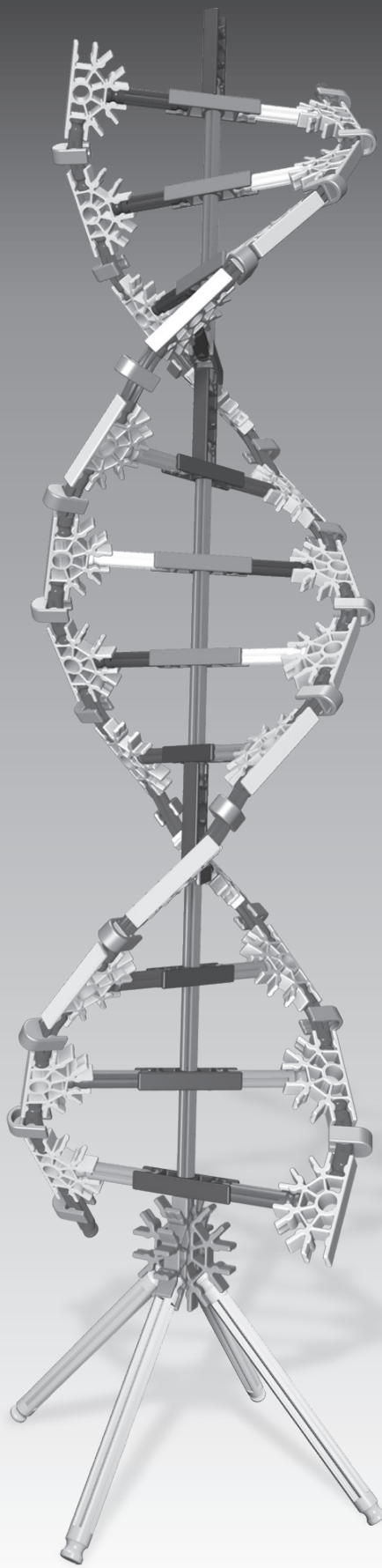
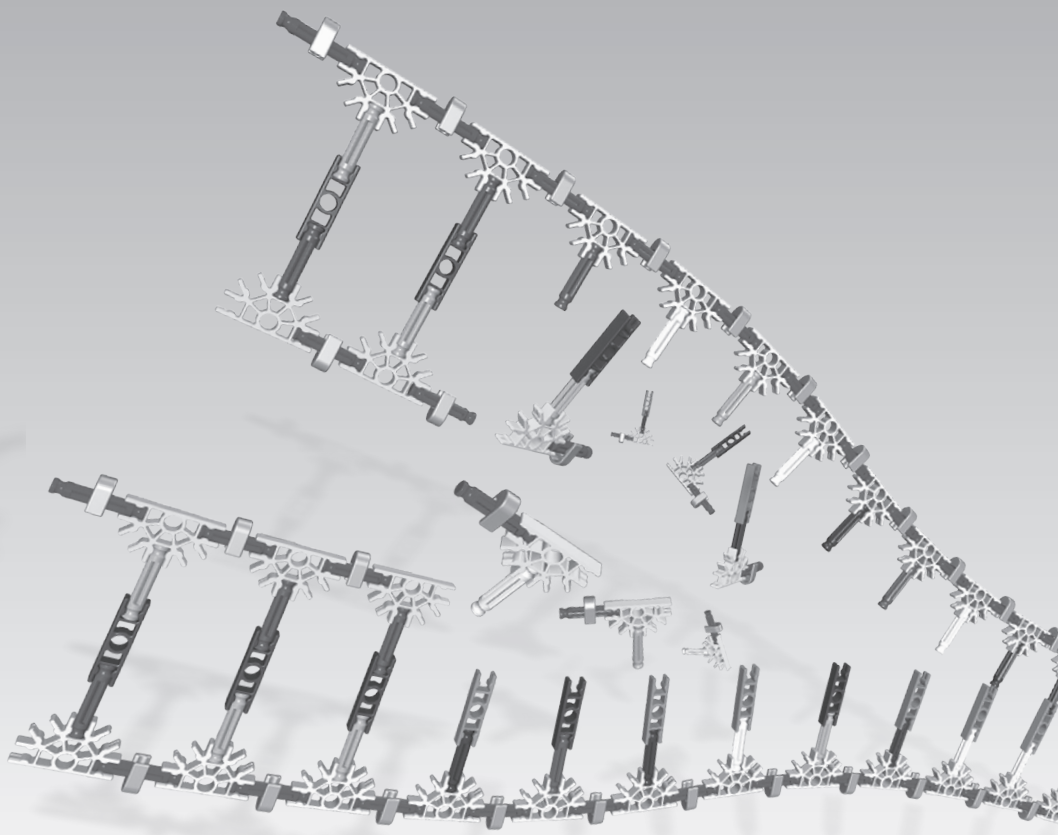
K'NeX Education®

TEACHER'S GUIDE

DNA, REPLICATION AND TRANSCRIPTION

DNA MOLECULE





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DNA, Replication and Transcription

Teacher's Guide

V4 - 11/14

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A NOTE ABOUT SAFETY:

Safety is of primary concern in science and technology classrooms. It is recommended that you develop a set of rules that governs the safe, proper use of K'NEX in your classroom. Caution students to keep hands, face, hair and clothing away from all moving parts.

⚠ WARNING:
CHOKING HAZARD – Small parts.
Not for children under 3 years.

⚠ ATTENTION :
RISQUE D'ÉTOUFFEMENT – Pièces de petite taille.
Ne convient pas aux enfants de moins de 3 ans.

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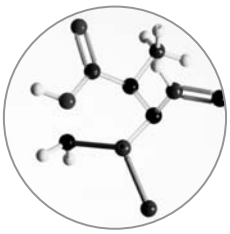
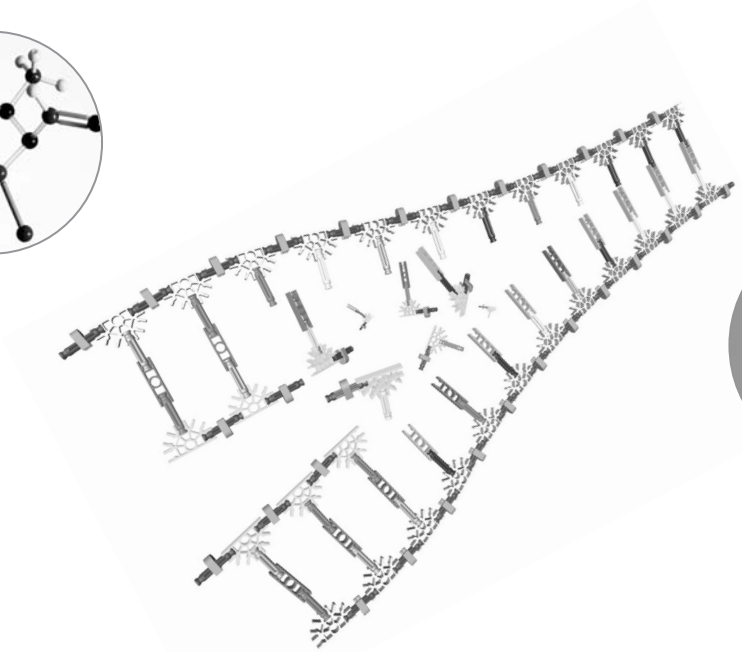
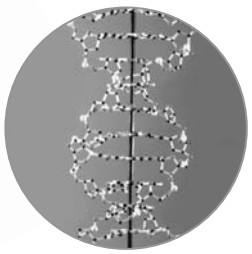
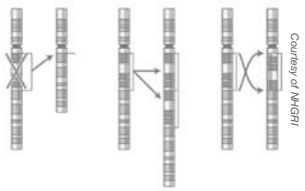








Courtesy of NHGRI

OVERVIEW

*The **K'NEX DNA, Replication and Transcription kit and this Teacher's Guide** are designed to aid in teaching the structure and function of the nucleic acid molecules that make up DNA (**d**eoxyribo**n**ucleic **a**cids) and RNA (**r**ibo**n**ucleic **a**cids). The **K'NEX DNA, Replication and Transcription kit** contains the materials needed to complete the basic lessons described by this manual.*

This Teachers Guide provides seven lessons that can be used to take students through three instructional modules:

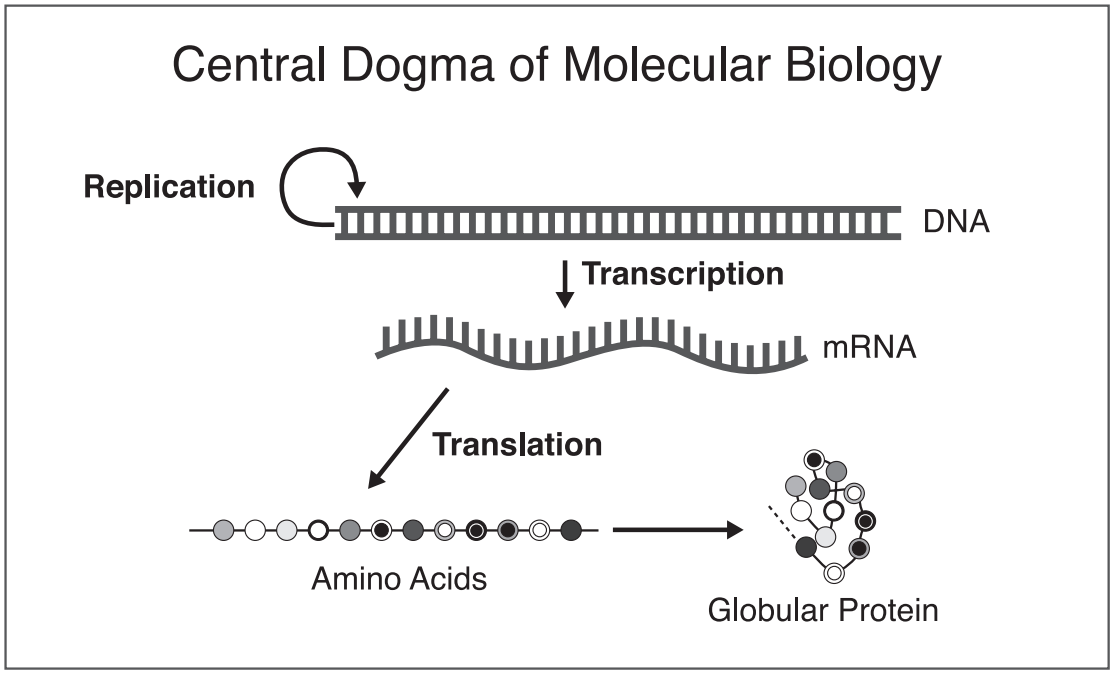
I. DNA Structure
II. Replication & Transcription
III. Coding, Translation, and Mutations.

Each basic lesson has been designed for a typical classroom period of 30-45 minutes. Suggestions for extension activities and advanced concept applications are provided and, where appropriate, easily accessed, supporting Internet resources are identified.

The use of student journals is encouraged for recording methodologies, observations, hypotheses, and results. Student journals may be considered the counterpart of laboratory notebooks, which are key to good science. Many patents and science misconduct hearings have been decided based on the records that scientists kept in laboratory notebooks or journals. Future scientific progress relies on accurately recording current discoveries.

OBJECTIVES

DNA replication and the processes required for the conversion of DNA to RNA to proteins are frequently referred to as the "Central Dogma of Molecular Biology." The key events in this central theory of life are **replication, transcription,** and **translation.**



This Teacher's Guide will support instruction on the structure of DNA and RNA and on their functions in the processes of replication, transcription and translation. While the kit itself provides an excellent demonstration tool at the elementary school level, the curriculum contained in this Guide is geared to middle and high school students. Content and activities appropriate only for more advanced or advanced placement (AP) students are enclosed in a text box and preceded by the symbol ∆.

Assessment In A Hands-On Environment

Assessment in a hands-on environment should provide opportunities for students to manipulate materials. For the **K'NEX DNA, Replication and Transcription kits**, such activities might include: building structures, transcribing DNA, or simulating mutation events. Throughout this Teacher's Guide, there will be activities labeled "Create/Assess" that provide ideas for inquiry-based extensions of the basic lesson. These activities will both challenge and reinforce student understanding of the basic concepts and, therefore, are suitable for use in assessment.

National Science Education Standards Alignment

The structure and function of DNA and RNA involve core concepts that are essential to fields of study ranging from medicine to forensics. Legal and ethical decisions that require an understanding of basic DNA-RNA concepts and related technology are made daily in our society, making this content an essential part of science education.

The **K'NEX DNA, Replication and Transcription kit** and Teacher's Guide can be used to support science curricula at Grades 5-8 and 9-12 levels with hands-on, inquiry-based instruction. This instruction aligns with the National Science Education Standards as shown in Table 1 below. Students and teachers are also encouraged to use the U.S. Department of Energy's genome web site at **http://www.doegenomes.org/** for easy access to a wealth of additional information and teaching resources on DNA, genomics and proteomics.

National Science Education Standards[1]		
	LEVELS 5 - 8	**LEVELS 9 - 12**
Unifying Concepts & Processes	• Evidence, models and explanation	
Science As Inquiry	• Abilities necessary to do scientific inquiry • Understanding about scientific inquiry	
Life Science	• Structure and function in living systems • Reproduction and Heredity • Diversity and adaptations of organisms	• Molecular basis of heredity • Biological evolution
Science & Technology	• Understanding about science and technology	
Science in Personal & Social Perspectives	• Personal health • Science and technology in society	• Personal and community health • Science and technology in local, national, and global challenges

Table 1 – Alignment of K'NEX DNA, REPLICATION AND TRANSCRIPTION lessons with the National Science Education Standards.[1]

KEY TERMS AND DEFINITIONS

Key Terms and Definitions are presented below in alphabetical order. Precise vocabulary has been chosen for clarity and correctness. Please refer to your text and curriculum for grade-level appropriate terms or definitions. Many of these definitions will be used in several different lessons. The teacher is urged to refer to this section as needed while preparing and presenting each of the seven lessons. It may also be helpful to provide students with this list.

1. **Anticodon** – a sequence of three (3) nucleotides found at a specific site on a transfer RNA molecule (tRNA) to complement a specific messenger RNA (mRNA) codon. The anticodon corresponds to a specific amino acid that is attached to the tRNA for transfer to a protein by that tRNA molecule.

2. **Antiparallel** – a term describing the two side rails of the ladder-like structure of a double-stranded DNA molecule. The ladder is formed when two strands of DNA lie parallel to each other and are hydrogen-bonded together through the nitrogen-containing bases that form the "rungs." Repeating deoxyribose sugar and phosphate groups make up the side rails of the ladder. The way in which the sugar and phosphate groups are connected is reversed (or is "anti") in one side rail of the ladder with respect to the other. This "anti" structure is frequently denoted by indicating that one side "goes in the **3'** to **5'** direction" and the other side "goes in the **5'** to **3'** direction." The " 3' " and " 5' " refer to the specific carbon atoms on the deoxyribose sugar that are connected by phosphates to form the side rails of the ladder.

