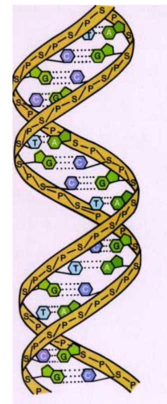
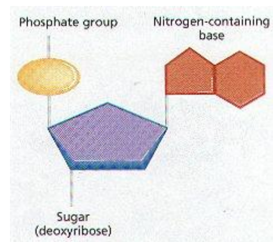
DNA
The Molecular Basis of Inheritance



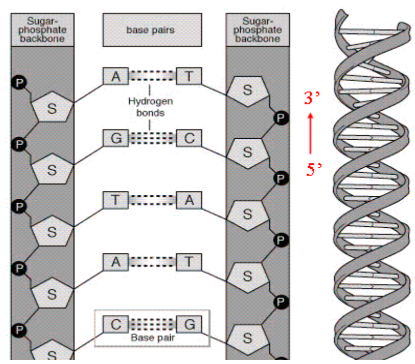
DNA Structure
- Deoxyribonucleic Acid
- Composed of 2 strands of nucleotides
- DNA is the genetic material

Nucleotides
- Recall that each nucleotide has three basic parts:
1. a phosphate group
2. a five carbon sugar (deoxyribose)
3. One of 4 nitrogen containing bases.



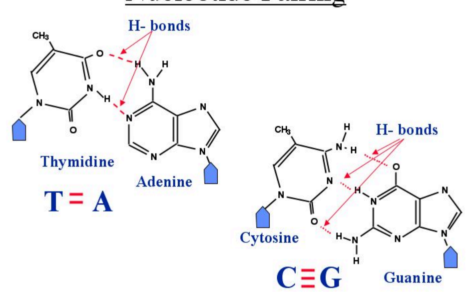
Bases
- There are four different nucleotides, each with one of four bases.
- The four bases are adenine, thymine, guanine and cytosine.
- The sequence of the bases determines an organism's characteristics.

DNA Structure
- DNA is composed of 2 long strands of nucleotides.
- The strands are joined together like a ladder.
- The strands twist to form a DOUBLE HELIX for protection, easy access and ease of copying.
- The double helix consists of two parts:
1. The sugar phosphate backbone (legs of the ladder).
2. The nitrogen containing base pairs which are joined by hydrogen bonds (the rungs of the ladder).



Complementary Base Pairing
- The strands are held together by weak hydrogen bonds between the bases
- Adenine always with Thymine (2 H bonds)
- Guanine always with Cytosine (3 H bonds)
- This is called complementary base pairing.

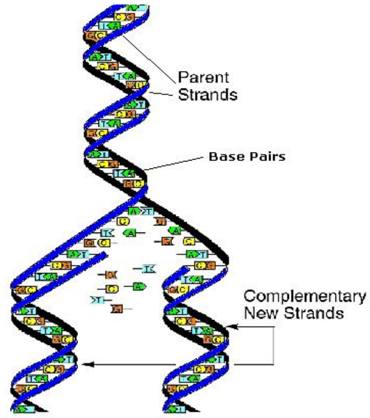
Nucleotide Pairing



DNA Replication
- Before division, DNA must replicate (copy) itself.
- This allows each new cell to have a complete set of chromosomes.
- Replication is carried out by a series of enzymes.
- There are 3 stages.

1. **"Unzipping"**
- Enzyme: DNA Helicase

- Attaches to DNA
- Breaks H bonds between complementary base pairs
- DNA "unzips" or unwinds



2. **Complementary base pairing**
   - Nucleotide monomers (always present in nucleoplasm) form H bonds with exposed nitrogenous bases by the process of complementary base pairing.
   - A binds to T
   - C binds to G
3. **Joining of adjacent nucleotides**
   - Enzyme: DNA Polymerase
   - Catalyzes formation of sugar-phosphate covalent bonds (dehydration synthesis) between each nucleotide

ie: creates new sugar-phosphate backbone

Semiconservative Replication
- Refers to each new double helix having one new strand and one old.
- Both new DNA molecules have the same base sequence as the original.

