Chapter 4 – DNA, RNA and Protein Synthesis

Objectives

Given the synopsis in this chapter, competence in each objective will be demonstrated by responding to multiple choice, matching, put-in-order, or fill-in questions, at the level of 85% or greater proficiency for each student.

A. To explain the process of transcribing DNA into RNA.
B. To explain the process of translating messenger RNA into protein.
C. To explain how cortisol, for example, controls gene expression.

Chromosomes and DNA

The nucleus of a cell contains deoxyribonucleic acid (DNA) and associated histone and non-histone proteins. DNA wraps around disks of histone proteins forming **nucleosomes**. During most of interphase (G1 phase), the nucleosomes are uncoiled and available for use by the cell. Later in interphase (S phase), the nucleosomes coil up to form loop domains which come together to form the chromatids of the chromosomes. After mitosis, the loop domains uncoil. The organization of the chromosomes and DNA is reviewed in Figure 4-1.

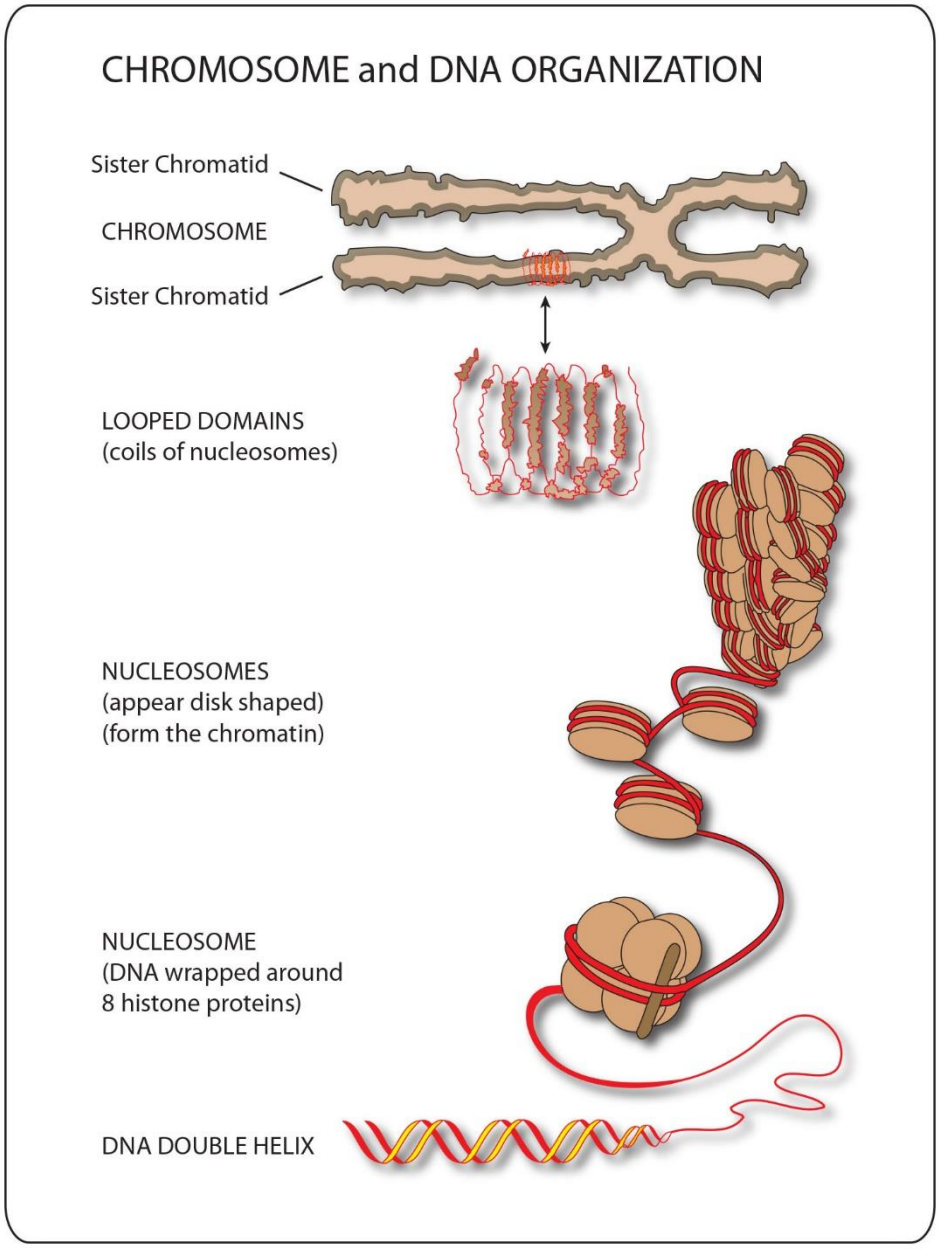

Figure 4-1 © 2014 David G. Ward, PhD

DNA is a double stranded molecule of deoxyribonucleic acid. One strand of DNA is the *template* strand (tDNA). The other strand of DNA is the *coding* strand (cDNA). DNA contains adenine (A), thymine (T), guanine (G), and cytosine (C). The strands of DNA are organized as a double helix, and the sequence of nucleotides in the *template* strand of DNA (tDNA) codes for particular sequences of nucleotides in the *codin*g strand of DNA (cDNA), as shown in Figure 4-2.