3. **Backbone** – the repeating sugar-phosphate sequence formed when nucleotides are joined together in long single strands of either DNA or RNA.

4. **Codon** – a sequence of three (3) consecutive nitrogen-containing bases on mRNA that code for an amino acid or a stop signal. Codons are the basic component of the genetic code.

5. **Complementary Base Pairs** – specific pairs of nitrogen-containing bases that always bond together when double stranded DNA is made or when RNA is formed from DNA. Specific bases have matching features that cause them to always form the same pairs. The matching pyrimidine-purine pairs found in DNA are cytosine-guanine and thymine-adenine. In RNA, uracil replaces thymine to create a uracil-adenine base pair.

6. **Covalent Bond** – a strong force that joins two atoms in a compound or molecule. The binding force results from the required sharing of electrons by two different atoms.

7. **Deoxyribose** – a simple sugar found in DNA (see Figure 1). This molecule is represented in the K'NEX DNA models by either the gray fan-shaped Connectors (parent DNA strands), or the yellow fan-shaped Connectors (daughter strands). Consistent with biochemical nomenclature, the "ose" at the end of this name signifies that this molecule is a sugar.

8. **Diamer** – two consecutive nitrogen-containing bases on one strand of DNA that have bonded together to form one molecule. This unusual bonding is often caused by exposure of DNA to ultraviolet light.

9. **DNA** – the abbreviation for **d**eoxy**r**ibonucleic **a**cid, the nucleic acid that makes up most genetic material (chromosomes). It is the genetic material that is passed from generation to generation (in most organisms) to code for the proteins that make up the organism.

10. **DNA Polymerase** – the enzyme that copies a DNA parent strand by adding nucleotides in a complementary fashion to a growing daughter strand. Consistent with biochemical nomenclature, the "ase" at the end of this name signifies that this molecule is an enzyme.

11. **DNA Coding Strand** – the strand of double-stranded DNA that is complementary to the template DNA strand, which is transcribed by RNA polymerase to form the complementary mRNA.

12. **DNA Template Strand** – the strand of double-stranded DNA that is transcribed and, hence, is complementary to mRNA.

13. **Exons** – sections of the mRNA template that code for amino acids.

14. **Genetic Code** – the 64 possible combinations of three nitrogen-containing bases (codons) found in DNA. Each specific sequence of three bases codes for a specific amino acid or "stop" command in the formation of proteins from DNA via RNA (see Table 2).

15. **Glucagon** – a small regulatory protein that plays a role in the conversion of sugar to energy (sugar metabolism) in humans and many other animals.

16. **Helicase** – a cellular enzyme that unwinds DNA and breaks the hydrogen bonds between paired nucleotides. Consistent with biochemical nomenclature, the "ase" at the end of this name signifies that this molecule is an enzyme.

17. **Helix or Double Helix** – the structure that native DNA takes in a chromosome when two complementary strands join together.

18. **Hydrogen Bond** – A special type of attraction that makes up the force holding two single strands of DNA together when a double-stranded helical ladder is formed. In DNA, this is a relatively weak bonding force found only between hydrogen and either nitrogen or oxygen atoms.

19. **Introns** – sections of the mRNA template that are cut out prior to translation into protein and, hence, do not code for amino acids.

20. **Lagging Strand** – the strand of double-stranded DNA that replicates later than, but immediately following, the leading strand.

21. **Leading Strand** – the strand of double-stranded DNA that is replicated first during the replication process.

22. **Ligase** – an enzyme that links together short fragments of DNA (Okazaki fragments) as they are synthesized on the lagging strand during DNA replication. Consistent with biochemical nomenclature, the "ase" at the end of this name signifies that this molecule is an enzyme.

23. **Methylation** – a chemical process involving the addition of a small, one-carbon unit to a larger molecule. Following DNA replication, certain nitrogen-containing bases are methylated because this makes them resistant to other cellular processes designed to destroy foreign and mutated DNA, which would be lacking the methylation pattern specific for that cell or organism.

24. **Molecule** – any group of atoms that are tightly bonded together to form a single structure.

25. **Nitrogen-containing Base** – a term used to refer to any one of the five nitrogen-containing molecules that make up a nucleotide (see Figures 2 and 3).

26. **Nucleotide** - the repeating structural unit that forms both DNA and RNA. It consists of three parts: 1) a nitrogen-containing base (purine or pyrmidine); 2) a phosphate group; and 3) a sugar (either deoxyribose or ribose – see Figure 1).

KEY TERMS AND DEFINITIONS

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27. **Okazaki Fragments** –segments of DNA that form on the lagging strand during replication. The fragments are later bonded together by ligase (an enzyme) to form one continuous new daughter strand.

28. **Phosphodiester Bond** – a special type of covalent bond that holds together the basic nucleotide units in DNA and RNA.

29. **Pitch** – the distance between any two points marking the beginning and ending of one complete turn of the helix when following either backbone making up a DNA double helix.

30. **Purine** – a class of chemical compounds built from a common double-ring structure containing five carbon and four nitrogen atoms. Examples found in DNA are adenine and guanine (see Figure 3). These are represented in the K'NEX model by white (adenine) and silver (guanine) connecting Rods

31. **Pyrimidine** – a class of chemical compounds built from a common single ring structure containing four carbon and two nitrogen atoms. Examples found in DNA are cytosine and thymine (see Figure 2). These are represented in the K'NEX model by teal (cytosine) and black (thymine) connecting Rods. Thymine is replaced by uracil in RNA. This is represented by a purple connecting Rod.

32. **Replication** – the process of creating additional copies of a piece of double-stranded DNA. This process is used by cells to duplicate their genetic material for distribution to new cells when organisms reproduce.

33. **Replication Fork** - a "Y" in a double-stranded DNA molecule where strands separate; the site in the parent DNA molecule where DNA replication occurs.

34. **Ribose** – a simple sugar found in RNA (see Figure 1). This molecule is represented in the RNA K'NEX model by the orange, flanged, fan-shaped Connectors. Consistent with biochemical nomenclature, the "ose" at the end of this name signifies that this molecule is a sugar.

35. **Rise** – The distance between two consecutive rungs of the DNA ladder (consecutive nitrogen-containing base-pairs) when DNA is in a double helical form.

36. **RNA polymerase** – the enzyme that creates RNA from DNA. Consistent with biochemical nomenclature, the "ase" at the end of this name signifies that this molecule is an enzyme.

37. **RNA** – the abbreviation for **r**ibo**n**ucleic **a**cid, the single-stranded molecule having a nucleotide sequence determined by DNA. Messenger RNA (mRNA) is used to make proteins within the cell. Other forms of RNA also exist and have other functions in the cell.

38. **Transcription** – the process of turning DNA into messenger RNA, which may then serve as a template for protein synthesis.

39. **Translation** – the process of turning messenger RNA into a protein.

40. **Triplet** – a sequence of three (3) nucleotides found on a strand of DNA or RNA to code for a specific amino acid.

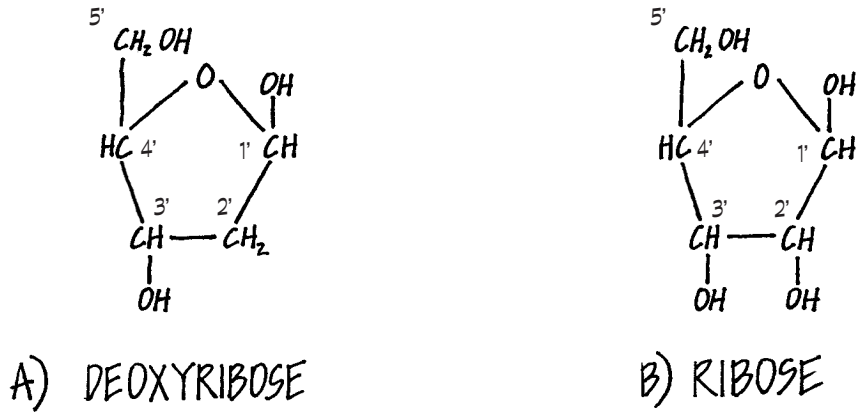

Figure 1 – Chemical structures and numbering systems for sugars found in DNA and RNA.

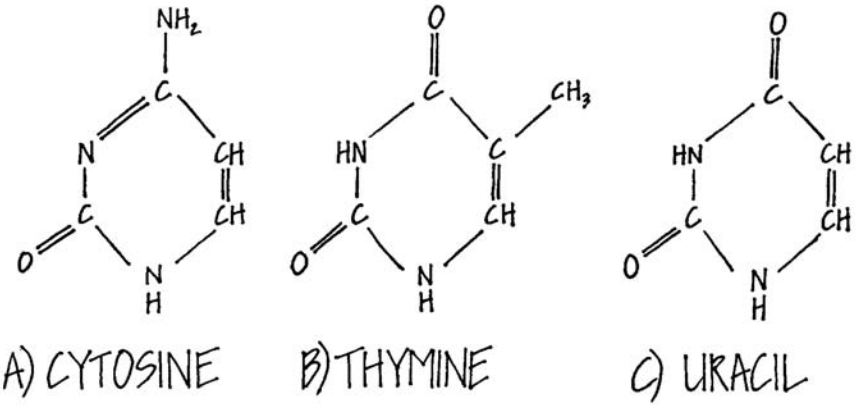

Figure 2 – Chemical structures for the pyrimidine class of nitrogen-containing bases found in DNA and RNA.

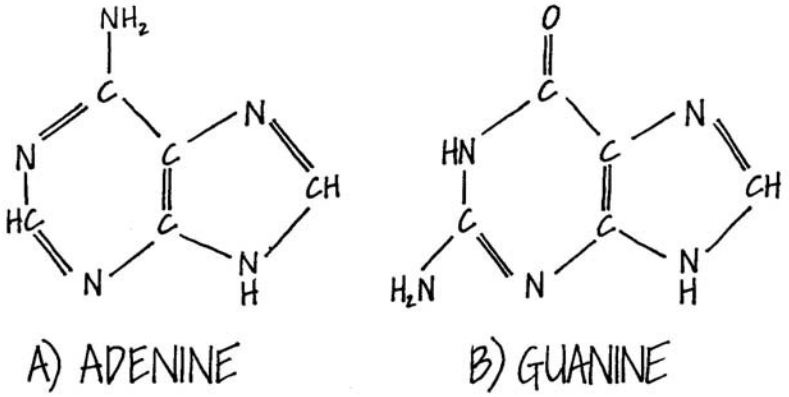

Figure 3 – Chemical structures for the purine class of nitrogen-containing bases found in found in DNA and RNA.

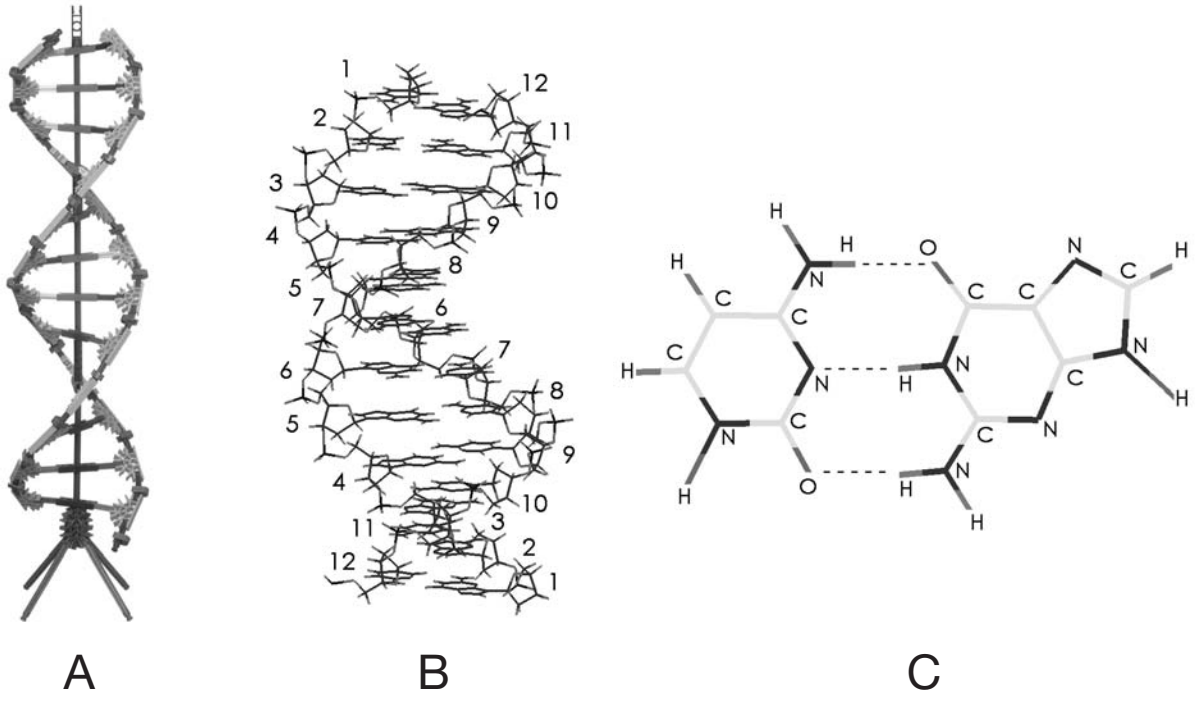

Figure 4 – (A) K'NEX double helix model; (B) corresponding computational representation of double-stranded DNA molecule with twelve base pairs; and (C) an individual cytosine-guanine base pair showing hydrogen bonds.

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Table 2 – The Genetic Code: the three-letter codes representing the possible sequences of nitrogen-containing bases found in mRNA to represent the 20 amino acids and stop codons. For each line of each cell of the table, the first three capital letters are the one-letter abbreviations for the nucleotide triplet making up the codon. Except for "stop" codons, the codon triplet is followed by both the three-letter and one-letter abbreviations for the encoded amino acid.

First Position	Second Position				Third Position
	UUU Phe F*	UCU Ser S	UAU Tyr Y	UGU Cys C	
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C	
	UUA Leu L	UCA Ser S	UAA Stop	UGA Stop	
	UUG Leu L	UCG Ser S	UAG Stop	UGG Trp W	
	CUU Leu L	CCU Pro P	CAU His H	CGU Arg R	
	CUC Leu L	CCC Pro P	CAC His H	CGC Arg R	
	CUA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R	
	CUG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R	
	AUU Ile I	ACU Thr T	AAU Asn N	AGU Ser S	
	AUC Ile I	ACC Thr T	AAC Asn N	AGC Ser S	
	AUA Ile I	ACA Thr T	AAA Lys K	AGA Arg R	
	AUG Met M	ACG Thr T	AAG Lys K	AGG Arg R	
	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G	
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G	
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gky G	
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G	

[Source for Table 2: Fig. 5.6, p.143 from BIOCHEMISTRY, 3rd ed. by Christopher K. Mathews, K.E. van Holde and Kevin G. Ahern. Copyright ©2000 by Addison Wesley Longman, Inc. Reprinted by permission of Pearson Education, Inc.]