Replication Animations
- DNA Replication
- http://www.fed.cuhk.edu.hk/~johnson/teaching/genetics/animations/dna_replication.htm

RNA
- Ribonucleic acid
- DNA does not DIRECTLY control protein synthesis (DNA is in nucleus, protein synthesis occurs in cytoplasm)
- Genetic info is passed on to RNA
- RNA serves a number of different functions

DNA/RNA
- There are some key differences between DNA and RNA

	DNA	RNA
Sugar	deoxyribose	ribose
Bases	adenine, thymine, guanine, cytosine	adenine, URACIL, guanine, cytosine
Strands	double stranded	single stranded
Shape	double helix	no helix

3 kinds of RNA
1. **Messenger RNA (mRNA)**
- single, uncoiled RNA strand
- carries genetic code from DNA (nucleus) to ribosomes (cytoplasm)
- formed during **TRANSCRIPTION**

1. **Ribosomal RNA (rRNA)**

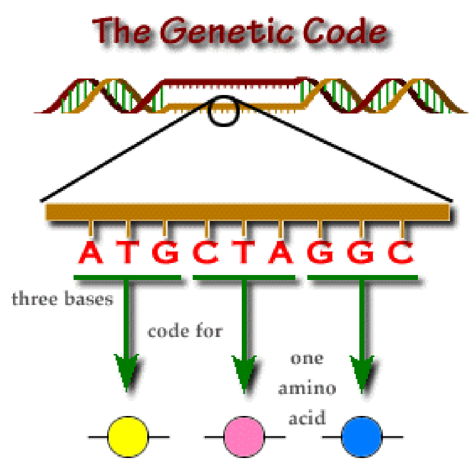
- Globular form of RNA
- Produced in the nucleolus
- Along with proteins, makes up the ribosomes (site of protein synthesis)

1. **Transfer RNA (tRNA)**

- Single RNA strand
- Folded back on itself in a "cloverleaf" fashion
- Carries individual amino acids to ribosomes for protein synthesis

The Genetic Code

- DNA has a particular base sequence.
- Proteins have a particular amino acid sequence.
- Suggests DNA contains coded information for protein synthesis.
- Organisms "genetic code" is determined by the order of bases in the DNA strand.
- # of bases = 4
- # of amino acids = 20
- How can 4 bases code for 20 amino acids?
  - one base/amino acid à 4 amino acids
  - 2 bases/amino acid à 42 = 16 amino acids
  - 3 bases/amino acid à 43 = 64 amino acids (more than enough)
- A group of 3 bases codes for each amino acid.
- This is referred to as a "**TRIPLET CODE**".



Codons

- A sequence of three transcribed mRNA bases is referred to as a **CODON**.
- Each codon codes for a particular amino acid.
- mRNA codons are presented in a table.

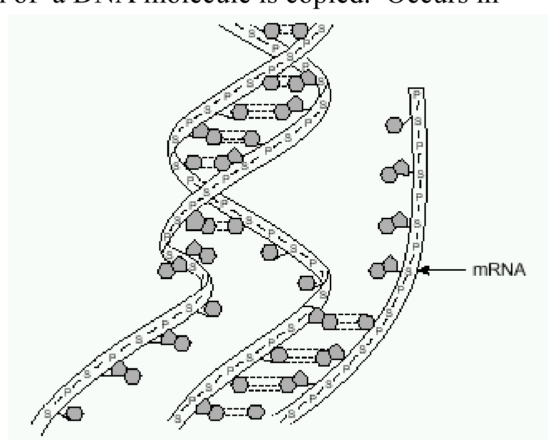
Table of mRNA Codons

- NOTE:
- There is more than one codon for each amino acid (remember: 20 amino acids, 64 codons)
- The triplet AUG can either specify for the amino acid METHIONINE or as a starter for protein synthesis. (Initiator or start codon)
- There are also three "stop" codons which signify the end of a polypeptide. These do not code for any amino acids.
- The genetic code is universal. That is the same codons stand for the same amino acids for most bacteria, animals, plants, fungi and so on.