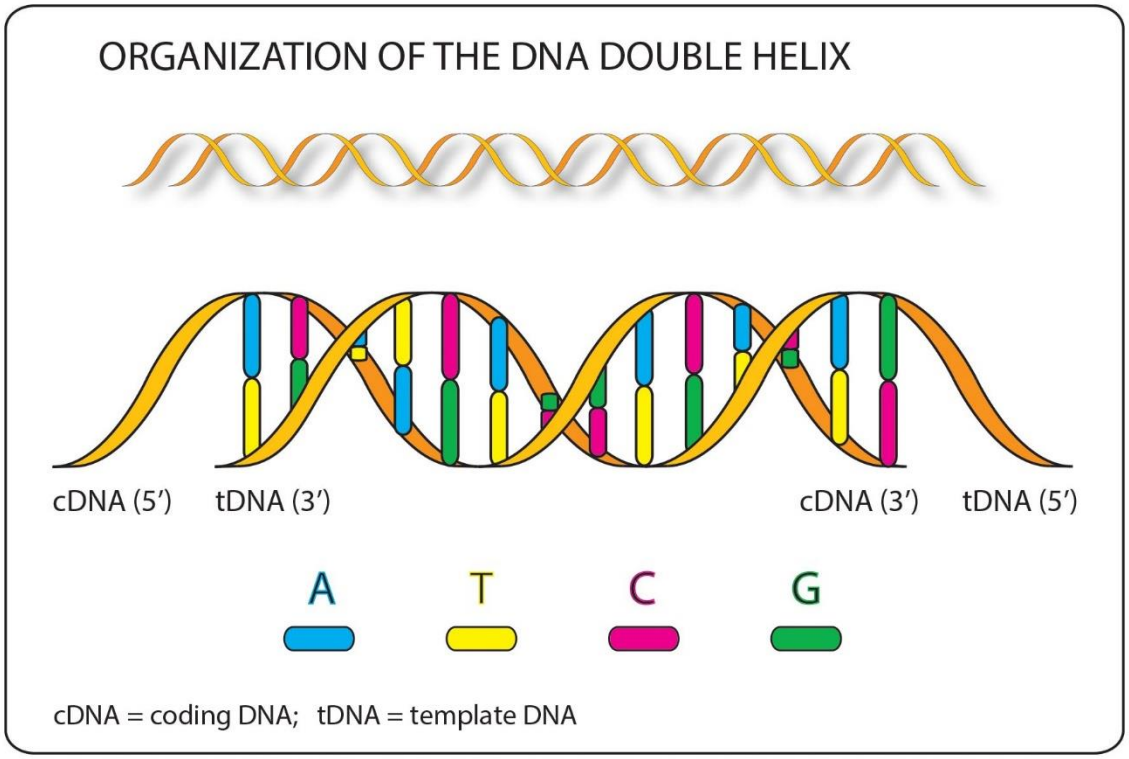

Figure 4-2 © 2019 David G. Ward, PhD

Each strand of DNA is composed of deoxyribose nucleotides linked lengthwise by covalent bonds between the 3' hydroxyl group of deoxyribose and the 5' phosphate group of the adjacent nucleotide. The nucleotides making up the template strand are aligned in the reverse direction of the nucleotides making up the coding strand. Please review chapter 3, Figure 3-10. The nitrogenous bases pair-up between strands by forming hydrogen bonds between a purine and a pyrimidine as shown in chapter 3, Figure 3-11, and summarized below:

- Adenine pairs with Thymine
- Thymine pairs with Adenine
- Guanine pairs with Cytosine
- Cytosine pairs with Guanine

Replication of DNA in chromosomes

During most of interphase (G phases) when somatic cells are carrying out their regular functions, each chromosome is comprised of a single chromatid. However, during a short period of interphase (S phase), in preparation for mitosis, the chromatid of each chromosome is duplicated to produce chromosomes with two identical sister chromatids. This duplication process is dependent on DNA replication, as shown schematically in Figure 4-3. The cDNA and tDNA strands are separated from each other, and the missing strands of DNA are replicated using DNA polymerase (not shown).