Amino Acid	Three-letter Abbreviation	One-letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

LESSON 1: Building the DNA Ladder

OBJECTIVES

Students will be able to:

1. Describe the DNA molecule.
2. Identify the component parts of the DNA molecule (bonds, nitrogen bases, deoxyribose sugar, phosphate).
3. Identify a nucleotide.
4. Understand the concept of base pairing in double-stranded DNA.

MATERIALS

Each student group should have the following:

- 1 **K'NEX DNA, Replication and Transcription kit** with building instructions booklet
- Student journals (1 per student)

PRELIMINARY ACTIVITY: Constructing the DNA Ladder

- Students should construct the DNA ladder-like structure by following the directions on Pages 2-5 in the **K'NEX DNA, Replication and Transcription kit** building instructions booklet. The flat DNA ladder that is constructed should be used as a reference for the following discussion.

- Students should **not** proceed with twisting the ladder (Step 3 on Page 4 of the building instructions booklet) until this first lesson has been completed.

INTRODUCTION: The Structure of the DNA Ladder

DNA exists most commonly in nature as a double helical structure. The double helix of DNA resembles a twisted ladder. A closer inspection of the chemical structure of the DNA ladder reveals that the side rails are made of alternating sugar and phosphate molecules. The sugar of DNA, deoxyribose, is represented by the gray piece on the K'NEX DNA model. The phosphate molecule is the light blue Clip on the K'NEX DNA model. These two molecules are connected by the purple Rod (the phosphodiester bond) on the K'NEX model.

Notice that the starting end of the DNA molecule always has a free phosphate group hanging on the 5'-end. This is demonstrated by the light blue Clip and purple Rod extending beyond the deoxyribose, (gray piece,) on each opposite side of the K'NEX double-stranded DNA model.

Δ The repeating sugar-phosphate groups making up a single strand of DNA are often referred to as the molecular backbone. The deoxyribose sugar (see Figure 1 below) has two connecting sites for the phosphate: the OH groups found on the 3'- and 5'- carbons (spoken "three-prime and five-prime carbons"). The reason these carbons are numbered with the prime system is because the attached nitrogen-containing bases are the starting point for the molecular numbering system. Carbons on attached molecules are numbered with the prime system.

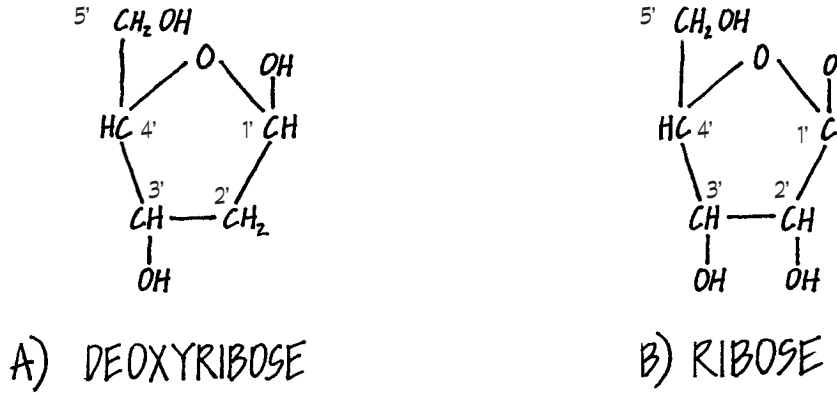
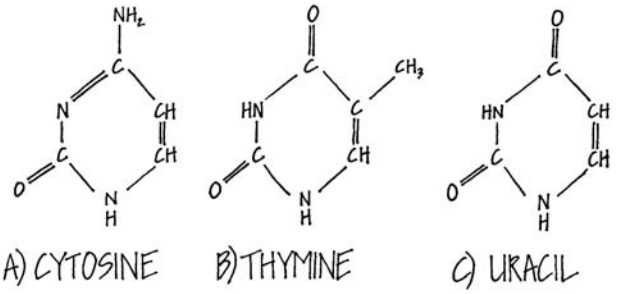


Figure 1 – Chemical structures and numbering systems for sugars found in DNA and RNA.

Δ Individual nucleotides are formed with the phosphate attached to the 5'- carbon. As new DNA strands are formed (see Module 2: Replication), new deoxyribose sugars with phosphates already attached to their 5'- carbons always connect to the growing strand by bonding to the OH of the 3'- carbon of the last sugar of the growing chain. To visualize this process, one can think of the phosphate on the 5'- carbon of the new nucleotide as a lasso that is used to grab the 3'- OH and attach the new unit to the growing chain. As a result, every strand of DNA has one free 5'- end with an unattached phosphate (the first unused lasso).

The "rungs" of the DNA ladder are composed of nitrogen-containing bases. There are four different nitrogen-containing bases in the DNA molecule: adenine (white Rod), thymine (black Rod), cytosine (teal Rod), and guanine (silver Rod). These nitrogen-containing bases pair in a very specific way to form the individual rungs of the ladder. Adenine always pairs with thymine, and cytosine always pairs with guanine. These pairs, adenine bonded to thymine and cytosine bonded to guanine, are called complementary base pairs. These pairings maintain the parallel sides of the DNA molecule because they have a common length. One larger purine base always pairs with one smaller pyrimidine base (see Figures 2 and 3 below).

Figure 2 – Chemical structures for the pyrimidine class of nitrogen-containing bases found in DNA and RNA.



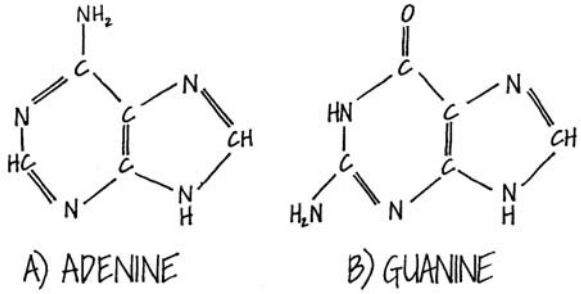


Figure 3 – Chemical structures for the purine class of nitrogen-containing bases found in DNA and RNA.

Adenine, thymine, cytosine, and guanine are usually referred to by one-letter codes, A, T, C, and G respectively, when recording their sequence in a single strand of DNA. When DNA forms a ladder or double-stranded structure, the sequence of one strand is always complementary to the other. For example, a strand with the sequence 5'-AACGGT-3' will bind to a strand with the complementary sequence 3'-TTGCCA-5'.

5'-AACGGT-3'

3'-TTGCCA-5'

The complementary base pairs forming each rung of the ladder are firmly held together by multiple hydrogen bonds. These are represented in the K'NEX model by brown Connectors (representing the three hydrogen bonds that hold together guanine and cytosine,) and orange Connectors (representing the two hydrogen bonds that hold together adenine and thymine.)

Important Note: The size of the Connectors representing the hydrogen bonds is not to scale. These bonding distances are actually a fraction of the length of the two nitrogen-containing bases being linked together (see Figure 4C).

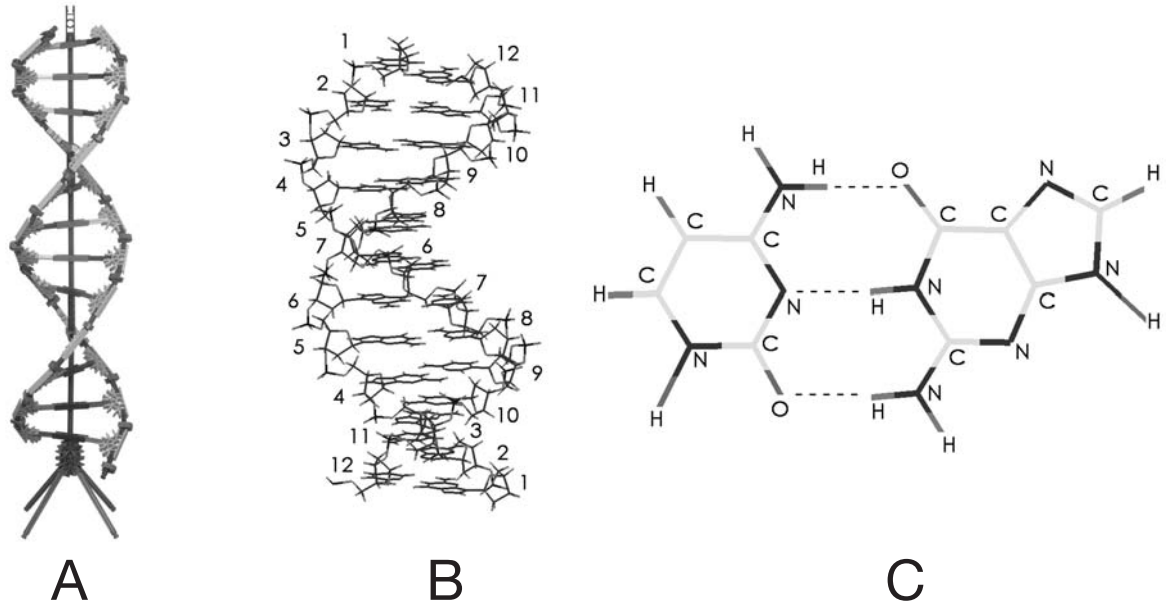


Figure 4 – (A) K'NEX double helix model; (B) corresponding computational representation of double-stranded DNA molecule with twelve base pairs; and (C) an individual cytosine-guanine base pair showing hydrogen bonds (dashed lines).

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Δ Adenine and guanine belong to a class of compounds called purines, all of which have a common 9-atom, double-ring structure. Thymine and cytosine belong to the class of compounds called pyrimidines, which are based on a common 6-atom, single ring structure. Advanced students having studied chemistry may be able to identify which atoms participate in the two hydrogen bonds between adenine and thymine and the three hydrogen bonds between cytosine and guanine. The difference in bonding energies required to form two versus three hydrogen bonds account for the increased amount of heat required to separate the two strands of a double-stranded DNA molecule when G-C combinations are found more frequently than A-T combinations.

In summary, there are three key concepts students should understand:

1) DNA is made up of sequences of nucleotides. In molecular terms, a DNA nucleotide consists of a phosphate group, a deoxyribose sugar molecule, and a nitrogen-containing base. Nucleotides are represented in the K'NEX model by the following pieces: 1 light blue Clip, 1 purple Rod, 1 gray Connector, and one of the Rods (silver, teal, white or black). Nucleotides are covalently bonded together in DNA by phosphodiester linkages (modeled by the purple Rod in the K'NEX model) to form the backbone of the molecules containing specific nucleotide sequences.

2) To create the ladder structure of the double-stranded DNA molecule, two single strands of DNA are held together by hydrogen bonds that form between complementary nitrogen-containing bases. Adenine complements thymine, and guanine complements cytosine.

3) The backbones of the two DNA strands making up the double-stranded molecule (ladder) are antiparallel. This means that one side rail lies in a 5' to 3' direction and the other lies in a 3' to 5' direction when they are linked together. Consequently, the free 5'-phosphate group (purple Rod with blue Clip) that is found on the starting end of every single-stranded DNA molecule appears on opposite ends of the two strands forming the ladder structure. A full understanding of this concept requires chemical background that is usually limited to advanced high school students (see AP notes above), but it is important for all students to note that 5' and 3' ends exist on the ladder if lessons on replication and transcription will be used.

CREATE/ASSESS

- Using the K'NEX DNA model, students should disconnect their previously built DNA molecule into nucleotide pieces and, in turn, create a single-stranded DNA molecule with the following sequence of twelve nucleotides:

5' - CACTCAGAAGGT - 3'

If several K'NEX kits are available in the classroom, each group may be given a different sequence to produce. In order to most accurately model and understand the cellular method of DNA synthesis, students should be encouraged to assemble DNA strands by first assembling the necessary nucleotides (with nitrogen-containing bases attached) prior to assembling the long backbone of repeating deoxyribose sugars and phosphate groups.

- Students should record in their journals the nucleotide sequence that they are assembling along with important notes about its construction. The following are key concepts that students should be asked:
  1. What components make up the strand?
  2. How many purines does your strand contain?
  3. How many pyrimidines does your strand contain?
  4. What clue indicates this is a DNA strand?

Students should now create a strand that is complementary to the single strand that was just completed and connect the two with the appropriate hydrogen bond pieces. The complementary strand sequence should be written in the student journals, showing the complementary base-pairing as follows:

5' - CACTCAGAAGGT - 3'

3' - GTGAGTCTTCCA - 5'

Student notes should also include hypotheses about why specific nucleotide sequences and complementary base-pairing are important to the function and structure of the DNA molecule.

Comment: Specific sequences of nitrogen-containing bases code for specific proteins. Complementary base-pairing maintains the parallel nature of the molecule. Notes about Chargaff's research could also be given or inquiry-based projects assigned at this time.

Students should record hypotheses in their journals about what consequences may occur if sequences of nucleotides in the DNA are altered.

APPLY

Questions/Activities for students:

- When the gray Connector is snapped together with the light blue Clip and the purple Rod, what bond is being simulated? (*The phosphodiester bond*)
- Explain complementary base pairing and how it affects the DNA molecule structure.
- Draw a picture of what the DNA ladder would look like if purines matched with purines and pyrimidines matched with pyrimidines.
- What type of bond is the phosphodiester bond? (*A covalent bond*)

- Δ - Draw the complementary nitrogen-containing base pairs and show the hydrogen bonding sites. There should be three hydrogen bonds for a G-C pair and two for an A-T pair.

EXTENSION ACTIVITIES

- Students may write a position paper concerning important issues and questions relating to DNA. Topics may include moral, ethical, and societal issues related to genetic engineering, genetically-modified organisms (GMOs), and the struggles of women and minority scientists (e.g., Rosalind Franklin and George Washington Caver). An excellent reading to accompany this assignment would be *The Double Helix: A Personal Account of the Discovery of DNA* by James Watson.[2] K'NEX models should be built to demonstrate points made in these papers.

Δ EXTENSION ACTIVITIES

- Students who are more advanced in their DNA structure knowledge should be presented with this lesson as a discovery lesson. The **K'NEX DNA, Replication and Transcription kit** instruction booklet may be withheld and only K'NEX piece identifications should be given to the students. Students should attempt to build the DNA model using their knowledge of nucleotide structure. The most advanced students may use the Internet to look up sections of specific known nucleotide sequences for proteins and create a corresponding DNA segment with their model (see web sites 2, 3, and 4 at the end of this lesson). This activity could serve as a lab practicum format for assessment.