Three-letter codons of messenger RNA and the amino acids specified by the codons			
AAU, AAC – Asparagine	CAU, CAC – Histidine	GAU, GAC – Asparatic acid	UAU, UAC – Tyrosine
AAA, AAG – Lysine	CAA, CAG – Glutamine	GAA, GAG – Glutamate	UAA, UAG – Stop
ACU, ACC, ACA, ACG – Threonine	CCU, CCC, CCA, CCG – Proline	GCU, GCC, GCA, GCG – Alanine	UCU, UCC, UCA, UCG – Serine
AGU, AGC – Serine	CGU, CGC, CGA, CGG – Arginine	GGU, GGC, GGA, GGG – Glycine	UGU, UGC – Cysteine
AGA, AGG – Arginine			UGA – Stop UGG – Tryptophan
AUU, AUC, AUA – Isoleucine	CUU, CUC, CUA, CUG – Leucine	GUU, GUC, GUA, GUG – Valine	UUU, UUC – Phenylalanine
AUG – Methionine			UUA, UUG – Leucine

Protein Synthesis

1. **<u>Transcription</u>**: the process by which a portion of a DNA molecule is copied. Occurs in the nucleus.



- RNA polymerase (enzyme) binds to DNA and causes a portion of it to "unzip".
- Free floating RNA nucleotides bind to complementary DNA bases on the DNA **TEMPLATE** strand forming a strand of messenger RNA (mRNA).

** NOTE: Uracil (U) in RNA binds with adenine (A) from DNA template.

- RNA polymerase moves along mRNA, covalently binding the nucleotides (dehydration synthesis rxn).
- Eventually RNA polymerase reaches a "stop" codon (UAA, UAG, UGA).
- This acts as a termination signal and the newly made mRNA strand is released.
- mRNA is small enough to leave the nucleus, so the protein "blueprints" can travel to the cytoplasm.
- Transcription is complete.

Transcription Animations

- www.ncc.gmu.edu/dna/mRNAanim.htm

- http://www-class.unl.edu/biochem/gp2/m_biology/animation/gene/gene_a2.html
- http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/transcription.swf
- http://www.dnai.org/a/index.html

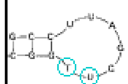
Protein Synthesis (continued)

2. **Translation**: the "decoding" of an mRNA strand into a polypeptide chain (protein). Occurs in the cytoplasm on a ribosome.
à Begins when mRNA moves through a nuclear pore into the cytoplasm and moves to a ribosome.

- Translation has 3 steps:

i. Initiation: mRNA binds to the small ribosomal subunit, then the larger unit joins.
ii. Elongation: lengthening of the polypeptide chain one amino acid at a time.

à mRNA moves along ribosome, presenting each codon to approaching tRNA molecules

tRNA Side Bar • Recall the structure of tRNA. • On one end of the cloverleaf is an amino acid. • On the other is an "anticodon". 3 bases which are complementary to the 3 base codon from mRNA. • The anticodon binds with the mRNA codon during translation.	

- A ribosome can accommodate 2 tRNA molecules: the incoming and outgoing tRNA.
- The incoming tRNA receives the peptide chain from the outgoing.
- An enzyme bind the new amino acid to the lengthening polypeptide (dehydration synthesis).
- The mRNA strand then moves laterally along the ribosome and another tRNA joins.
- In this manner, the polypeptide chain lengthens.

1. **Termination**: elongation continues until a "stop" codon on the mRNA is reached.

- There is no tRNA molecule for this molecule.
- The ribosome subunits break apart.
- The mRNA strand is released and disintegrates.
- The resulting protein (polypeptide) is available for further modification.

Polysomes

- Several ribosomes can move along one strand of mRNA at a time.
- This allows for the production of several of the same proteins at once.

Translation Clips

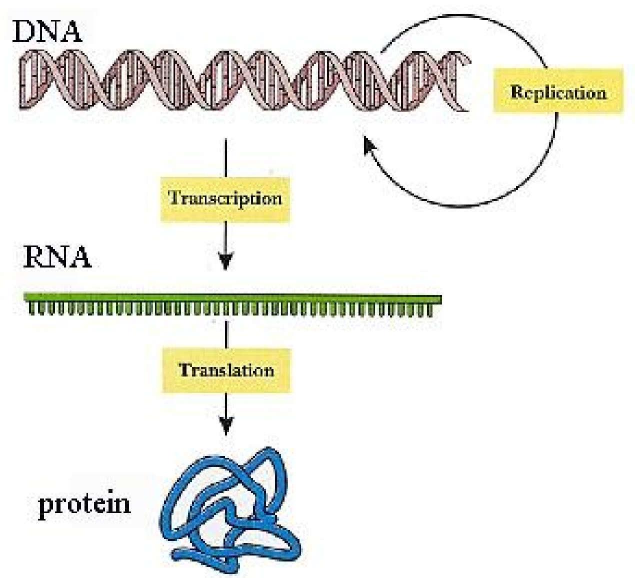
- http://www.sp.uconn.edu/~terry/images/movs/prtsynth.mov
- http://www.ncc.gmu.edu/dna/ANIMPROT.htm
- http://www-class.unl.edu/biochem/gp2/m_biology/animation/gene/gene_a3.html
- http://www.dnai.org/a/index.html