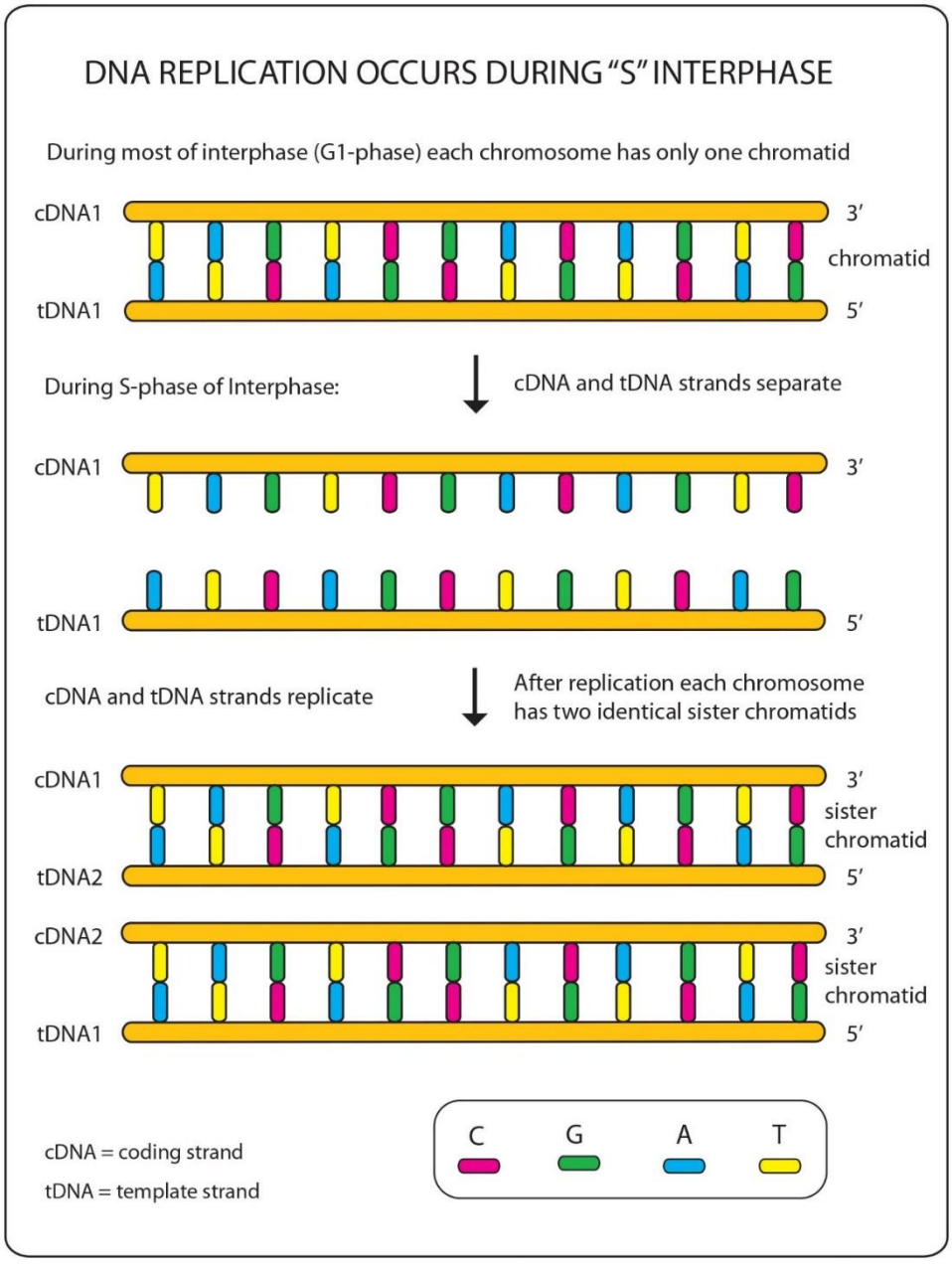

Figure 4-3 © 2015 David G. Ward, PhD

Gene Expression

In physiology, we are especially interested in the portion of a cell's life (G phases) when *somatic cells are carrying out their functions*. These functions depend on gene expression, the process in which genetic information is used to make end products of **protein** or **RNA**. Specifically, the production of protein involves transcription of tDNA to pre-mRNA, the processing of pre-mRNA to **mRNA**, and the translation of mRNA to **protein**. The production of **ribosomal RNA** (rRNA) involves, transcription of tDNA to pre-rRNA and its processing to **rRNA**. The production of **transfer RNA** (tRNA) involves, transcription of tDNA to pre-tRNA and its processing to **tRNA**.

Organization of a Gene - the human Vasopressin Gene

Within the chromosomes are chromatids, and within the chromatids are **genes**. The organization of the cDNA (coding strand) of the human vasopressin gene is shown in Figure 4-4.

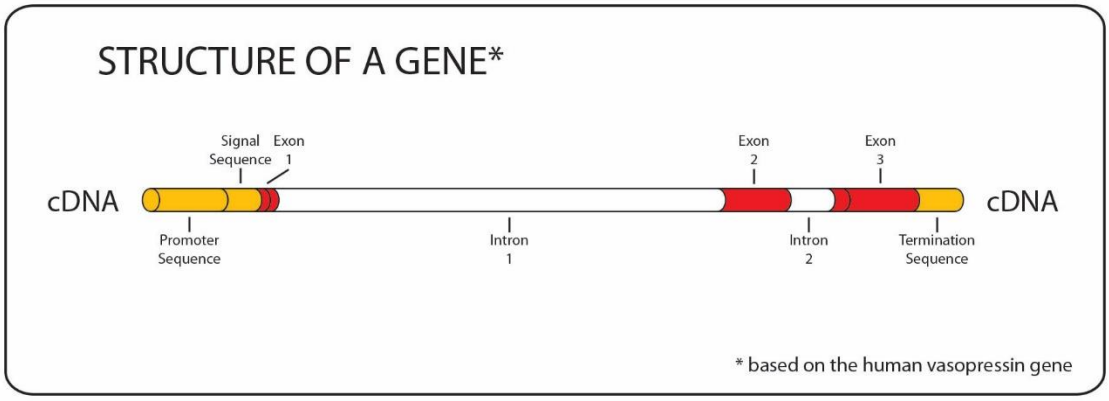

Figure 4-4 © 2016 David G. Ward, PhD

A gene is characterized as a section of DNA where the cDNA strand begins with a promoter sequence of nucleotides, continues with a signal sequence of nucleotides and various sequences of nucleotides called exons and introns, and ends with a termination sequence of nucleotides. The portion of the gene after the promoter sequence is sometimes called the transcription unit.

Transcription of tDNA to pre-mRNA

The cDNA, except for the promoter, is replicated as a **pre-mRNA** (precursor RNA), as shown schematically in Figure 4-5. Transcription of tDNA to pre-mRNA is similar to replication of tDNA and cDNA.