- Students may enjoy researching Rosalind Franklin and her X-ray crystallography studies that helped to determine the structure of the DNA double helix. A short essay may be written describing how Watson and Crick made their conclusions on the structure of DNA using her research and her pictures of the molecule. Examples of how the molecule is not structured (based on earlier proposals by Watson and Crick) should be constructed using the **K'NEX DNA, Replication and Transcription kit.** An excellent resource is James Watson's autobiographical

account of the discovery of the double helix, The Double Helix: A Personal Account of the Discovery of DNA[2], which is listed in the references at the end of this lesson.

- Essay/Research: Students may study and research PCR (polymerase chain reaction). The steps of PCR may be detailed and practical uses of PCR discussed. **K'NEX DNA, Replication and Transcription kits** could be used to demonstrate the steps of PCR.

- Students may use HyperChem® or other computational programs to make a computer model of DNA and compare the exact chemical structure and dimensions to their K'NEX model. A free trial version of HyperChem® may be downloaded at the Hypercube, Inc. web site. Alternatively, students may find Chime/Protein Explorer graphical representations of DNA molecules on the Internet for exploration and comparison with the K'NEX models. (These activities may use web sites 6, 7, or 8 at the end of this lesson.)

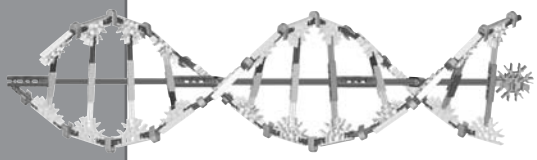
Useful Related Web Sites

1. **http://knexeducation.com//** - *K'NEX Education Homepage*
2. **http://knex.com//** - *K'NEX homepage*
3. **http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome&itool=toolbar** – *Entrez Genome NCBI Search Site (U.S. National Institutes of Health search site)*
4. **http://www.ebi.ac.uk/embl/** - *EMBL (European Bioinformatics Institute) Nucleotide Sequence Database*
5. **http://www.doegenomes.org/** - *Human Genome Program official web site.*
6. **http://www.rcsb.org/** - *Molecular Structure Databank*
7. **http://www.hyper.com//** - *Hypercube homepage*
8. **http://www.umass.edu/microbio/chime/whatis_c.htm** - *What is Chime?*
9. **http://www.proteinexplorer.org** - *1 Hour QuickTour for Protein Explorer*

References

[1]National Research Council, National Science Education Standards. National Academy Press, Washington, DC. 1996.

[2]Watson, James, The Double Helix: A Personal Account of the Discovery of DNA. New York, Atheneum, 1968.



LESSON 2: Forming the Double Helix Structure

Objectives

Students will be able to:

1. Form right-handed double helixes with ten base pairs per turn using the DNA ladder from Lesson 1 and either a stand (rigid molecule) or tubing (flexible molecule).
2. Form and identify right-handed and left-handed helixes.
3. Identify the basic structural features of the double helix: rise, pitch, and grooves.
4. Understand how double-helical DNA exists in bacteria and humans.

Materials

Each student group should have the following:

- 1 **K'NEX DNA, Replication and Transcription kit** with building instructions booklet
- Student journals (1 per student)

** NOTE: Each student group should have completed Lesson 1 and have built a flat DNA ladder before proceeding with this lesson.*

Preliminary Activity: Forming the Double Helix

- Students should construct the DNA Double Helix following the directions on Pages 6 through 9 in the **K'NEX DNA, Replication and Transcription** building instructions booklet.

Introduction: The Double-helical Structure

DNA is most commonly found in a double helical structure in living cells. This means that when two pieces of single-stranded DNA bond together (through complementary base pairing) to form the ladder structure discussed in Lesson 1, the ladder tends to twist into a coil or "helix." This coiling occurs naturally due to the chemistry of the atoms involved. Basic chemical principles tell us that any three atoms in a row will arrange themselves to form a specific bond angle that is determined by the atoms involved and any other atoms to which they are bonded. The net result of all the atoms in a DNA molecule selecting their preferred bond angles is the double helix. For students who have not yet had chemistry, this structural preference probably can be best explained by summarizing that the chemistries of the elements making up DNA cause it to form a double helix more easily than any other three-dimensional structure.

Double-stranded DNA most frequently coils into a right-handed helix. This preference again is due to the chemistry of the atoms involved. The **K'NEX DNA, Replication and Transcription** building instructions booklet describes how to create a right-handed double helix. Students should inspect their DNA double-helixes as they wind them onto the vertical stands and before threading them onto the flexible purple tubes to be sure that they are right-handed. A student can double-check the "handedness" of the coil by holding his or her right hand in a "thumbs up" position beside the vertical model. When in the thumbs-up position, an imaginary string connected to the pointer finger of the right hand would wind around the thumb in a right-handed helix if the winding is initiated in the same direction the finger is pointing. Thus, the thumb of the right hand corresponds to the central red rod used to support the K'NEX double helix and the backbones should wrap in the direction pointed by the fingers.

A final consideration for students is how tight to make the DNA helix. The tightness of the coil formed by double-stranded DNA also is predetermined by the chemical properties of the atoms. The most "comfortable" twist is one in which ten base pairs can be counted in one complete turn of the backbone. Note the arrows on Page 7 of the **K'NEX DNA, Replication and Transcription** building instructions booklet, which show one turn of the helix. One complete turn means the DNA backbone that forms one side rail of the DNA ladder, when viewed looking down the center of the helix, outlines a full circle. This can be difficult to visualize, however, because the paths of the two side rails (backbones) cross over each other in the formation of the helix.

When the double helix is formed, two grooves can be traced down opposite sides of the helix. These grooves are important binding sites for enzymes and other molecules in the cell that initiate DNA replication and transcription. In order to demonstrate that the double helix has two separate, non-intersecting grooves, tie a ribbon to one end of the molecule and guide the ribbon along the center of one groove running down the length of the helix. This will allow students to readily visualize the separate grooves as shown in Figure 5 below.

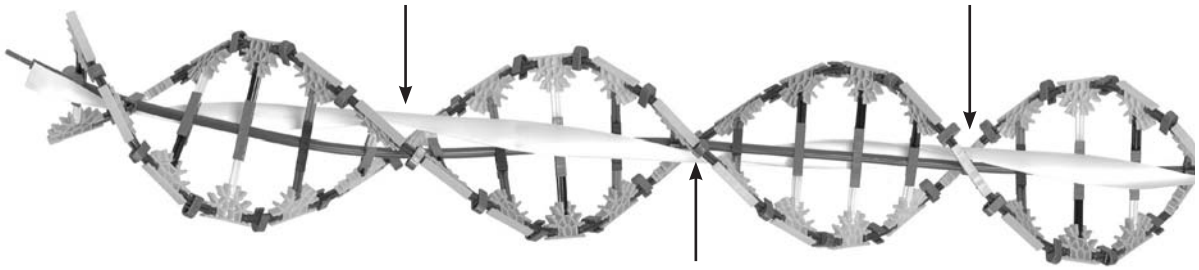


Figure 5 – Double-stranded DNA molecule with ribbon tracing one of two non-intersecting grooves. Arrows point to the groove traced by the ribbon.

Δ In the **K'NEX DNA** Double Helix model, the two grooves turn out to be the same width because the rungs of the DNA ladder are perfectly flat. However, the true DNA ladder is not flat. The base pairs forming the bridge between the two backbones meet at an angle that is somewhat less than 180 degrees, such that each rung of the ladder is v-shaped. When this "creased" ladder twists into a double helix, the result is the formation of two grooves, one of which is significantly larger than the other. Advanced texts refer to these two grooves as the "major" and "minor" grooves of the DNA Double Helix (see Figure 6 on Page 19).

The rise and pitch of the double helix can also be demonstrated. These are most easily examined while the molecule is still on the vertical stand. Rise refers to the vertical distance between two sets of complementary base pairs. Pitch refers to the number of base pairs making up one complete turn of the helix. The most commonly encountered form of DNA forms a helix with a pitch of ten (10) base pairs per turn. This means that the rise is 1/10th the length of the pitch.

Students often have a difficult time understanding the length and sub-microscopic width of the DNA molecule. For example, in man, DNA molecules are about one meter long (each molecule containing several thousand million base pairs)—this in contrast to the DNA length of simple bacteria which is around 2 millimeters long.[2] Despite this length, a single double-stranded DNA molecule cannot be seen with a normal light microscope because they are only nanometers (one billionth of a meter) wide. Chromosomes can be seen with a light microscope because a chromosome is actually a very long piece of double-stranded DNA wrapped many, many times around a string of protein "beads." Thus, the width of a leg of a chromosome is many, many times the width of double-stranded DNA.

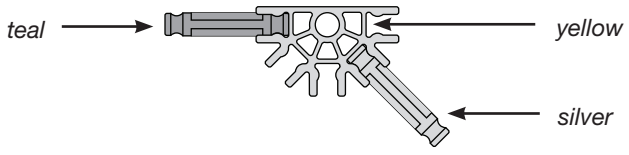
Δ - Advanced students should be able to appreciate the three levels of structure that are used to describe how DNA exists in a cell.

- The *primary* structure of DNA is simply the linear order of the consecutive nucleotides that make up the two single strands of DNA that form a double-helix. This primary structure was evident in the flat DNA ladder that was formed in Lesson 1.
- However, the twisted 3D-structure that is naturally assumed by the molecule when it forms a double helix is called the *secondary* structure of the DNA molecule.
- The *tertiary* structure, or the third level of structure, is encountered when long pieces of double-stranded DNA coil about themselves or around other molecules. For example, mammalian chromosomes are made of up chains of nucleosomes, which themselves are long sections double-stranded DNA helixes coiled around proteins called "histones." Thus, when a "chromosome spread" is examined with a light microscope, what students are actually viewing are long chains of nucleosomes. As mentioned previously, a single piece of double-stranded DNA is not visible with a light microscope.

CREATE/ASSESS

- Students should shape their DNA ladders into double helixes with ten base pairs per turn using the vertical stand. While in the more rigid vertical stand, students should identify the two grooves of the helix. Students may more easily recognize the two separate grooves by tying a wide ribbon or cord to the top of the molecule and winding the ribbon down one groove to the bottom of the molecule.
- Students should pair up so that two DNA helixes are present in the group. Students should discuss right-handedness amongst themselves to confirm understanding. After students are comfortable with identifying right-handedness, one of the DNA molecules should be removed from the stand and twisted into a left-handed helix. Students should sketch these structures in their journals with the aid of colored pencils and arrows, making sure to highlight right-handed DNA as the primary form found in organisms. Students who struggle with 3-D drawings should have reference pages of texts that demonstrate the right-handed DNA structure (if not both right and left-handed structures) documented in their journals.

Δ - Advanced students may create a DNA Double Helix with a **major** and **minor** groove by replacing all of the straight brown and orange Connectors (used to form the straight rungs of the ladder of a DNA daughter molecule – Page 14 Building Instructions booklet) with available gray and yellow Connectors. Replace the orange Connectors with gray Connectors and replace the brown Connectors with yellow Connectors. Then attach the nitrogen-containing bases from one strand into the first opening of the appropriate yellow or gray Connectors and attach their complementary nitrogen-containing bases into the fourth openings on the corresponding Connectors as shown in the drawing below. When all of the orange and brown Connectors have been replaced, a V-shaped ridge will run down the length of the DNA. After all of the rungs have been replaced, rewinding the molecule, using the red Rod stand or the purple tubing, will allow visualization of the major and minor grooves. Refer to the diagram below for help on constructing this model.



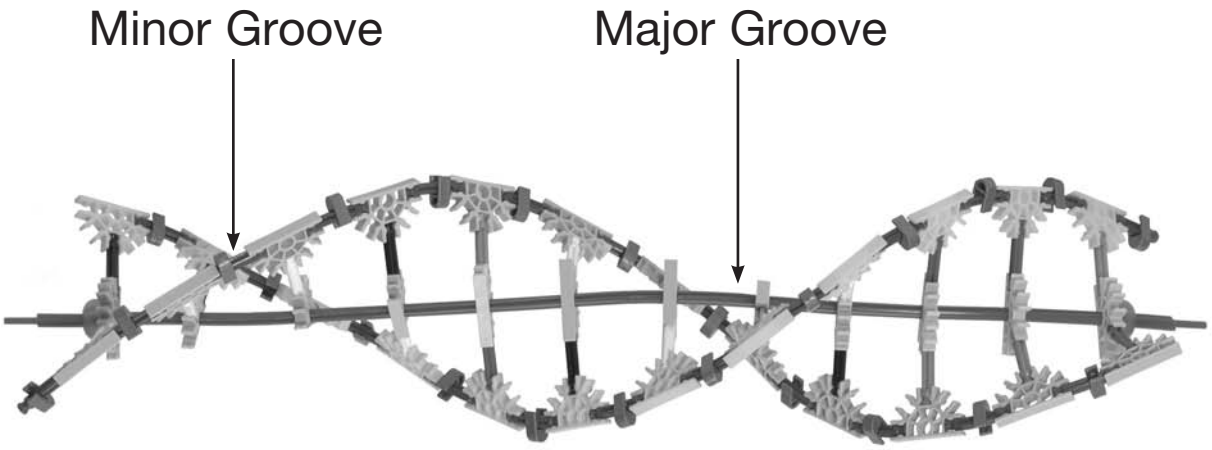


Figure 6 - DNA double helix with major and minor grooves created by constructing the model with 135-degree Connectors for H-bonds.

APPLY

Questions/Activities for students:

- This is a good time to combine some basic math and geometry with science.
  1. A full circle is 360 degrees. Ask students to calculate the number of degrees that are spanned by one base pair when the standard DNA Double Helix is formed with a pitch of 10 base pairs per turn. (Answer: 36 degrees/base pair.)

  2. Nanotechnology is one of the latest frontiers in science. Generally speaking, this term refers to technology that uses materials having special properties because the materials are present in quantities that amount to only a few atoms. "Nano" is a prefix that means one billionth (0.000000001) of whatever unit it precedes. Students should be encouraged to perform calculations to help them understand nanoscale measurements and unit conversion; these exercises may help students understand the submicroscopic size of a DNA molecule. Here are some suggested calculations with detailed answers to help demonstrate the calculations. They use the common scientific practice of unit cancellation to confirm proper conversions.

  Questions:

  a. A nanometer is equal to 10 Angstroms. There are 10^9 nanometers in one meter. How many Angstroms are in one meter?

  b. The pitch of the standard double helix is approximately 34 Angstroms. What is the pitch of the double helix in nanometers? In meters?

  c. If the standard DNA double helix has 10 base pairs per turn, what is the rise of the double helix in nanometers/base pair?

