RNA virus tends to have more replicating errors—and as a result, more mutations than a DNA virus, as will be discussed in Chapter 2.

Another enzyme involved with DNA replication is known as *primase*, which is an enzyme that allows DNA polymerase to bind to a formerly single strand of DNA. Primase has the ability to form a double-stranded segment that allows for the binding of DNA polymerase through laying a complementary fragment of RNA on top of the single strand of DNA. (Geer and Messersmith 2002).

Because DNA replication is performed in the cell's nucleus, viruses must gain entry before taking advantage of DNA polymerase and other enzymes to replicate their own genomes and divide further (Louten 2016).

1.2.3 RNA Structure and Role

DNA and RNA are known to have both positive and negative strands. The positive strand of DNA is found within a single-stranded DNA virus and is referred to as any strand that has the same base sequence as a negative DNA strand. Meanwhile, a negative strand of DNA will have a base sequence complementary to that of the positive strand. The positive strand of RNA has the same polarity as viral mRNA while also containing codon sequences, "trinucleotide sequences of DNA or RNA that correspond to a specific amino acid…" [that may be translated to viral proteins] (genome.gov). Negative RNA strands are noncoding and must be copied by RNA polymerase in order to produce mRNA that is translatable (King et al. 2014). This background information is important for understanding key characteristics of various categories of viruses, (e.g., (+) or (-) sense DNA viruses).

DNA is able to use mRNA, transfer RNA (tRNA), and ribosomal RNA (rRNA) to replicate itself and repair mistakes during the process of replication. Messenger RNA is a single stranded temporary copy of a DNA molecule that is to be translated by the ribosome. Ribosomal RNA is known to help translate the information available in mRNA and change it into a protein to be used by the cell. The mechanism is illustrated below in Figure 1.4 below. The process of DNA replication and the transcription of DNA into mRNA occurs in the nucleus of the cell while mRNA is translated in the cell's cytosol by ribosomes, leading to the creation of a protein. RNA acts as a copy of a DNA molecules' hereditary genetic information. Ribosomes have the ability to create proteins through the use of amino acids using a sequence of nucleotides present in the RNA (Louten 2016).

As depicted in Figure 1.4, *transcription* refers to the creation of an RNA model from an original DNA sequence, while translation occurs when RNA goes through the process of being made up of nucleotides to becoming made up of proteins consisting of amino acids during the process of protein *translation*. These processes will be described in detail in the next subsection.

Nucleic acids have the ability to code for proteins through a series of three nucleotides that code for a specific amino acid. Because proteins are made up of amino acid chains, the nucleic acids within each amino acid directly impact each protein. mRNA of a previously known arrangement can be used to determine the amino acid sequence by directing the synthesis of a selected protein. In this manner, the genetic code could be decoded by comparing the original order of the mRNA with the synthesized proteins' amino acid sequence (Smith 2008).

1.2.4 Protein Synthesis

Protein synthesis is the process of creating protein strands through the use of ribosomes, mRNA, tRNA, and amino acids – mainly through transcription and translation, as shown in Figure 1.4.