- The cDNA and tDNA strands are separated from each other using RNA polymerase.
- The tDNA is transcribed to construct a pre-mRNA replicate of the cDNA using RNA.
- The original tDNA and cDNA come back together, and the pre-mRNA is released.

As a result of this process ***the pre-mRNA will have the same base sequence as the cDNA****, except the pre-mRNA will have Uracil wherever the cDNA has Thymine*

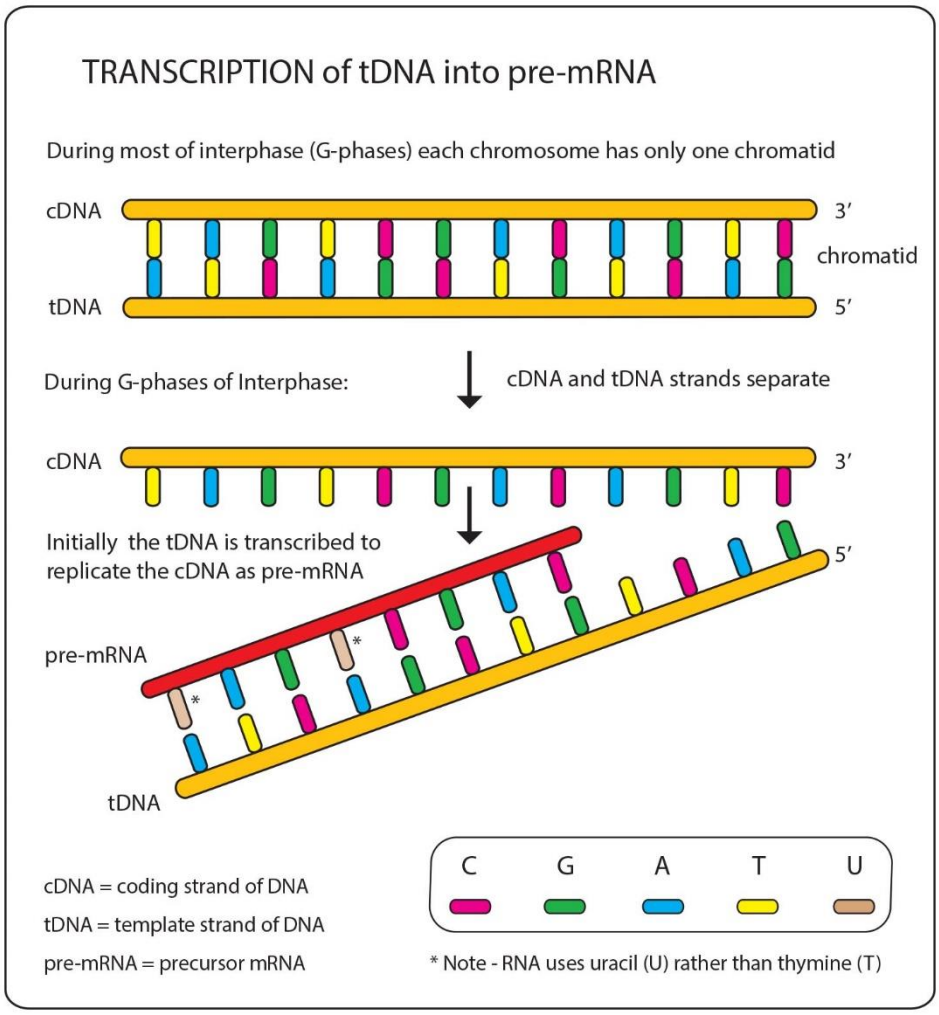

Figure 4-5 © 2019 David G. Ward, PhD

Transcription factors and RNA polymerase

The transcription process can not start until an RNA polymerase binds to the promoter sequence of a gene. Promoter sequences also define the direction of transcription and indicate which DNA strand will be transcribed (this strand is the template strand).

- ***Transcription factors (chemical messengers) allow RNA polymerase II (RNAPII)*** to recognize the proper DNA sites at the promoter region of **protein coding genes**, and ***to initiate transcription*** at the appropriate nucleotide.

At the start of transcription, RNA polymerase II (RNAP-II) separates the DNA strands and reads the transcription unit of the template strand of DNA (tDNA), adding nucleotides to the 3′ end of the growing chain of pre-mRNA, as shown in Figure 4-6.