Answers:
a. Angstroms per meter:

$$\frac{10^9 \ \cancel{nanometers}}{meter} \times \frac{10 \ Angstrom}{\cancel{nanometer}} = \frac{10^{10} \ Angstroms}{meter}$$

b. Pitch: 34 Angstroms

$$34 \ \cancel{Angstroms} \times \frac{1 \ nanometer}{\cancel{10 \ Angstroms}} = 3.4 \ nanometers$$

$$3.4 \ \cancel{nanometers} \times \frac{1 \ meter}{10^9 \ \cancel{nanometers}} = 3.4 \times 10^{-9} \ meters$$

c. Rise per base pair with 10 base pairs per turn:

$$\frac{3.4 \text{ nanometers } \cancel{\text{per turn}}}{10 \text{ base pairs } \cancel{\text{per turn}}} = 0.34 \text{ nanometers/base pair}$$

3. What happens to the pitch and shape of the grooves when the long, flexible model of the Double Helix is arched like a rainbow? How might this be important when enzymes are replicating DNA?

   Students should observe that grooves on the outside of the arc become larger and those on the inside of the arc become smaller. Such changes in groove size and shape sometimes form the "recognition sites" for enzymes and other proteins that help in DNA replication and transcription. For example, an expanded groove may form the perfectly-sized pocket that fits the hand of an enzyme trying to find the right spot to begin to pry apart double-stranded DNA for replication.

Extension Activity:

- Bacteria have circular chromosomes. Have several student groups combine their DNA molecules to make a single Double Helix that is long enough to form a circle. Ask the students to explore what happens to DNA when the chromosome is joined together and is either under-wound or over-wound. This can be demonstrated by twisting the DNA strand when bringing the two ends together to form a closed loop.

Δ Extension Activities

- Below are the parameters for A, B and Z forms of double-stranded DNA. What would you have to do differently to model these forms of DNA with K'NEX? A web site with visuals for these forms is listed below.

Parameters of Polynucleotide Helixes[1]

	A Form	B Form	Z Form
Handedness of Helix	Right	Right	Left
Base Pairs per Turn	11	10	12
Rotation per Base Pair	33°	36°	-30°
Rise per Base Pair	0.26 nm	0.34 nm	0.37 nm
Pitch of Helix	2.8 nm	3.4 nm	4.5 nm

[Source: Table 4.3. p.99 from BIOCHEMISTRY, 3rd ed. by Christopher K. Mathews, K.E. van Holde and Kevin G. Ahern. Copyright ©2000 by Addison Wesley Longman, Inc. Reprinted by permission of Pearson Education, Inc.]

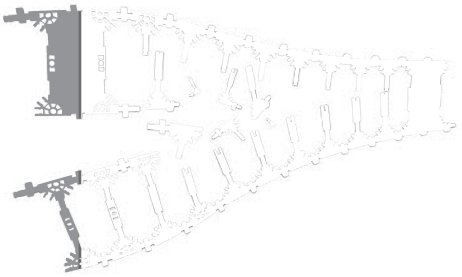
Useful Related Web Sites

http://www.lmb.uni-muenchen.de/groups/Biostruc/chap-08/chap-08-slides.html - *A, B, and Z DNA structure*

References

[1] Mathews, Christopher K., van Holde, K. E.; Ahern, Kevin G, *Biochemistry, Third Edition*. San Francisco: Benjamin Cummings. 1999.

[2] Marks, John, *Science and the Making of the Modern World.* Oxford: Heinemann Educational. 1997.



LESSON 3: The Basic Replication Process

OBJECTIVES

Students will be able to:

1. Understand the DNA replication process.
2. Identify reasons for replication of DNA.
3. Understand the need for an exact copy of DNA.
4. Understand the function of complementary base-pairing.

MATERIALS

Each student group should have the following:

- 1 **K'NEX DNA, Replication and Transcription kit** with building instructions booklet
- Student journals (1 per student)

* *NOTE: Each student group should have completed Lesson 1 and have built a flat DNA ladder before proceeding with this lesson. It is also recommended that students complete Lesson 2 so that they understand that DNA exists in a twisted, or helical, state.*

PRELIMINARY ACTIVITY: Replication of a DNA Molecule

- Students should replicate the DNA double helix following the directions on Pages 10 through 13 in the **K'NEX DNA, Replication and Transcription** building instructions booklet.

* *NOTE: If Lesson 2 has been performed, the DNA model should be uncoiled (and taken off its stand or tubing) to perform the steps on Pages 10 - 13.*

- Once the construction shown on Pages 10 - 13 has been completed and understood, students can continue by building a large, flexible DNA molecule shown on Pages 14 –17 of the building instructions.

INTRODUCTION: The Basic Replication Process

When a cell divides by either mitosis or meiosis, each new cell must have an *exact* and *complete* copy of the DNA from the original cell. The process by which each cell receives an exact copy of the original cell's DNA is called replication. The word replication implies "replica," reinforcing the concept of a process to form an *exact* duplicate of the DNA molecule.

DNA is duplicated using a "semiconservative" replication process to help ensure that exact copies of the DNA are formed. This semiconservative process means that each new DNA molecule contains one strand from the original or parent molecule (the parent strand). The other strand of the new DNA molecule that is created by using the parent strand as the template is called the daughter strand. Thus, DNA replication produces two identical DNA molecules, each consisting of one parent and one daughter strand as shown in Figure 1.

Semiconservative DNA Replication

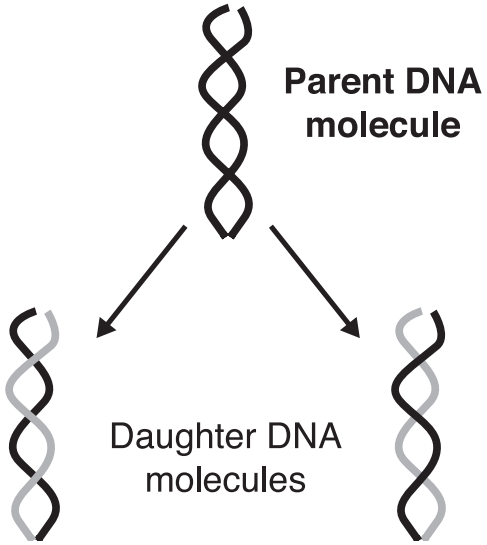


Figure 1: Semiconservative DNA replication means that one of each of the two strands found in the parent DNA molecule (black) appear in each daughter molecule. Each daughter molecule contains one new strand (gray) formed by complementary base pairing with the strand from the parent molecule.

DNA replication is a systematic process. Assisted by specific enzymes, the process is as follows:

1. **Helicase**, an enzyme, unwinds DNA and breaks the hydrogen bonds between the nucleotides creating a replication fork (a "Y") in the DNA molecule. This process is sometimes referred to as an "unzipping" of the double-stranded molecule. The replication fork or "Y" is the site in the parent DNA molecule where DNA replication occurs. The nitrogen-containing bases are now exposed for complementary pairing (DNA separates into single-strands at the fork).

2. **DNA polymerase** moves along each open side of the DNA at the replication fork, attaching free nucleotides to the exposed nitrogen-containing bases on both of the separated strands by complementary base pairing.

   [Δ - The free nucleotides are called "dNTPs" for deoxy-nucleotide-triphosphates because they actually have three phosphate groups attached before they are incorporated in the DNA.]

   DNA polymerase only works in a 5' to 3' direction, so these bases are added in a complementary fashion to the original DNA molecules in a 5' to 3' direction along the replication fork. This creates two phenomena: the leading strand and the lagging strand. These strands are represented in the **K'NEX DNA, Replication and Transcription** building instructions booklet on Pages 10 and 11 and are represented diagrammatically in Figure 2.

DNA Replication Fork

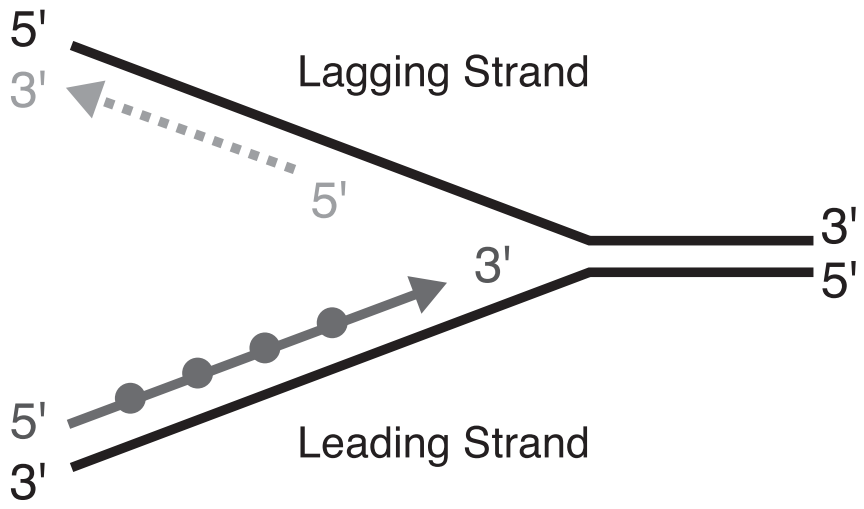


Figure 2: The replication fork of a parent DNA molecule (represented by solid lines) with 5' to 3' synthesis of the daughter of the leading strand (represented by a solid line with dots) and the daughter of the lagging strand daughter strand (represented by a dashed line). This figure should be compared with the ***K'NEX DNA, Replication and Transcription*** *building instructions booklet Pages 10 and 11.*

3. The leading strand begins to be built first in a continuous 5' to 3' direction toward the replication fork. The lagging strand (or opposite side) is built in pieces [Δ - Okazaki fragments] that are also being built in the 5' to 3' direction. Because the overall direction of the lagging strand of the replicating molecule is 3' to 5', large segments of new DNA are assembled in the 5' to 3' direction as the replication fork moves down the lagging parent strand, and these fragments are later joined together by an enzyme to form the lagging daughter strand of the replicating DNA molecule.

   [Δ - The enzyme joining these Okazaki fragments is called DNA ligase.]

   Because the fragment assembly is not continuous and begins only after a large enough segment of the parent molecule is exposed, completion of the strand's replication lags behind the leading strand. Thus, this side of the replication fork is named the lagging strand because of this contrast with the continuously building leading strand.

4. It should be noted that this sequence of events is happening at numerous sites throughout the original DNA molecule. Several replication forks may be present during the replication of any one DNA molecule. Remember, a DNA molecule may be one meter in length.

5. The end result of the replication of a single DNA molecule is two DNA molecules, identical in nucleotide base sequence. Each of the newly replicated DNA molecules consists of a daughter strand and a parent strand.

CREATE/ASSESS

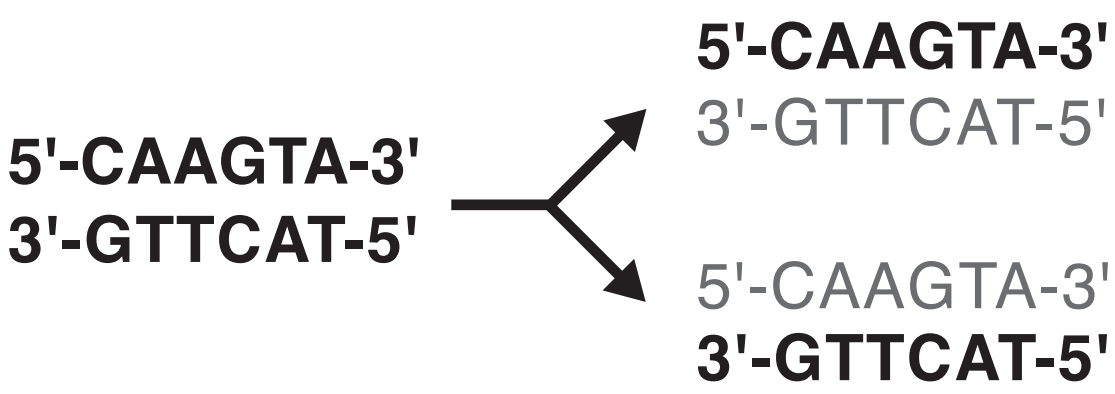
Student groups should make DNA molecules with short nucleotide sequences of their own design and then trade them with another group. Both groups should perform the necessary steps to complete one cycle of DNA replication, but with at least one intentional error in the process. Replicated DNA molecules should be exchanged back between partners and any "errors" should be identified and recorded in their student journals. Students may begin to form hypotheses about how replication errors may occur or be corrected. These hypotheses should also be recorded in the student journals.

- Δ Advanced students may create a large DNA molecule by using multiple **K'NEX DNA, Replication and Transcription kits** or the **K'NEX DNA, Replication and Transcription Class Pack**. Replication should then be demonstrated to include Okazaki fragments, applicable enzyme labels or simulated enzyme molecules, and labels for lagging and leading strands.

APPLY

Questions/Activities for students:

- Students should (diagram) create and correctly label a poster showing their preliminary DNA replication activity using different colors or shading to indicate the parent and daughter strands as shown below.



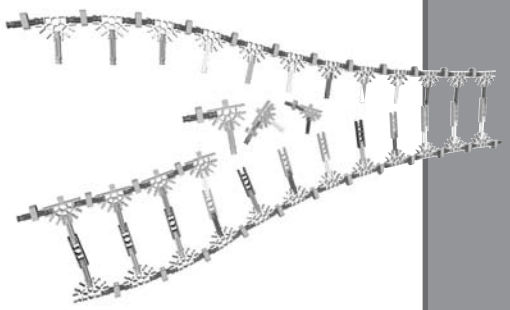
- Students should draw the cell cycle and indicate where replication occurs. (The cycle should be drawn in the student journals with the phase where replication occurs highlighted.) Students should be asked why is it important that DNA replication take place at that stage of the cell cycle.

* *NOTE: most textbooks present mitosis and the cell cycle before DNA. If this material has not been previously presented, please refer to the appropriate section of the text before assigning this activity to students.*

EXTENSION ACTIVITIES (Δ Primarily for advanced students)

- Δ- Ask students to compare and contrast DNA replication in eukaryotes to DNA replication in prokaryotes.
- Δ - Ask students to discuss the classic Meselson and Stahl experiment[1] and how their research led to the concept of semiconservative replication.
- Δ - Students may research and discuss the terms: proto-oncogene and tumor-suppressor gene. Students may explore how these genes work in the cell and how they tie in with DNA replication.
- Δ - Students may research, discuss, and give examples of how cancer drugs can affect DNA replication. Specific drugs/drug-types should be identified and their effects noted in the student journals. Questions approached during this exercise may include:
  - How do these drugs target cancer cells?
  - What new research is on the horizon that is improving the effectiveness of cancer-treating drugs?
  - In your opinion, what would be the perfect cancer-treating drug?
  - How might that drug work?
  - What type of testing would need to be done to see if your drug was working as you had hoped?

LESSON 4: mRNA Production



OBJECTIVES

Students will be able to:

1. Create mRNA from a DNA template.
2. Compare/contrast DNA and RNA.
3. Understand the genetic code and know how to read and interpret the codons.
4. Understand the function of mRNA.