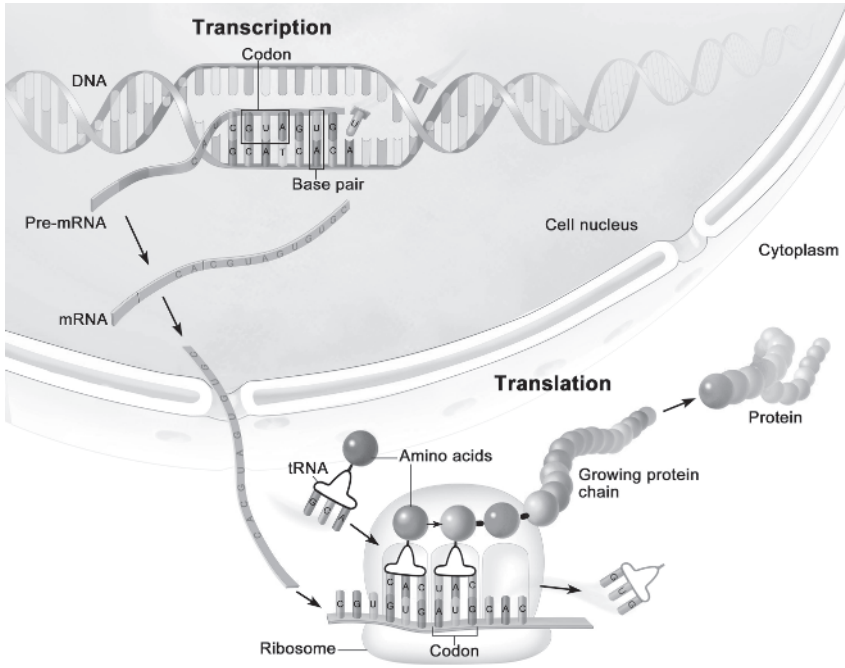

Figure 1.4 Protein Synthesis: Transcription and Translation https://nci-media.cancer.gov/pdq/media/images/761782.jpg

Transcription occurs when a section of DNA is copied into mRNA. The template strand of the two strands of DNA is known to act as a template for transcription. RNA polymerase II is the enzyme that can synthesize DNA from that template, which is recruited to the complex by transcription factors binding to the promoter sequence. RNA polymerase II then reads the template strand of DNA using complementary base pairing rules specifically in the 3′ to 5′ direction (Louten 2016).

Translation is a three-part process consisting of initiation, elongation, and termination. Each of these three parts, discussed below, allows for the assembly of a protein through amino acids from the ribosome (Geer and Messersmith 2002).

- *Initiation*
  This consists of a ribosome attached to a 5′ cap of mRNA transcript which then scans until a start codon is recognized. Corresponding tRNA along with a ribosomal subunit join the complex and elongation begins.
- *Elongation*
  During elongation, tRNA delivers amino acids to form a growing chain for each additional codon. Once the tRNA delivers its amino acid to the mRNA, it is then released from the ribosome and recharged by enzymes within the cell to be reused.

- *Termination*
  This third and final stage of translation occurs when the moving ribosome encounters a stop codon, causing the ribosome to leave the mRNA when the protein is released as a result.

1.3 CELLULAR TRANSPORT

Cellular transport refers to the movement of resources or supplies through the plasma membrane of the cell. There are two general categories of transport: passive and active. Passive transport does not require any energy, while active transport does require it.

1.3.1 Plasma Membrane

The plasma membrane is the main layer of separation between a cell and its surrounding environment. Because of this, the plasma membrane is the first layer of contact a virus encounters while infecting a cell. The plasma membrane is most commonly represented by the fluid mosaic model, which portrays the integral proteins as being suspended in the lipid bilayer. The lipid bilayer is composed of amphipathic phospholipid molecules that contain both a hydrophobic tail and a hydrophilic head portion. The fatty tails of the phospholipids face together when placed in an aqueous solution, while the heads will be positioned on the outside. This way, the bilayer is able to form a barrier between the inside of the cell and the extracellular environment, creating a plasma membrane sufficient for cellular activities.

Integral membrane proteins (IMPs) are located in the lipid bilayer of the cell membrane. These integral proteins are necessary for a variety of extracellular functions, and, among other functions, can act as receptors or as cellular adhesion molecules (CAMs), which are used to adhere neighboring cells to each other. In addition to integral proteins are peripheral membrane proteins located on the surface of the plasma membrane associated with intracellular activities (Louten 2016).

Integral proteins also help with transporting substances, including those such as ions and other small molecules from one side of the cell to the other. However, some of these substances may be too large to fit between the channels and carrier proteins situated in the lipid bilayer and must be exported through processes such as exocytosis – a form of active transport out of a cell.

1.3.2 Cell Signaling

Also known as transduction, cell signaling is a vast network where the cell has the ability to communicate with other cells by releasing hormones and

with other signaling molecules. Transduction would not be possible without the signal-transduction pathway, which allows for signals to be transmitted throughout the cell and results in a cellular response (Nair et al. 2019).

In order to communicate successfully with other cells, messages are first transferred from the *ligand* (a molecule that binds to a receptor) to the appropriate receptor, then decoded through a series of reactions of second messengers otherwise known as ions, kinases, and other small molecules. Afterward, the same message travels to the nucleus from the cell membrane, where several processes occur, including gene expressions, subsequent translations, as well as protein targeting. These processes target both the cell membrane and other organelles, all from the original message communicated. An intermediate is formed by a combination of intracellular signaling. This process is initiated by the response brought to the cell's ligands.