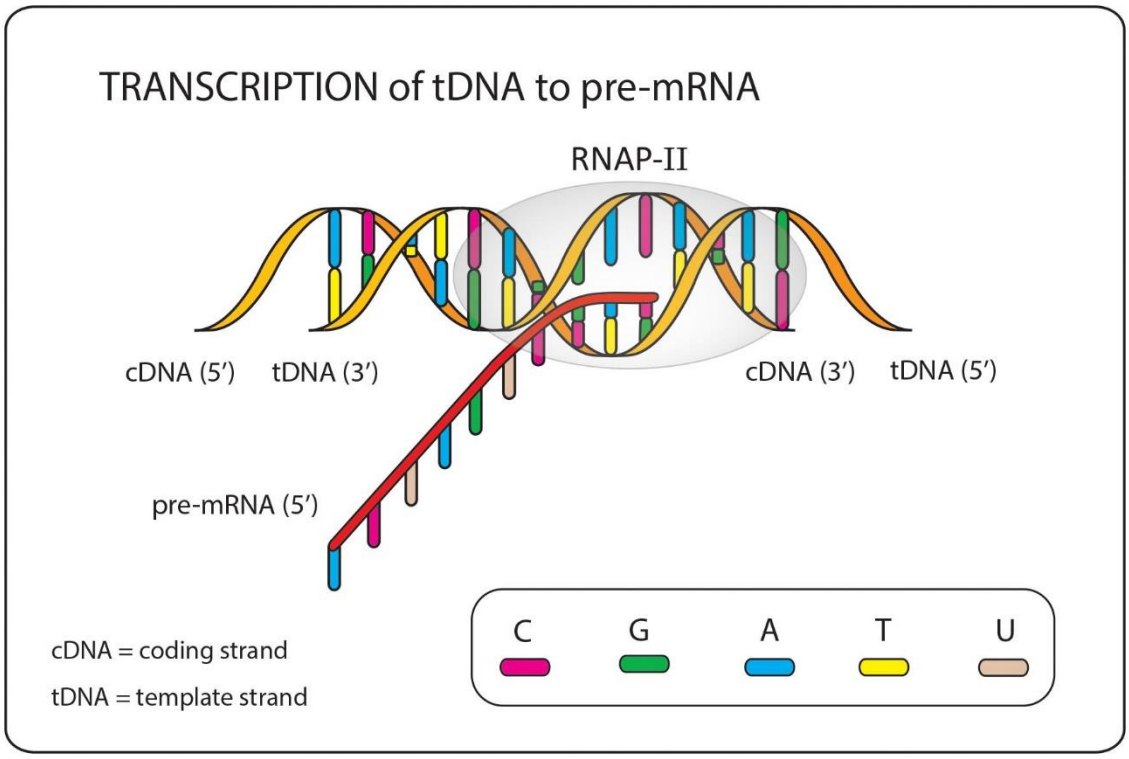

Figure 4-6 © 2019 David G. Ward, PhD

Processing of pre-mRNA to mRNA

In the pre-mRNA, nucleotide segments with instructions for making proteins (called **exons**) are often intermingled with nucleotide segments (called **introns**) that do not have instructions for making proteins.

Precursor RNA (pre-mRNA) is processed in the nucleus into messenger RNA (mRNA) by a series of cut-and-paste reactions, as shown in Figure 4-7.

- Introns are cut out and the exons are reassembled.
- Messenger RNA (mRNA) is produced by the reassembly of the nucleotides in the signal sequence, exons, and termination sequence

Transcription of DNA to pre-rRNA and pre-tRNA, and Processing to rRNA and tRNA

Chromosomes not only contain genes for protein coding, they also contain genes for production of ribosomal RNA (rRNA) and genes for production of transfer RNA (tRNA).

The production of **ribosomal RNA** (rRNA) requires transcription of tDNA to pre-rRNA by RNA polymerase I or III (RNAP-I, RNAP-III), followed by the processing of pre-rRNA to **rRNA**. The production of **transfer RNA** (tRNA) requires transcription of tDNA to pre-tRNA by RNA polymerase III (RNAP-III), followed by the processing of pre-tRNA to **tRNA**.

Ribosomal RNA is critical for the construction of ribosomes. Transfer RNA is critical for transporting amino acids. Both are needed for the synthesis of proteins.