Reading the Code

- You must be able to read the code from DNA sequence to mRNA codon to tRNA anticodon to amino acid. For example:

DNA sequence	mRNA codon	tRNA anticodon	Amino acid
TGG	ACC	UGG	threonine
ACA	UGU	ACA	cysteine

Protein Synthesis Summary
http://www.johnkyrk.com/er.html



Gene Mutations

- Gene Mutation: any alteration in the code of a single gene or any change in its expression.
- mistakes during replication or environmental factors can cause a change in the genetic code
- effects may be positive or neutral but are usually NEGATIVE
- usually changes in one or more DNA nucleotides or an alteration in the sequence of nucleotides
- results in incorrect transcription and translation
- resulting polypeptide may be non functioning
- affects protein functioning and can have a drastic effect on the normal functioning of the body

Point Mutations

- incorrect nucleotides replace correct nucleotides
- also called **SUBSTITUTION** mutations

- use the sentence “**THE FAT CAT ATE THE RAT**” to see the effects
- changes single codons only
- may have no effect

GAT à GAC – both code for leucine

- may result in a new amino acid in the polypeptide chain

CTT à CAT – valine replaces glutamate (sickle cell anemia)

- may result in a premature end to the polypeptide change

AGT à ATT – serine replaced by STOP

ORIGNAL SEQUENCE

- UGUAC AUG UAU ACG UCU CAA UGA UCCA
  Met Tyr Ser Thr Gln STOP

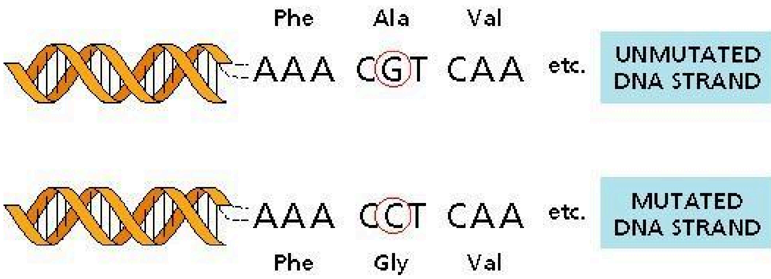
POINT MUTATIONS

- UGUAC AUG UAU ACG UCU CAG UGA UCCA
  Met Tyr Ser Thr Gln STOP
- UGUAC AUG UAU ACG CCU CAA UGA UCCA
  Met Tyr Ser Pro Gln STOP
- UGUAC AUG UAA ACG UCU CAA UGA UCCA
  Met STOP

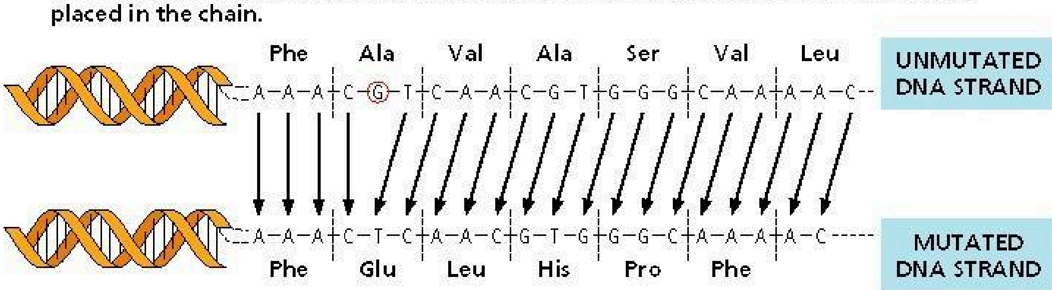
Frameshift Mutations

- nucleotides are ADDED to or DELETED from DNA
- results in change to all codons after mutation
- results in nonfunctional protein/enzyme due to altered codon sequence
-

(A) Point mutation. When, for example, a C is substituted for a G in the DNA strand, the mRNA codon matches with the tRNA carrying the amino acid glycine rather than the tRNA carrying the amino acid alanine.