The process of cell signaling has the ability to control various multicellular activities including cell growth, differentiation, and other cell-specific functionalities. Due to this variability, signaling can be used in multiple areas, including endocrine, paracrine, juxtracrine, autocrine, and in neuronal neurotransmission. In addition to the various signals, the chemical makeup of the ligands is also differentiated in the inclusion of smaller molecules such as lipids, nucleic acids, and proteins, among others (Nair et al. 2019).

1.4 IMMUNE DEFENSE

The human body has various ways to defend itself against assault from infectious diseases and toxins. The immune system has the ability to determine the difference between "self" and "foreign" elements, and to mount an effective immune defense by acting against all "foreign" invaders. There are different protective mechanisms at the body's disposal, consisting of *innate* and *adaptive immunity*, both of which are explained below.

1.4.1 Innate Immunity

The innate immune system acts against all nonspecific invaders such as antigens and other harmful microorganisms rather than just attacking any one specific infectious microparasite. This nonspecific protective mechanism consists of three separate components: *physical barriers*, the *inflammatory response*, and *phagocytosis*.

Physical (and chemical) barriers that act as the body's primary line of defense are surrounded by epithelia. Epithelial surfaces have the ability to separate the body from pathogens present in the external environment and consist of the skin,

respiratory and urogenital tracts, as well as the mucus membranes. When these barriers are penetrated by invaders and pathogens enter the body, an immune response occurs to rapidly eliminate the infection.

The inflammatory response occurs at the infected area of tissue injury caused by an influx of foreign invaders or from other bodily trauma. An influx of specialized cells to the injured tissue results in an inflammatory reaction leading to a release of chemicals. These specialized phagocytic cells, known as macrophages, have the ability to isolate and identify invasive cells while releasing active molecules. The surrounding capillaries then dilate as a result of the released inflammatory cytokines, which then increases blood flow, eventually causing fluid leakage that accounts for the side effects of inflammation. These effects may include swelling, redness, and pain.

Phagocytosis, the last step in innate immunity, is used to engulf and digest cells as a form of receptor-mediated endocytosis. This phase is oftentimes referred to as the cleansing or "healing" phase as it is where the inflammatory response is diminished, and the side effects of inflammation are reduced. Phagocytosis plays a role in protecting the body against viruses as macrophages and other specialized phagocytic cells help to digest bacteria and dead cells in an effort to defend against pathogens and other harmful microorganisms. In some instances, however, certain viruses are able to gain entry and take over a cell through phagocytosis.

1.4.2 Adaptive Immunity

Adaptive immunity is a specific protective mechanism that has two main components and five important characteristics. As compared to innate immunity, adaptive immunity allows the body to recognize and identify specific antigens and pathogens while also retaining their genetic information for future attacks. Because this immunity can recognize specific antigens, it activates lymphocytes (a type of white blood cell) with a plan of attack unique to that antigen. As a result, the humeral and the cell-mediated immune responses—the two main components of adaptive immunity—are put into place. Both these responses are discussed below.

1.4.2.1 Humoral Immunity

The *humoral* immune response mainly corresponds with the function of B cells and their production of immunoglobulins, or antigen-specific antibodies. Immunoglobulins are a type of antigen receptors found on B cells, and each immunoglobulin is only able to identify a single antibody. Because of this, the immune system contains a variety of antigens in order to create a successful defense against the wide array of pathogens and other foreign invaders the immune system encounters on a daily basis.

B cells have the ability to produce more cells with their particular antibodies when they identify and collide with their specific, corresponding antigens. Several of the new B cells created from this collision will transform into plasma cells, which are known to produce a high quantity of antibodies. The main function of these antibodies within the process of adaptive immunity is to bind with extracellular pathogens present in the blood or other bodily fluids.

1.4.2.2 Cellular Immunity

In addition to the humoral aspect of adaptive immunity is the *cell-mediated* immune response. Rather than relying on the function of B cells, cellular adaptive immunity focuses more on the work of T cells within the body in response to foreign invaders and potentially harmful substances. Much like B cells, T cells also have the ability to recognize antigens; however, their receptors are able to identify a large number of antigens through their similarities to immunoglobulins. B cells are only able to recognize one specific antigen.