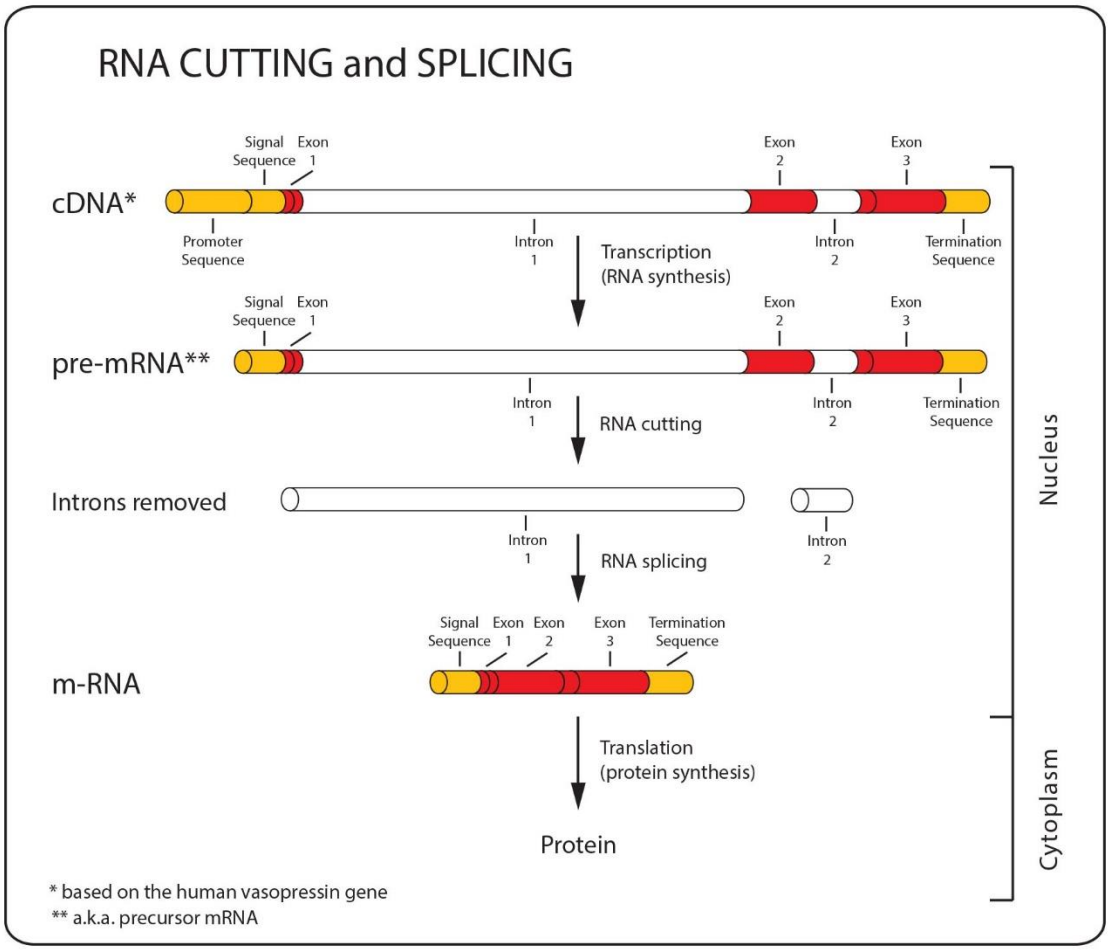

Figure 4-7 © 2019 David G. Ward, PhD

Translation: Using Messenger RNA, Ribosomal RNA, and Transfer RNA to Synthesize Peptides and Protein

Information for the sequencing of amino acids into a peptide, polypeptide, or protein is encoded in the sequence of triplet codons in the messenger RNA (mRNA). Translation uses ribosomes (made of ribosomal RNA (rRNA)) to house the mRNA; transfer RNAs (tRNAs) to transport amino acids to the ribosomes; mRNA to sequence the amino acids; and enzymes and GTP to connect the amino acids into a peptide.

mRNA Genetic code

The mRNA genetic code is based on triplet codons of three nitrogenous bases each.

- **Codons** are triplets of nitrogenous bases of mRNA. Each triplet code corresponds to a particular amino acid (or to stop or start)
- **Anticodon** are triplets of nitrogenous bases of tRNA that complement the triplets of nitrogenous bases of the mRNA. Each tRNA molecule possesses a triplet code that corresponds to a particular amino acid.

A listing of mRNA codons and their corresponding amino acids are shown in Table 4-1. For example: AUG codes for the amino acid methionine (**Met**), also an initiation (start) code; GAU codes for the amino acid aspartate (Asp).

Table 4-1. The Genetic Code (mRNA Codons)

1st position (5' end)	2nd position (middle)				3rd position (3' end)
	U	**C**	**A**	**G**	
U	Phe Phe *Leu* *Leu*	*Ser* *Ser* *Ser* *Ser*	Tyr Tyr STOP STOP	Cys Cys STOP **Trp**	**U** **C** **A** **G**
C	*Leu* *Leu* *Leu* *Leu*	Pro Pro Pro Pro	His His Gln Gln	*Arg* *Arg* *Arg* *Arg*	**U** **C** **A** **G**
A	Ile Ile Ile **Met**	Thr Thr Thr Thr	Asn Asn Lys Lys	*Ser* *Ser* *Arg* *Arg*	**U** **C** **A** **G**
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	**U** **C** **A** **G**

Initiation

In humans at least, many polypeptide messengers are needed to prepare ribosomes for attachment to **messenger RNA** (mRNA). Several of these polypeptides and an initiator transfer RNA bind to the 40S ribosome subunit (**small ribosomal unit**, Figure 4-8), forming the 43S complex, and allow the subunit to find the 5' end of the mRNA. The 43S complex scans along the mRNA until the initiation codon (typically Methionine, Met) is reached, which causes the release of critical initiation factors. Upon the release of these critical factors, the 60S ribosome subunit (**large ribosomal unit**, Figure 4-8) joins the complex to complete initiation.

Elongation

In RNA translation, as shown in Figure 4-8, molecules of **transfer RNA** with the appropriate **anticodons** attach to adjacent **codon** sites of the mRNA. Transfer RNA (tRNA) carries a particular **amino acid** (AA) to the mRNA. The amino acid carried in by each tRNA is attached to the previous amino acid by a peptide bond. Step by step, a protein is formed.