MATERIALS

Each student group should have the following:

- 1 **K'NEX DNA, Replication andTranscription kit** with building instructions booklet
- Student journals (1 per student)

* *NOTE: Each student group should have completed the following before proceeding with this lesson: Lesson 1, including the construction of a flat DNA ladder; Lesson 3 and understand the value of complementary base pairing. Lesson 2 would also be advisable so that students understand that DNA exists in a twisted or helical state.*

PRELIMINARY ACTIVITY: mRNA Production

- Students should create mRNA following the directions shown on Pages 18 and 19 of the **K'NEX DNA, Replication and Transcription** building instructions booklet.

* NOTE: *If Lesson 2 has been performed (Pages 6 and 7 in the building instructions booklet), the DNA model will need to be uncoiled (and taken off its stand) in order to perform the steps shown on Pages 18 and 19.*

INTRODUCTION: mRNA Production and Its Function

RNA exists in nature as a single-stranded nucleic acid. The chemical structure of RNA has several similarities to DNA, but it also has a few remarkable differences. Like DNA, RNA nucleotides are made up of a 5-carbon sugar, a nitrogen-containing base, and a phosphate group. RNA's single-stranded backbone of alternating sugar and phosphate molecules (as opposed to DNA's double-stranded structure) is represented by the following K'NEX pieces: the phosphate molecule is a light blue Clip just as it is in the K'NEX DNA molecule, and the sugar and the phosphate are connected by the purple Rod (the phosphodiester bond) just as on the K'NEX DNA model. Unlike DNA, the 5-carbon sugar in RNA is ribose. Ribose is represented in the K'NEX RNA model by the orange fan-shaped Connector with a side flange. The last difference between DNA and RNA involves the nitrogen-containing bases. Both DNA and RNA have the purines, adenine and guanine, and the pyrimidine cytosine, but RNA contains uracil instead of thymine as the second pyrimidine option.

To recap, there are three differences between DNA and RNA:

1. RNA is single-stranded, but DNA is double-stranded.
2. RNA contains ribose (from which it derives its name) as its 5-carbon sugar, but DNA contains deoxyribose (from which it derives its name).
3. RNA contains uracil in place of thymine, which is present in DNA.

LESSON 4

There are three major forms of RNA:

1. messenger RNA (mRNA)
2. ribosomal RNA (rRNA)
3. transfer RNA (tRNA)

Each type of RNA has its own specific function, and mRNA is important because it carries the DNA "message" from the nuclear area, where the genetic code is replicated, to a cellular site where it can be translated into proteins. The location and organization of the cellular machinery for mRNA production is somewhat different in prokaryotic cells (bacteria) and eukaryotic cells (plants and animals). This lesson will center on the production and function of mRNA in eukaryotic cells.

DNA is the molecule of life that codes for all of the proteins produced by the organism. mRNA is made in the nucleus from the DNA template in a process called transcription, but ribosomes in the cytoplasm of the cell are the site of protein synthesis. DNA, therefore, is the ultimate code, but it is housed and protected in the nucleus of the cell, while protein synthesis occurs in the cytoplasm of the cell (on the ribosomes). There needs to be a vehicle or messenger to transmit the code from the DNA in the nucleus to the cytoplasm so that protein synthesis may occur. mRNA is that vehicle.

When a certain protein is needed by the cell, transcription begins with the two strands of the double-stranded DNA separating or "unzipping" to expose its nitrogen-containing bases in a limited region of the double helix. The exposed nitrogen-containing bases on one of the two complementary strands of the DNA act as a template for making mRNA by complementary base pairing, as shown on Pages 18 and 19 of the **K'NEX DNA, Replication and Transcription** building instructions booklet. Two major differences can be noted between the processes of DNA *replication and transcription*:

1. In contrast to replication, only one DNA strand of the double-stranded DNA molecule is transcribed to mRNA. The other strand, the coding strand, is inactive until after transcription when the DNA molecule zips back together.

2. *Thymine* pairs with adenine during ***replication***; however, *uracil* pairs with adenine during ***transcription***.

Transcription is very systematic and is assisted by specific enzymes in the following sequence:

1. Complex signaling mechanisms in the cell send a message to the nucleus that a specific protein is needed.

2. RNA polymerase (officially called RNA polymerase II when synthesizing mRNA) binds to DNA at the promoter region (Δ- a region 40-100 base pairs upstream from the coding region and methionine start codon) and unwinds a small section of the DNA. The enzyme separates a small section (about 10 nucleotides long[1]) into two single-stranded segments (opening DNA by breaking the hydrogen-bonds between nucleotides) to expose DNA's nitrogen-containing bases and thus the gene that codes for the protein needed.

3. With assistance from RNA polymerase II, free RNA nucleotides pair up with the exposed DNA nitrogen-containing bases on the template side of the DNA molecule by using complementary base-pairing rules. Remember that uracil on RNA binds with adenine on DNA. The other complementary base pairs remain the same, as demonstrated in DNA replication in Lesson 3. RNA polymerase II can only add RNA nucleotides onto the 3' end of the growing mRNA molecule; therefore, mRNA synthesis occurs in the 5' to 3' direction just as DNA replication occurs in the 5' to 3' direction, as shown in Figure 1 below.

4. RNA polymerase II continues down the DNA template building the mRNA molecule, which peels away and trails behind the RNA polymerase II-DNA complex. Messenger RNA synthesis occurs at a rate of about 60 nucleotides per second[1], and several RNA polymerase II molecules may be attached to the DNA template at one time. The result can be *many* mRNA molecules encoding for the same protein, thereby multiplying the number of protein molecules of this type that can be synthesized.

5. Completion of transcription is indicated by a termination site that includes a "stop" codon being transcribed to the mRNA. Upon reaching the termination site, RNA polymerase II detaches from both the DNA molecule and the mRNA molecule. The mRNA molecule released in eukaryotic cells is actually a pre-mRNA molecule that needs to be "edited."

6. Δ - Pre-mRNA is edited by enzymes. These excise the introns from the molecule leaving only the exons behind, as shown in Figure 2.

RNA Transcription from DNA

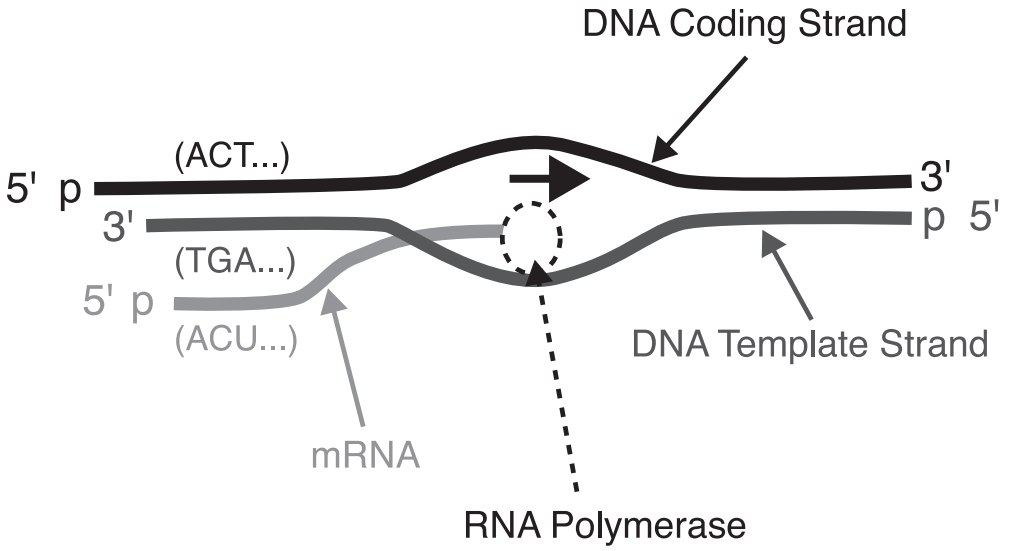
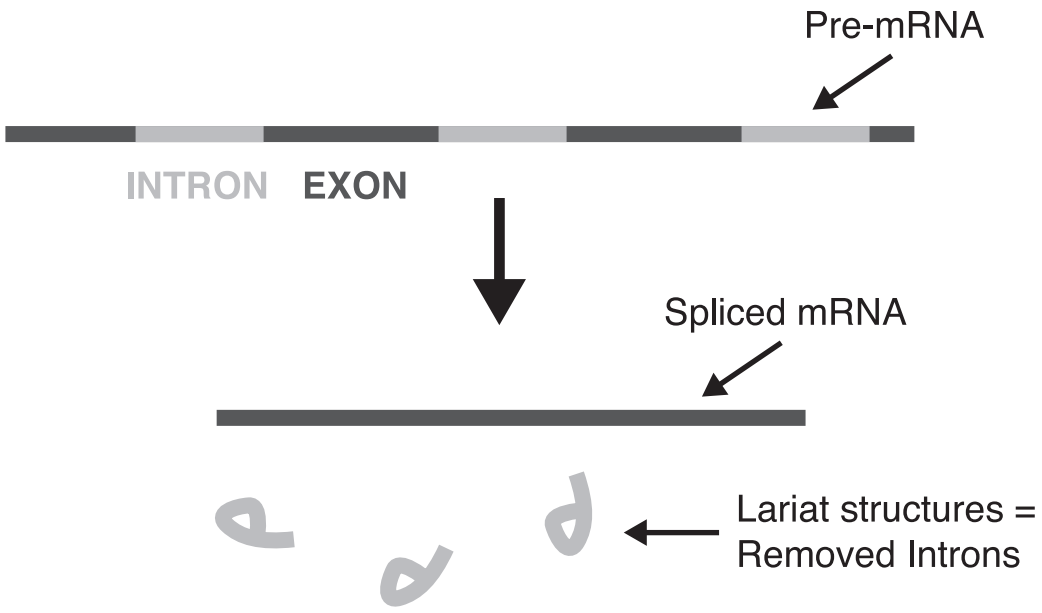


Figure 1 – mRNA synthesis from double-stranded DNA.

Splicing



Δ *Figure 2 – Editing of pre-mRNA to remove introns.*

Overall, the end result of transcription is an mRNA molecule that has been edited, is small enough to travel through the pores of the nuclear membrane to enter the cytoplasm of the cell, and carries the DNA code, or gene sequence, for the protein that is needed by the cell.

But how does mRNA become the code for a protein? The answer lies not only in the sequence of the mRNA nucleotides, but also in the grouping of that sequence. Sequences of three consecutive mRNA nucleotides, called codons, code for a specific amino acid (the building units of proteins) or for a stop signal, and herein lies the genetic code. Table 1 gives the sequences of mRNA codons that code for each of the 20 amino acids. It should be noted that more than one codon may code for an amino acid but each codon codes for only one amino acid. In other words, each codon is specific for one and only one amino acid.

The codon AUG, (which codes for methionine,) always appears at the translation start site in each mRNA segment. Methionine is like a start signal for building the protein molecule; however, this amino acid may not be present in the final protein product due to cleavage during post-translational processes. Likewise, one of the three “stop” codons found in Table 1 ends each segment of mRNA that is translated into protein. (For more details, please refer to Lesson 6, which details the rest of the protein synthesis process).

First Position	Second Position				Third Position
	UUU Phe F*	UCU Ser S	UAU Tyr Y	UGU Cys C	
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C	
	UUA Leu L	UCA Ser S	UAA Stop	UGA Stop	
	UUG Leu L	UCG Ser S	UAG Stop	UGG Trp W	
	CUU Leu L	CCU Pro P	CAU His H	CGU Arg R	
	CUC Leu L	CCC Pro P	CAC His H	CGC Arg R	
	CUA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R	
	CUG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R	
	AUU Ile I	ACU Thr T	AAU Asn N	AGU Ser S	
	AUC Ile I	ACC Thr T	AAC Asn N	AGC Ser S	
	AUA Ile I	ACA Thr T	AAA Lys K	AGA Arg R	
	AUG Met M	ACG Thr T	AAG Lys K	AGG Arg R	
	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G	
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G	
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gky G	
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G	

Table 1 – The Genetic Code: the three-letter codes representing the possible sequences of nitrogen-containing bases found in mRNA to represent the 20 amino acids and stop codons. For each line of each cell of the table, the first three capital letters are the one-letter abbreviations for the nucleotide triplet making up the codon. Except for Stop codons, the codon triplet is followed by both the three-letter and one-letter abbreviations for the encoded amino acid.

CREATE/ASSESS

- Students should make a sample mRNA molecule of their own and then trade it with a partner. The partner should perform the necessary steps to work back to the original double-stranded DNA molecule that coded for the mRNA. The original template DNA strand *must be labeled* - mark with tape, yarn, or an extra K'NEX piece - to distinguish it from its complementary coding DNA strand. (Refer to Figure 1). All sequences, (mRNA, and both DNA strands,) should be recorded and labeled in the student journals.

- After performing the previous task, students should re-create a (different) sample mRNA molecule, this time making sure that actual codons are in the mRNA strand. (The strand should include appropriate "start" and "stop" codons as well.) Students should again exchange mRNA molecules and work their way back to the original DNA template and complementary strand. All sequences and the amino acids coded for in the mRNA strand and the DNA template and complementary strands should be recorded and identified in the students' journals.

APPLY

Questions/Activities for students:

- The genetic code is generally considered universal, meaning that any given codon will code for the same amino acid in all living things. Ask students to propose examples of how this has been shown to be true.
  Examples:
  1. Genetically Modified Organisms (GMOs) - DNA from one species is used to insert a gene and produce a gene product in another species.

2. Human insulin is produced by bacteria using the human DNA coding sequence.
3. Identical segments of DNA coding sequences and corresponding amino acid sequences can be found in different animals that produce similar proteins, e.g., hemoglobin.

- Δ The first codon to be deciphered was UUU, which codes for phenylalanine. This codon was deciphered by M. N. Niremberg in 1961 by creating a mRNA composed only of uracil nucleotides so that the same codon was produced, regardless of the reading frame of three nucleotides.[1] Using this example, ask students to devise experiments to decode other codons found in Table 1.

EXTENSION ACTIVITIES (Δ Primarily for advanced students)

- Ask students to research mitochondrial DNA (mtDNA) and mRNA synthesis in the mitochondria, having them compare the similarities with nuclear DNA replication and transcription. Some scientists have presented these mitochondrial activities as evidence that mitochondria were once independent organisms that became engulfed by larger cells, which then became dependent on the assimilated structures.

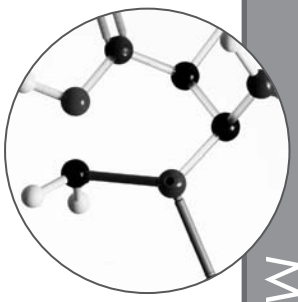
- Students should research and report on the "one gene – one enzyme" hypothesis of Beadle and Tatum.

- Δ Advanced students may proceed to Lesson 5.