(B) Frameshift mutation. When, for example, a G is deleted from the DNA strand, the codon that had the G and all subsequent codons are misread and different amino acids are placed in the chain.



Mutagens

- a mutagen is defined as any agent that causes mutations in humans
- there are 2 broad categories

High Energy Radiation: x-rays, gamma rays, UV light
Chemical Mutagens: benzenes, dioxins, some substances in cigarette smoke

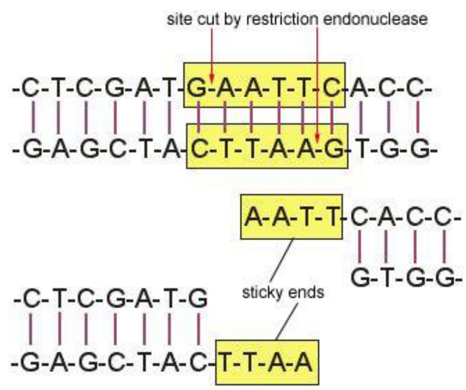
Recombinant DNA

- formed when the DNA from 2 different species is combined

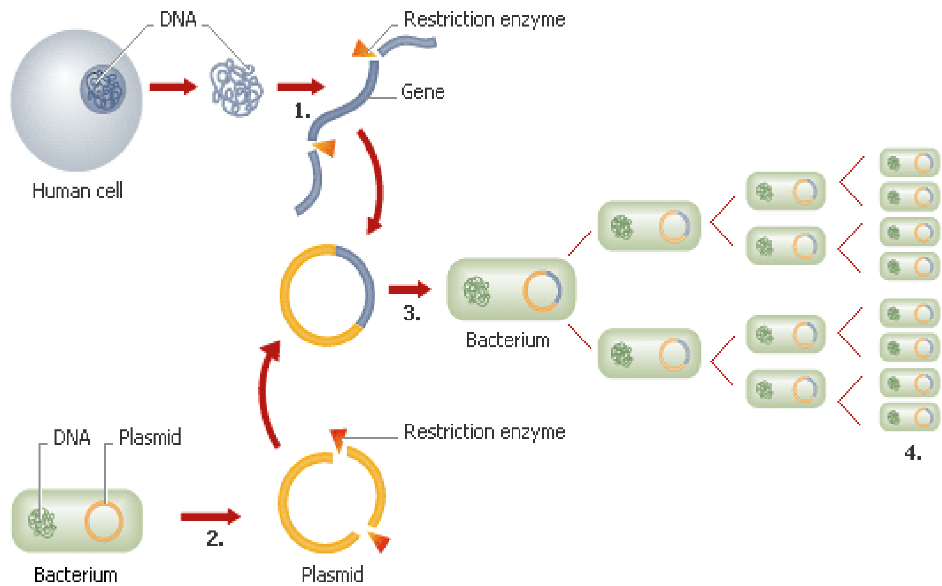
Uses:

- Gene cloning: using bacteria to make large quantities of a desirable piece of DNA (human insulin – hormone (protein) to treat diabetes, interferon – protein that blocks viral growth)
- Genetic engineering: insertion of genes from one organism into another (insecticide producing plants, increased milk production in animals)

Steps in rDNA production



1. Extract DNA from donor organism.
2. Restriction enzymes (endonucleases) "cut" the DNA at specific points leaving "sticky ends" (unpaired bases) which will bind to their complementary bases.
3. A vector (carrier) is required to introduce the foreign gene into the host cell. The vector is usually a PLASMID (circle of bacterial DNA). If the same restriction enzyme is used to cut the plasmid, the sticky ends of the gene will match the sticky ends of the vector.
4. "Recombination" takes place. Donor DNA binds to the sticky ends of the plasmid strand. DNA ligase seals the donor DNA to the plasmid.
5. Treated bacteria take up the plasmids. These bacteria are allowed to reproduce, thus many copies of the recombinant DNA are made. The proteins produced by these bacteria are collected and purified.


DNA
Restriction enzyme
Gene
1.
Human cell
3.
Bacterium
DNA
Plasmid
Restriction enzyme
4.
Bacterium
2.
Plasmid