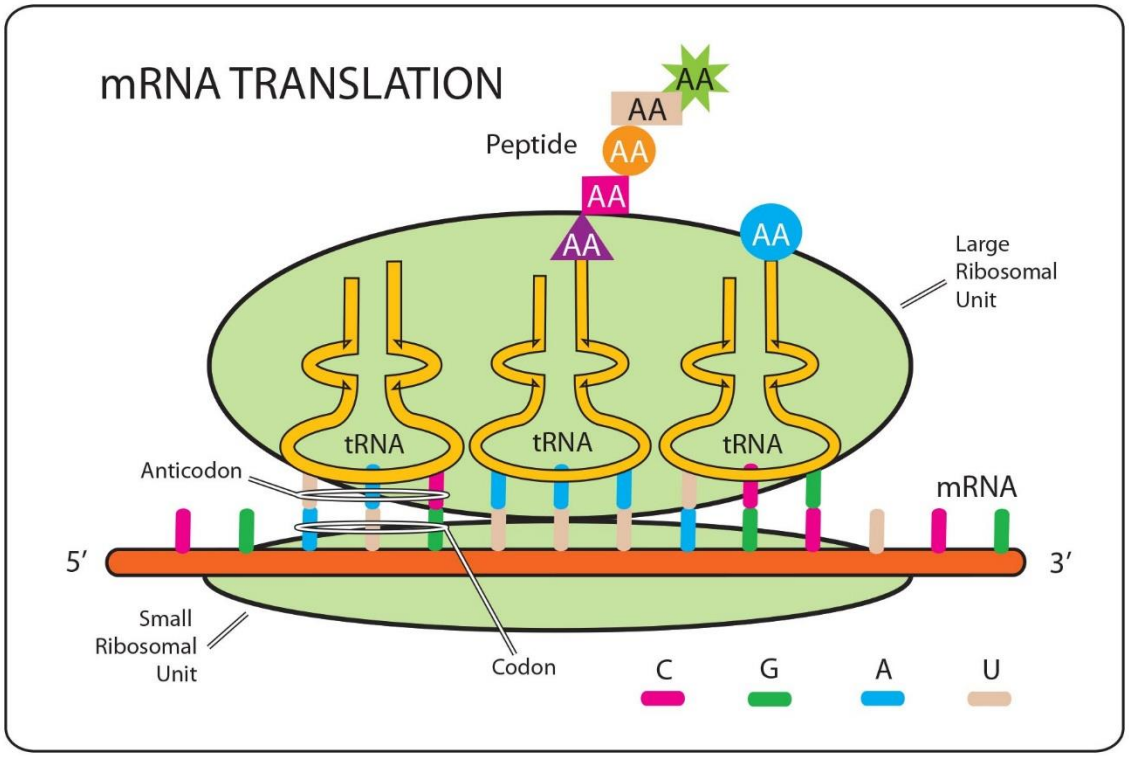

Figure 4-8 © 2015 David G. Ward, PhD

Termination

Elongation continues until a termination codon is reached at the end of the mRNA.

Destination of proteins

All translation begins on free ribosomes. At the beginning of elongation, the signal sequence of the mRNA produces a **signal protein**. The signal protein directs the ribosome to remain free in the cytosol or to attach to the endoplasmic reticulum.

The signal protein will direct ribosomes to remain ***free*** if the ribosome is synthesizing the following classes of proteins.

1) Proteins destined to remain in the cytosol
2) Proteins that will attach to the inner surface of the plasma membrane
3) Proteins that are transported into the nucleus
4) Proteins to be incorporated into peroxisomes, and mitochondria

The signal protein will direct ribosomes to ***attach to the endoplasmic reticulum*** if the ribosome is synthesizing the following classes of proteins.

1) Proteins secreted from the cell
2) Integral transmembrane proteins
3) Soluble proteins that will reside in the ER, Golgi complex, vesicles, and lysosomes.

As shown in Appendix D, Figure D.8, proteins in the ER typically leave in transport vesicles produced by budding of the ER membrane. Newly synthesized proteins from the ER enter the Golgi complex. As the proteins pass through the Golgi they are modified in specific ways:

1) The protein may be trimmed by proteolytic enzymes
2) Amino acids may be modified
3) Carbohydrates may be modified or added

Proteins in the Golgi complex typically leave in vesicles produced by budding of the Golgi membrane.

Regulation of Gene Expression

Different genes are expressed by cells at different stages of development, by cells in different tissues, and by cells exposed to different chemical messengers and stimuli.

- ***In a given cell, only a fraction of genes are expressed at any given time***.

Gene expression is the use of a gene to make a protein. Gene expression can be regulated through transcriptional control, processing control, and translational control.

Transcriptional Control

The transcription of the tDNA of a gene is critically dependent on the binding of RNA polymerase II to the core (proximal) promoter region of a gene. In transcriptional control, numerous chemical messengers (transcription factors) must bind to **proximal promoter elements** to allow binding of the RNA polymerase. Accordingly, there are a large number of ways that chemical messengers enhance or inhibit expression of a gene.

In addition, binding sites further removed from the core promoter region of a gene can influence the initiation of transcription. These binding sites are often referred to as distal promoter elements, and include the **response elements** (**RE**) and enhancer elements. Notable transcription factors that bind to response elements include cAMP response element binding protein (**CREB**), glucocorticoid response element binding protein (**GREB**), mineralocorticoid response element binding protein (**MREB**), thyroid hormone response element binding protein (**TREB**), and insulin response element binding protein (**IREB**). Response elements seem to be involved in orchestrating the expression of distinct and separate genes.

The role of response elements and response element binding proteins in the regulation of gene expression by the hormone cortisol is illustrated in Figure 4-9. Cortisol binds to GREB and forms a cortisol-GREB complex which then passes through the nuclear pores. The cortisol-GREB complex then binds to the glucocorticoid response elements (GRE) of various genes and allows transcription of these genes by RNA polymerases.

- ***Response element binding proteins together with their chemical messenger are transcription factors***.