Unlike B cells, T cells are only able to identify and attack an antigen or other harmful cell after it has already been processed by particular "antigen-presenting" cells such as macrophages. After the cell is processed, antigens on the cell's surface bind to the T-cell receptor, which then signals the T-cell to grow and divide while also going through cellular differentiation. The activated T cells are now able to travel to the site where the antigen entered the body and release *cytokines* – small proteins involved in the inflammatory process – while working together with other T cells, B cells, and phagocytic cells to rid the body of infectious invaders. Cytokines or "chemical messengers" are generated by a variety of cells and can have many different functions including initiating cellular activities and processes.

The above two immune systems work together to successfully eliminate and protect the body from foreign material and pathogenic invaders. Both adaptive and innate immunity are able to initiate the body's immune response, prompting the release and activation of *lymphocytes* – the inflammatory response and phagocytosis among other methods to eliminate the attacking antigens and to prepare the body for future invasions of hazardous microorganisms.

1.5 APPLICATIONS

The following four illustrative examples are intended to complement the above material and to provide a better understanding of the aspects discussed within the chapter.

Illustrative Example 1.1 CYTOKINES

Examine the role of cytokines in inflammation.

Solution
There are many types of cytokines produced by the body under stress or injury. The common inflammatory response induces heat, redness, swelling, and pain, as well as impaired function. The majority of cytokines are formed by activated macrophages and T cells. Some are proinflammatory, such as IL-1β, IL-6, and TNF-α, while others are activated in order to counteract the inflammatory process. Anti-inflammatory cytokines include IL-4 and IL-10, among others.

Illustrative Example 1.2 RNA
Describe the functions of mRNA, tRNA, and rRNA.

Solution
While there are other types of RNA, the main three are:

- Messenger RNA (mRNA), which is transcribed directly from DNA, goes on to produce proteins with the help of tRNA.
- Transfer RNA (tRNA) transcribes mRNA into proteins.
- Ribosomal RNA (rRNA) forms ribosomes, and are, therefore, critical for the synthesis of proteins.

Illustrative Example 1.3 Lymphocytes
Explain the role of lymphocytes in adaptive immunity.

Solution
The lymphocytes are a part of the lymphoid system and are composed of B and T cells. The adaptive immune system is activated from these lymphocytes, and pathogens and other foreign invaders are disposed of before entering the bloodstream. The activation of large numbers of lymphocytes can occur as a result of antigen recognition, and the activated lymphocytes will have specified roles against particular antigens. This specific immunity can be seen in the humoral and cell-mediated immune responses against pathogens.

Illustrative Example 1.4 Translation and Transcription
Differentiate between translation and transcription in protein synthesis.

Solution
Translation accounts for the assembly of a protein through amino acids from the ribosome, and is composed of three parts, including initiation, elongation, and termination. Through the use of these three processes, translation is able to assemble a protein made up of an amino acid sequence from RNA in the cell's ribosome. Meanwhile, transcription in protein synthesis takes place when mRNA is produced through the copying of a DNA section.

1.6 CHAPTER SUMMARY

- A highly complex system of organelles is present within each eukaryotic cell.
- One of the most important organelles is the cytoplasm, which has the ability to secure the organelles together, while also being the area many cellular processes occur by suspending other organelles and molecules in its characteristic gel-like composition.
- When DNA replication occurs, the cell is able to create a direct replicate copy of itself and its chromosomes during synthesis of the cell cycle.
- Protein synthesis utilizes mRNA and tRNA in addition to amino acids and ribosomes when creating protein strands through transcription and translation.
- The main layer of separation between cells and the outside extracellular environment is the plasma membrane, which also acts as the first layer of contact encountered by a virus or foreign invader when infecting a cell.
- Transduction, or cell signaling, has the ability to facilitate multiple processes such as the communication between cells.
- The immune system acts against all foreign invaders in different protective mechanisms consisting of innate and active immunity.

1.7 PROBLEMS

1 How many antigens are B cells able to recognize?

2 What are immunoglobulins? Cytokines?

3 When identifying nonspecific foreign invaders, which immunity is used?

4 How many nucleotides is each amino acid encoded by?

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