REFERENCES

[1] Campbell, Neil A. *Biology, 4th Edition.* San Francisco: Benjamin/Cummings. 1996.

LESSON 5: Coding Glucagon: A Small Protein



[Δ - Advanced Lesson]

OBJECTIVES

Students will be able to:

1. Create a piece of double stranded DNA that encoded for a known protein.
2. Clearly distinguish between coding and template strands of DNA.
3. Understand the relationship of mRNA to the coding and template strands of DNA.

MATERIALS

It is recommended that four* student groups work together for this exercise. They should have the following:

- 4 kits from the **K'NEX DNA, Replication and Transcription Class Pack** containing 20 foot flexible tubing (recommended)
  OR
  4 **K'NEX DNA, Replication and Transcription kits**
- 1 building instructions booklet per group
- Student journals (1 per student)
- Copies of the Genetic Code Table (Table 1 below)
- Copies of the Amino Acid Sequence for Glucagon (Figure 1 below)

* If there are fewer than 4 groups, these should work to create as many glucagon segments as possible, creating a partial molecule.

First Position	Second Position				Third Position
	UUU Phe F*	UCU Ser S	UAU Tyr Y	UGU Cys C	
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C	
	UUA Leu L	UCA Ser S	UAA Stop	UGA Stop	
	UUG Leu L	UCG Ser S	UAG Stop	UGG Trp W	
	CUU Leu L	CCU Pro P	CAU His H	CGU Arg R	
	CUC Leu L	CCC Pro P	CAC His H	CGC Arg R	
	CUA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R	
	CUG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R	
	AUU Ile I	ACU Thr T	AAU Asn N	AGU Ser S	
	AUC Ile I	ACC Thr T	AAC Asn N	AGC Ser S	
	AUA Ile I	ACA Thr T	AAA Lys K	AGA Arg R	
	AUG Met M	ACG Thr T	AAG Lys K	AGG Arg R	
	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G	
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G	
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gky G	
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G	

Table 1 – The Genetic Code: the three-letter codes representing the possible sequences of nitrogen-containing bases found in mRNA to represent the 20 amino acids and stop codons.

[Source for Table 1: Fig. 5.6, p.143 from BIOCHEMISTRY, 3rd ed. by Christopher K. Mathews, K.E. van Holde and Kevin G. Ahern. Copyright ©2000 by Addison Wesley Longman, Inc. Reprinted by permission of Pearson Education, Inc.]

NH_3^+ - His - Ser - Glu - Gly - Thr - Phe - Thr - Ser - Asp - Tyr - Ser - Lys - Tyr - Leu - Asp -
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Ser - Arg - Arg - Ala - Gln - Asp - Phe - Val - Gln - Trp - Leu - Met - Asn - Thr - COO^-
16 17 18 19 20 21 22 23 24 25 26 27 28 29

Figure 1 – Amino Acid Sequence of Glucagon with amino acid order indicated by numbers.[1]

Preliminary Activity:
Creating a Glucagon Code and Building the DNA Template

- Using the Decoding Table (Table 1), students should refer to the amino acid sequence of glucagon in Figure 1 and create a possible mRNA coding sequence. A start codon (methionine) and stop codon may be added.

- These sequences should be recorded in the students' journals.

- Students should then record the complementary DNA sequence that would be required in a piece of template DNA (this is the "antisense" or "noncoding strand"). The "coding strand" (or "sense strand") of the DNA should be written complementary to the template DNA sequence. Figure 2 may be useful in demonstrating the relationship of the coding DNA strand, the template DNA strand, and the corresponding mRNA strand. Figure 3 shows the beginning of a typical student journal entry.

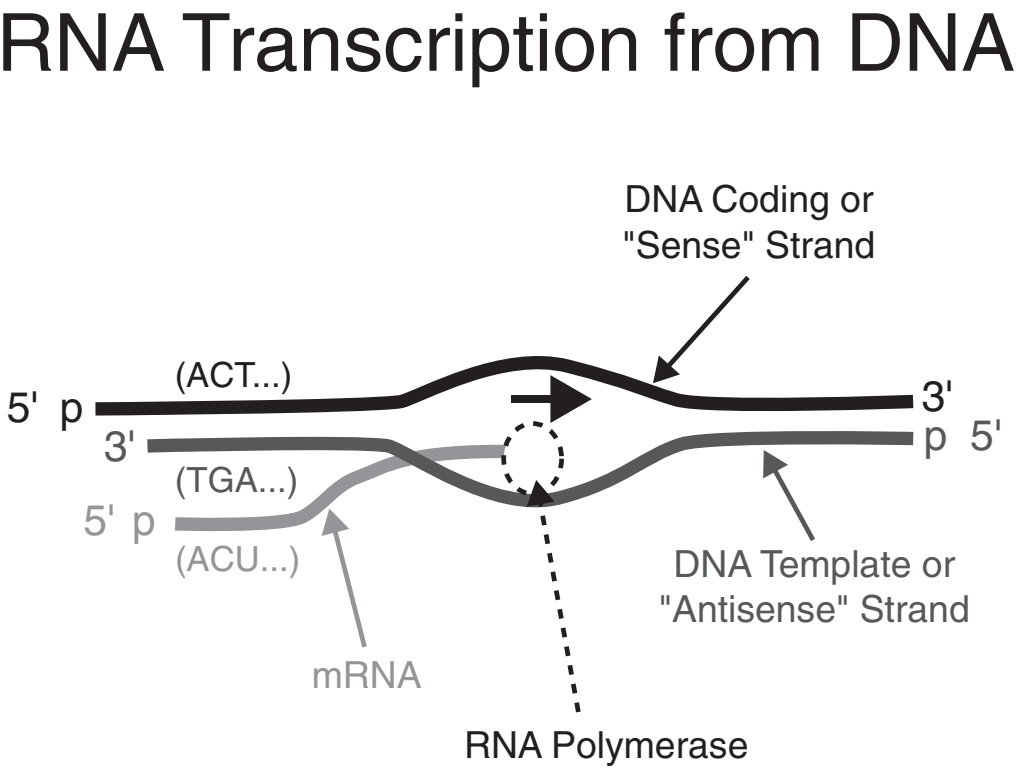


Figure 2: Double-stranded DNA being transcribed to mRNA.

Amino Acids	Met	His	Ser	Glu	Gly	Thr	Phe	Tyr	Ser...
mRNA	AUG	CAC	AGC	GAA	GGA	ACC	UUU	UAC	UCC...
Template DNA	TAC	GTG	TCG	CTT	CCT	TGG	AAA	ATG	AGG...
Coding DNA	ATG	CAC	AGC	GAA	GGA	ACC	TTT	TAC	TCC...

Figure 3: Possible student journal entry showing mRNA, template DNA and coding DNA sequences for glucagon.

- After the double helical segment coding for glucagon is determined by defining both the template and coding strand, divide the sequence into 24-base pair segments and have different student groups make as many of the pieces of the full DNA structure as possible. Glucagon has 29 amino acids; therefore, 87 base pairs are required to code the entire protein. If start and stop codons are added, the entire sequence will have 93 base pairs; therefore, four student groups should be able to create the "gene" sequence. The entire glucagon "gene" may then be mounted as a twisted double helix on the single piece of flexible tubing found in the **K'NEX DNA, Replication and Transcription Class Pack.**

Introduction

The amino acid sequences of many different proteins can be found on the Internet. The addresses for several of these web sites are provided at the end of this lesson. Several well-known web sites serve as repositories for sequences and structures of proteins that have been published by scientists following strict "peer review" procedures that are designed to check the work for accuracy. Despite the care taken to ensure the validity of published sequences, students may find several different proposed DNA sequences and different amino acid sequences for a given protein such as glucagon. This is because the same protein may have slightly different amino acid sequence in different animals. Thus, the source of a protein or DNA sequence usually is listed with any sequence information that is provided.

Although the "same" protein may vary somewhat in amino acid composition between species, the composition never varies enough to totally interfere with the protein's major function in the cell. For example, hemoglobin, the protein that carries oxygen in your blood, will likely have slightly different DNA sequences in the genomes of humans and pigs. Proteins with such homology between species can sometimes, but not always, be switched between species and still remain functional.

Most proteins are very large. They may contain hundreds of amino acids formed from several separate chains. The DNA coding sequences for these proteins may easily be over ten thousand base-pairs long. For this reason, students should target small proteins, often called peptides, for exercises suggested in this lesson. Glucagon is a good example of such a small protein or peptide.

Create/Assess

- After creating the glucagon DNA, transcribe the DNA back to mRNA, taking care to remember which of the DNA strands is the coding and which is the template strand.
  - What variations are possible in both the DNA and mRNA sequence and why?
  - What other possibilities exist?

[Assessment Note: Teachers may use this as a lab practicum activity.]

- Divide the students into eight groups. Using the **K'NEX DNA, Replication and Transcription Class Pack**, assign each group a number from 1-8. Each group should design and build separate, unique DNA sequences of 8 codons (24 base pairs). Students should record their own group's sequence in their journals and label their coding strand. Prior to beginning this activity, one group should be designated to write a sequence that would include a start codon (Met) and one group should be asked to write a sequence that would include a stop codon. After the 24 base-pair sequences have been built, complementary template or antisense strands should be built to complete the double helix. The groups should then work cooperatively to put all eight sequences together as a single double-helical molecule using the 20-foot piece of flexible purple tubing and using the general assembly instructions for the 24 base-pair model shown on Pages 16 and 17 of the **K'NEX DNA, Replication and Transcription** building instructions booklet. Students should then use the sequence code to determine the amino acid sequence of their new "mystery" peptide.

Apply

Questions/Activities for students:

- Use a biology text or the Internet to identify a small protein that is of interest to the student group. One interesting group of small proteins (peptides) is a family of antimicrobial peptides that include magainins, melittin, and cecropins. Go to one of the reliable web sites below and find either the amino acid sequence and/or DNA coding sequence for one of these proteins and create the DNA counterpart.

- Animal species that are closely related tend to have very similar DNA coding sequences for specific proteins. In fact, similarities and differences in these DNA sequences are often used as a measure of "genetic drift" or an "evolutionary process." Research the DNA sequences for a given peptide in several closely related and unrelated species. Compare the degree of "homology" among the species.

- Ask students to form hypotheses about what would happen if the DNA for glucagon was present in the cell but never transcribed. What would be the overall effect in the body?

Useful Related Web Sites

1. **http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome&itool=toolbar** – *Entrez Genome NCBI Search Site (U.S. National Institutes of Health search site)*
2. **http://www.ebi.ac.uk/embl/** - *EMBL (European Bioinformatics Institute) Nucleotide Sequence and Homology Search Database*
3. **http://www.rcsb.org/** - *Molecular Structure Databank*
4. **http://history.nih.gov/exhibits/rodbell/4_1_rodbell.htm** - *NIH Glucagon History*

References

[1] Amino Acid Sequence of Glucagon. **http://www.www.anaspec.com/content/pdfs/c_literature123.pdf**

LESSON 6: Translation of an mRNA Transcript

Objectives

Students will be able to:

1. Recognize and discuss the tRNA structure.
2. Compare/contrast tRNA and mRNA.
3. Understand how the genetic code translates into a protein.
4. Understand the function of tRNA.

Materials

Each student group should have the following:

- 1 **K'NEX DNA, Replication and Transcription kit** with building instructions booklet
- Student journals (1 per student)

* *NOTE: Each student group should have completed (at least) Lessons 1 through 4 before doing this lesson.*

K'NEX DNA, Replication and Transcription kits at this time do not provide models for tRNAs and the associated amino acids. This lessons suggests that students may combine the K'NEX mRNA model with tRNA and amino acid models that they construct from paper, or other material, to demonstrate the process of translation.

Preliminary Activity: tRNA Recognition and Translation

- Students should research the cloverleaf tRNA molecular shape and identifying characteristics in their texts or another reliable peer-reviewed source. This molecule should be sketched in their student journals with the identifying characteristics appropriately labeled.

- Students should construct an mRNA molecule from their K'NEX kits using random nitrogen-containing bases, as shown on Pages 18 and 19 of the **K'NEX DNA, Replication and Transcription** building instructions booklet. Each student should then build a corresponding protein molecule by "translating" the mRNA code into a specific amino acid sequence. Students should be creative in designing their own manipulatives to represent chains of amino acids. One possibility would be to create a paper chain with each "link" labeled as the appropriate amino acid corresponding to their mRNA code.

Introduction: tRNA and Its Function

In Lesson 4, students learned the process of transcription and how the template DNA molecule transfers its code to a "portable" mRNA molecule, which then exits the nucleus and enters the cytoplasm. This lesson will address the functions of the other types of RNA molecules (also produced in the nucleus) and the rest of the protein synthesis or "translation" process that occurs in the cytoplasm of the cell. Translation seems to be very appropriately named in that it *translates* the initial code from the DNA template into a protein molecule.

Transfer RNA molecules are one of the three major forms of RNA found in animal cells. This lesson on translation will center on transfer RNA (tRNA) and, briefly, ribosomal RNA (rRNA). When mRNA exits the nucleus and enters the cytoplasm, it must travel to a ribosome in order for its code to be translated

into the protein needed by the cell. Ribosomes exist in two places in the cytoplasm. Some ribosomes are in the cytosol unattached to any other organelle, and some ribosomes are attached to the endoplasmic reticulum (termed rough endoplasmic reticulum due to the ribosomes attached). Ribosomes themselves are made up of two sub-units each consisting of rRNA and associated proteins. One sub-unit is larger than the other and they are only functional when they are attached to each other and to the mRNA molecule.

A transfer RNA molecule is a piece of single-stranded RNA that loops back over itself in a rather distinguishable, cloverleaf-like shape. The middle "loop" of the tRNA molecule (analogous to the middle cloverleaf) possesses a triplet sequence of nitrogen-containing bases called an anticodon, which pairs with the codon on the mRNA. At the opposite end of the molecule (analogous to the stem of the cloverleaf) an amino acid is bound to the tRNA molecule. As we saw in the genetic code in Table 1 (shown again below), each codon and therefore each anticodon is specific for one and only one amino acid. This distinguishes each type of amino acid-carrying tRNA molecule from others.

First Position	Second Position				Third Position
	UUU Phe F*	UCU Ser S	UAU Tyr Y	UGU Cys C	
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C	
	UUA Leu L	UCA Ser S	UAA Stop	UGA Stop	
	UUG Leu L	UCG Ser S	UAG Stop	UGG Trp W	
	CUU Leu L	CCU Pro P	CAU His H	CGU Arg R	
	CUC Leu L	CCC Pro P	CAC His H	CGC Arg R	
	CUA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R	
	CUG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R	
	AUU Ile I	ACU Thr T	AAU Asn N	AGU Ser S	
	AUC Ile I	ACC Thr T	AAC Asn N	AGC Ser S	
	AUA Ile I	ACA Thr T	AAA Lys K	AGA Arg R	
	AUG Met M	ACG Thr T	AAG Lys K	AGG Arg R	
	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G	
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G	
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gky G	
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G	

Table 1 – The Genetic Code: the three-letter codes representing the possible sequences of nitrogen-containing bases found in mRNA to represent the 20 amino acids and stop codons.