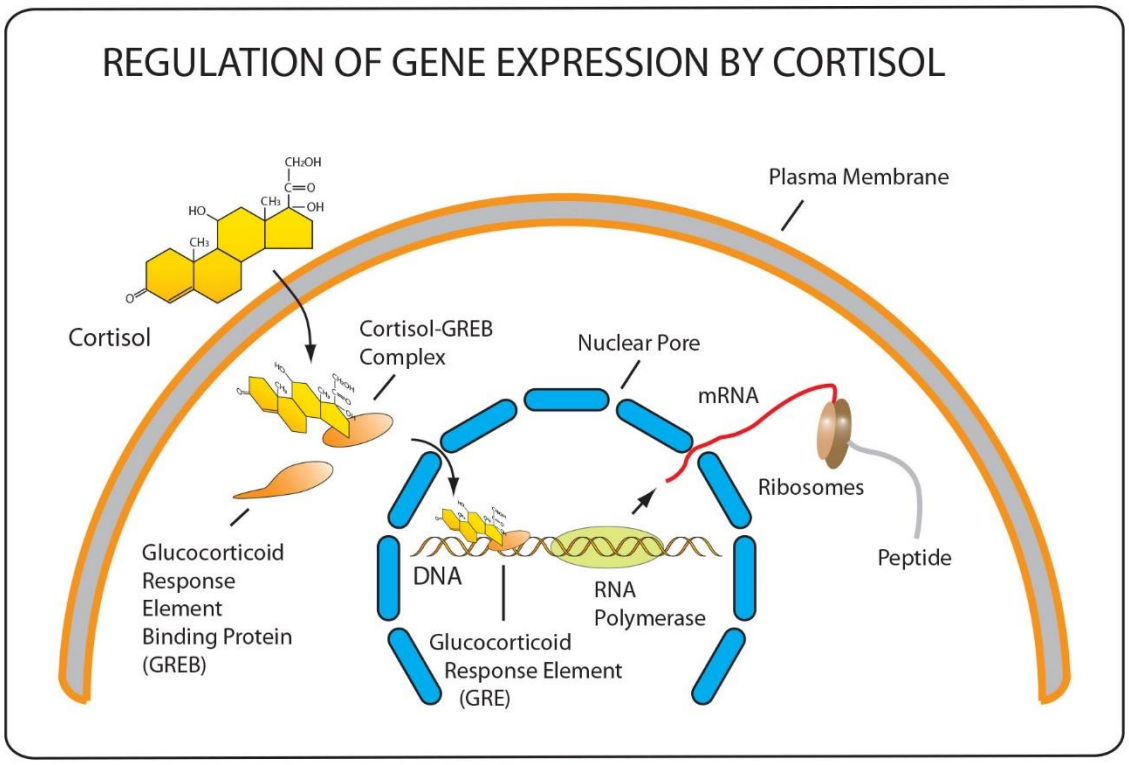

Figure 4-9 © 2014 David G. Ward, PhD

- ***Response element binding proteins are critical for much of the regulation of gene expression by hormones***.

Enhancer sequences act to increase the rate at which genes are transcribed, and their effects can be quite powerful. Enhancers can be thousands of nucleotides away from the promoters with which they interact, but they are brought into proximity by the looping of DNA (e.g. looped domains, Figure 4-1). This looping is the result of interactions between the proteins bound to the enhancer and those bound to the promoter. The proteins that facilitate this looping are called activators, while those that inhibit it are called repressors.

Other Transcriptional and Processing Control

Alteration of promoter strength can have deleterious effects upon a cell, often resulting in disease. For example, some tumor-promoting viruses transform healthy cells by inserting strong promoters in the vicinity of growth-stimulating genes, while translocations in some cancer cells place genes that should be "turned off" in the proximity of strong promoters or enhancers.

Furthermore, transcription of portions of DNA can be prevented by methylation of the DNA or by incorporating the DNA into nucleosomes (see, Figure 4-1).

Finally, the processing of pre-mRNA can be controlled to produce mRNA with different combinations or sequences of exons, and thus to produce different peptides.

Quiz Yourself

1-5. Matching

A)	Purine	Uracil	1) _____
B)	Pyrimidine	Cytosine	2) _____
C)	none of the above	Thymine	3) _____
		Adenine	4) _____
		Guanine	5) _____

6-10. Matching

A)	adenine	pairs with adenine	6) _____
B)	guanine	pairs with guanine	7) _____
C)	thymine	pairs with thymine	8) _____
D)	cytosine	pairs with cytosine	9) _____
		pairs with uracil	10) _____

11-15. Place the following steps of Transcription in order.

A)	introns are removed	first	11) _____
B)	messenger RNA moves to cytoplasm of cell	second	12) _____
C)	RNA polymerase binds at the promoter sequence of the DNA	third	13) _____
D)	precursor RNA formed from DNA - includes introns and exons	fourth	14) _____
E)	messenger RNA formed by splicing together exons of the precursor RNA	fifth	15) _____

16-20. Place the following steps of Translation in order.

A)	tRNA with an appropriate anticodon carries in a specific amino acid	first	16) _____
B)	adjacent amino acids are linked by peptide bonds	second	17) _____
C)	a termination sequence is reached	third	18) _____
D)	mRNA attaches to ribosomes	fourth	19) _____
E)	mRNA leaves the nucleus	fifth	20) _____

Fill in

21. Ribose or deoxyribose, a nitrogenous base and phosphate make a _____.

22. RNA nucleotides contain _____ oxygen than DNA nucleotides.

24- The strands of the DNA double helix are held together by hydrogen bonds between _____ and _____.

24. The tDNA (template DNA) nucleotide sequence Cytosine, Adenine, Thymine will lead to the messenger RNA nucleotide sequence _____ _____ _____.

25. In order to act, RNA polymerase must attach to the _____ region of the gene.

Study Questions

1. Compare and contrast the composition and organization of DNA and RNA.
2. Explain what is meant by “gene expression” and explain how transcription factors (chemical messengers) control gene expression.
3. Describe the major steps in transcribing DNA into mRNA, in translating mRNA into protein.