[Source for Table 1: Fig. 5.6, p.143 from BIOCHEMISTRY, 3rd ed. by Christopher K. Mathews, K.E. van Holde and Kevin G. Ahern. Copyright ©2000 by Addison Wesley Longman, Inc. Reprinted by permission of Pearson Education, Inc.]

Translation is very systematic, takes place on a ribosome, and is assisted by specific proteins. Three steps: initiation, elongation, and termination describe the translation process.

1. The **initiation** step is the first step in translation. This step combines the sub-units of the ribosome, the mRNA initiation codon, and the initiator tRNA. The mRNA initiation codon is AUG which complementary base-pairs with the tRNA anticodon UAC. The UAC tRNA anticodon is specific for the amino acid methionine. Therefore, as stated in Lesson 4, the code AUG, methionine, is the start signal for the synthesis of a polypeptide chain. This first step is assisted by proteins called initiation factors.

2. The second step in translation is **elongation**. This step is assisted by proteins called elongation factors. Elongation of the polypeptide chain occurs as the ribosome sub-units move down the mRNA molecule in the 5' to 3' direction. As the ribosome moves down the mRNA, two major events occur. First, each mRNA codon is paired with its complementary anticodon on a tRNA.

Second, the amino acids carried to mRNA by tRNA are covalently bonded together to form the growing polypeptide chain. A growing polypeptide chain is often referred to and is graphically represented as a growing string of beads (similar to a bracelet).

His - Ser - Glu - Gly - Thr - Phe - Tyr - Ala - Ile - Gln - Asp - Asn

3. Step two, elongation, continues until a stop codon is translated. The final step in translation is **termination**.

   [Δ - When a stop codon on mRNA is reached, a protein called a release factor binds to the stop codon and brings a water molecule to the end to the growing polypeptide chain. This water molecule frees the polypeptide chain from the last tRNA molecule and from the ribosome.]

   Once the polypeptide chain is free, the ribosome sub-units separate and synthesis of that polypeptide is complete. It should be noted that several ribosomes can be translating the mRNA molecule at the same time, thereby greatly increasing the number of protein molecules produced by a single translated mRNA molecule.

CREATE/ASSESS

- Students should go back to Figure 1 – Amino Acid Sequence of Glucagon shown on Page 34 in *Lesson 5 - Coding Glucagon*, and record in their student journals the possible anticodons that would be associated with the amino acid sequence given. After recording their anticodon sequences, students should compare their results with a partner and see if they "agree." This activity will point out the redundancy in the genetic code; it is possible that students may have encoded the same protein using slightly different DNA sequences.

- Students may be asked to uncode the short nucleotide sequences of each strand of the DNA molecules shown in the **K'NEX DNA, Replication and Transcription** building instruction booklet. Note that the strands should be read in the 3' to 5' direction to correspond with the mRNA 5' to 3' synthesis direction. For example, the molecule shown on Pages 4 and 5 of the building instructions booklet would yield these results:

DNA Strand as Template	mRNA Strand	Amino Acid Sequence
5'-GCCAGATCATTT-3'	5'-AAAUGAUCUGGC-3'	Lys-Stop(-Ser-Gly)
3'-CGGTCTAGTAAA-5'	5'-GCCAGAUCAUUU-3'	Ala-Arg-Ser-Phe

- Δ Advanced students may research other small protein molecules (other than glucagon), record their codon sequence in their journals and then translate them with anticodons of tRNA. The web sites listed at the end of this lesson will be useful for this activity.

APPLY

Questions/Activities for students:

- Students should research the processes by which different types of proteins created on: 1) ribosomes free in the cytoplasm, and 2) ribosomes bound to the endoplasmic reticulum. Generally, cytoplasmic proteins (enzymes, etc.) are created on ribosomes that are free in cytoplasm. Membrane-bound proteins are synthesized by ribosomes attached to the endoplasmic reticulum.

- Students could undertake an activity demonstrating biochemical evolution. This can be shown by comparing similarities and differences in amino acid sequencing of a common molecule from several closely related species. For example, the hemoglobin molecule has been sequenced for humans as well as many other species. Students can readily compare the sequences of the different species by looking for phylogenic similarities and differences.[1] The web sites listed at the end of this lesson will assist in this activity.

- Proteins may serve both structural and functional purposes. Enzymes are examples of proteins that serve specific functions. Collagen (skin and bone tissue) and keratin (hair and nails) are examples of structural proteins. Ask students to research the many functions that proteins may serve.

- Δ Certain antibiotics such as tetracycline and streptomycin react specifically with bacterial ribosomes and not with eukaryotic ribosomes. Ask students to research how these antibiotics work to selectively inhibit bacterial growth.

EXTENSION ACTIVITIES (Δ Designed primarily for advanced students)

1. Students should research and discuss the P site and the A site on ribosomes. Formal names for the sites, functions and locations should be included in the discussion. A description of how they work in the translation process should be described in the student journals.

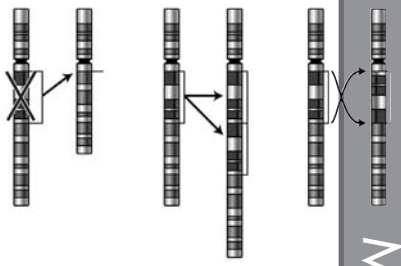
USEFUL RELATED WEB SITES

1. **http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome&itool=toolbar** – *Entrez Genome NCBI Search Site (U.S. National Institutes of Health search site)*
2. **http://www.ebi.ac.uk/embl/** - *EMBL (European Bioinformatics Institute) Nucleotide Sequence and Homology Search Database*
3. **http://www.rcsb.org/** - *Molecular Structure Databank*

REFERENCES

[1] Miller, K. and Levine, J. Laboratory Manual Biology. Prentice Hall. 1991.

LESSON 7: DNA Mutations



OBJECTIVES

Students will be able to:

1. Recognize basic types of errors in DNA replication.
2. Predict altered gene products resulting from errors in replication.
3. Understand how and when errors in replication may lead to permanent mutations.

MATERIALS

Each student group should have the following:

- 1 **K'NEX DNA, Replication and Transcription kit** with building instructions booklet
- Student journals (1 per student)

PREBUILD

- Each student group should begin with one replicated DNA ladder that has been coiled into a double helix. The double helix should have one parent strand made with gray Connectors for deoxyribose sugars and one daughter strand made with yellow Connectors for deoxyribose sugars, as described in Lesson 3 and as shown on pages 10 – 17 of the **K'NEX DNA, Replication and Transcription** building instructions booklet.

- Student groups should be encouraged to use different, random base-pair sequences.

PRELIMINARY ACTIVITY: Simulating a Mutation Event

- Students should record the base sequence of both the parent and daughter strands of their DNA molecule in their journals. A complementary strand of mRNA should be built as described in Lesson 4 using the daughter strand as the template. (Reminder: this means that the parent strand is the coding strand.) The mRNA sequence should also be recorded in the students' journals and the corresponding amino acid sequence should be determined and recorded.

- Instruct students to mutate the *daughter strand* of their double helical DNA molecule by using one of three methods:
    1. **Substitution:** Change the nitrogen-containing base in any one deoxyribonucleotide sub-unit on the daughter strand. Any teal, black, white or silver rod may be exchanged for one of the three remaining colors.
    2. **Deletion:** Remove one deoxyribonucleotide sub-unit from any point on the daughter strand, reconnecting the backbone by linking the remaining adjacent nucleotides.*
    3. **Insertion:** Insert one additional deoxyribonucleotide sub-unit at any point in the backbone of the daughter strand.*

** NOTE: Students will need to carefully stretch and bend the double-stranded DNA molecule to accommodate the resulting unmatched nitrogen-containing base in the backbone on one side of the molecule.*

Students should now change the sequence of their mRNA molecule to represent the mutational event just simulated and record the new sequence in their journals.

Acknowledgement: The graphic used in the title and as a design element throughout this Guide has been provided courtesy of the National Human Genome Research Institute.

LESSON 7

INTRODUCTION

DNA can be mutated by a number of natural environmental and chemical sources. One of the most potent natural mutagens is ultraviolet light. Most people are now aware of the potential for ultraviolet light to cause mutations that result in skin cancer, and sunblocks are now used by most sunbathers to prevent such mutational events. Ultraviolet light causes mutations because it may cause neighboring nitrogen-containing bases in a single strand of DNA to bond together to form a single molecule called a "diamer." This diamerization results in a local distortion of the normal DNA structure, causing replication and transcription enzymes to "stall" and make errors in these processes. This results either in premature termination or in mismatching of nitrogen-containing base pairs on the complementary DNA or RNA strand being formed. Replication errors may become permanent errors in the genome because the "mutation" is then accurately copied during subsequent rounds of replication.

Chemicals in the environment can also cause mutagenic events by bonding or interacting with specific nitrogen-containing bases of DNA, such that normal base-pairing is altered during replication. Occasionally, whole sections of DNA may be deleted. Sometimes random segments of foreign DNA may be inserted into the middle of a gene by events such as a "cross-over" in which two pieces of double-stranded DNA exchange segments.

All cells have proteins that are specifically designed to seek out and remove altered sequences of DNA. It is easy to imagine how altered DNA segments might be located by virtue of abnormal base pairing. For example, an adenine positioned complementary to a guanine (instead of a thymine) would readily stand out due to the abnormal base-pair length or bulk, as shown in Figure 1 below.

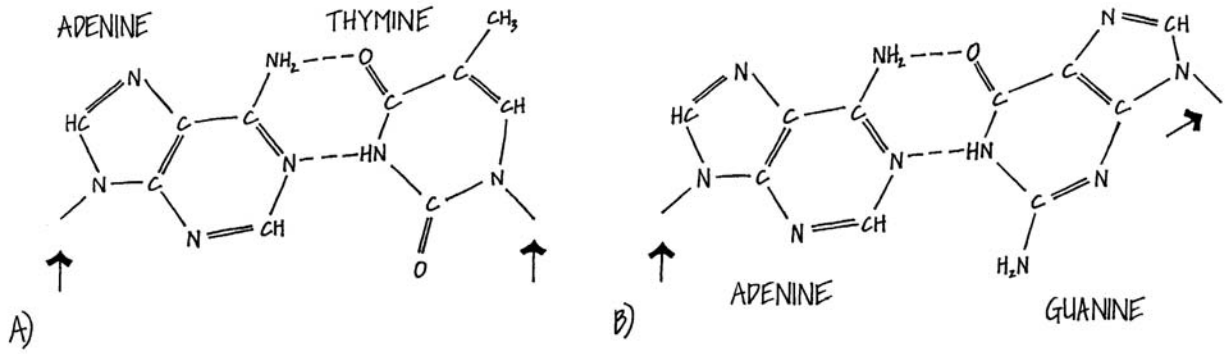


Figure 1: (A) Normal adenine-thymine base-pair and (B) adenine-guanine mismatch representing a mutational event. Notice the differences in overall base-pair diameters as well as the altered position and angle for connection to the sugar-phosphate backbone (arrows).

When base mismatches occur, repair enzymes that exist in the cell must determine which of the mismatched bases represents the un-mutated sequence and which represents the result of a mutation.

How can a cellular enzyme determine which is which? Most cells have a method for tagging a strand of DNA after it is correctly formed and "proofread" so that when the new strand next becomes the parent strand during subsequent replication, it is ignored and the new daughter strand becomes the target for any repair mechanisms. The "tag" that cells put on DNA strands after proofreading is usually a series of methyl groups added to certain nitrogen-containing bases; thus, "methylation" is the process that often follows DNA replication.

CREATE/ASSESS

- Using the models created in the preliminary activity, begin with the first nucleotide of the mRNA as the start of the reading frame and use Table 1 – The Genetic Code, shown on Page 38 to predict an amino acid sequence for both the un-mutated and mutated strands of mRNA.

- Explore how each type of event proposed in the Preliminary Activity will lead to varied types of mutations. For example, many of the mutational events will result in "frame shift" mutations. This means that the insertion or deletion of an extra base results in a shift of the triplet reading frame and shown in Table 1.

Table 1 – Mutational Events Outcomes: The four possible transcription and translation sequences below reflect: (1) the unmutated processes; (2) a substitution mutation in which the fourth base of the template strand, "T", was substituted with an "A"; (3) a deletion mutation in which the fourth base of the template strand, "T", is missing; and (4) an insertion mutation in which an extra "T" is inserted before the expected fourth base of the template strand, "T".

1. Unmutated:	GCC	AGA	TCA	TTT	Coding DNA
	CGG	TCT	AGT	AAA	Template DNA
	GCC	AGA	UCA	UUU	mRNA
	Ala	Arg	Ser	Phe	Amino Acid Sequence
2. Substitution:	GCC	**A**GA	TCA	TTT	Coding DNA
	CGG	(A)CT	AGT	AAA	Template DNA
	GCC	UGA	UCA	UUU	mRNA
	Ala	Stop			Amino Acid Sequence
3. Deletion:	GCC	**A**GA	TCA	TTT	Coding DNA
	CGG	_CTA	GTA	AA	Template DNA
	GCC	_GAU	CAU	UU	mRNA
	Ala	Asp	His		Amino Acid Sequence
4. Insertion:	GCC	_AGA	TCA	TTT	Coding DNA
	CGG	**T**TC	TAG	TAA A	Template DNA
	GCC	AAG	AUC	AUU U	mRNA
	Ala	Lys	Ile	Ile	Amino Acid Sequence

Note that the insertion or deletion of three bases would insert or delete one entire amino acid in the protein but all other amino acids would remain the same.

Apply

Questions/Activities for students:

- Cellular repair mechanisms exist for each of the types of mutation events described above. Discuss the importance of the timing of the repair event with respect to replication and transcription.

- How might a single, point mutation result in the deletion of an entire portion of a protein? *(Change of an amino-acid codon to a stop codon.)*

  How might a single point mutation result in a protein of twice the length? *(Change of a stop codon into an amino-acid codon with continued read-through to an unrelated DNA sequence/gene.)*

- Some mutational events may lead to beneficial outcomes for the organism or animal. For example, bacteria often become resistant to antibiotics through mutations that change the target site for the antibiotic without otherwise affecting the essential functions of the bacteria. Such a mutation, however, is likely to happen to only one out of a billion bacteria. Discuss how quickly one resistant bacteria might replicate to outnumber a billion others of the original strain if the antibiotic is present. Assume the bacteria can double every 20 minutes. How long would it take for one mutated bacterium to replicate to become 1,000,000? *(About 30 doublings, which is only 10 hours!)*

Useful Related Web Sites

http://www.nih.gov/ - *NIH Homepage*