Carbohydrate Review (C,H,O)

• All carbs are made of sugar.
• They are polymers of many sugar units.
• Carbs are the bodies most important energy source.
• Carbs can be classified according to the number of sugar units they contain.

[Glucose molecular structure image]
Monosaccharides

• These contain only a single sugar unit.
• Glucose, Fructose and Deoxyribose are examples.
Disaccharides

• Are a combination of two monosaccharides.
• Sucrose, Maltose, and Lactose are examples.
Polysaccharides

• A carb formed when 3 or more sugars are linked together.
• There are 3 main examples of polysaccharides.
• 1) Starch – a sugar storage molecule found only in plants
• 2) Cellulose – a carb based building molecule plants use to construct cell walls.
• 3) Glycogen – a sugar storage molecule found mainly in animal cells (muscle and liver).
Starch

• Starch is a sugar storage molecule found only in plants.
• Starch can be composed of 1000-6000 sugar units linked together.
Glycogen

• The main sugar storing molecule in our bodies.
• Glycogen is formed when excess sugars are linked together in our muscle and liver cells forming huge chains of sugars.
Lipid Review (C,H,O)

• Lipids are the only molecule organisms can use to store vast quantities of energy (calories).
• Fat is also important as an insulator, and it protects and cushions internal organs.
• Fat can be modified into hormones, phospholipids (for cell membranes), waxes.
Triglycerides

- Triglycerides are formed by the union of glycerol (a 3 carbon chain) and three fatty acids (themselves long chains of carbon atoms).
- Excess carbs are converted into fatty acids and then into triglycerides before being sent into the blood to a fat cell.
Protein Review (C,H,O,N)

• Proteins are **structural** molecules that give most cells, and therefore organisms their shape and appearance.

• **Enzymes** are protein molecules that help almost every chemical reaction in the body take place.
The building blocks of proteins are Amino Acids.
“R” Groups of Amino Acids

Nonpolar

Glycine (Gly)
Alanine (Ala)
Valine (Val)
Leucine (Leu)
Isoleucine (Ile)

Methionine (Met)
Phenylalanine (Phe)
Tryptophan (Trp)
Proline (Pro)
Peptide Bond
Nucleic Acids (C,H,O,N,P)

- **Nucleic acids** are biological molecules essential for life.
- Examples include DNA and RNA.
- They function in encoding, transmitting and expressing genetic information.
- The functional units (or building blocks) of nucleic acids are Nucleotides.
Nucleotides

- They are the functional units (or building blocks) of nucleic acids.
- Each nucleotide contains:
  - a 5 carbon sugar
  - a phosphate group
  - a nitrogen base
Nucleotides are linked together to form Nucleic Acids.

- These are the 4 DNA nucleotides.
- A pairs with T
- G pairs with C
Structure of DNA

• First described by James Watson and Francis Crick who won the Nobel prize in 1953 (although they stole the work of Rosa Franklin who used X-ray diffraction to work out the exact structure.)
RNA

• RNA is similar to DNA in that it is a string of nucleotides.
• There are however 3 key differences:
  • 1) RNA is single stranded.
  • 2) RNA has no Thymine (T). A pairs with U (Uracil).
  • 3) RNA has a ribose sugar.
• RNA plays a role in 3 processes we’ll examine:
  • 1) As RNA primer in DNA replication
  • 2) As mRNA in protein synthesis
  • 3) As tRNA in protein synthesis
Comparing DNA to RNA

- **Cytosine** (C)
- **Guanine** (G)
- **Adenine** (A)
- **Uracil** (U)

**Nucleobases of RNA**

**RNA** (Ribonucleic acid)

**DNA** (Deoxyribonucleic acid)

- **Base pair**
- **Helix of sugar-phosphates**

**Nucleobases of DNA**

- **Cytosine** (C)
- **Guanine** (G)
- **Adenine** (A)
- **Thymine** (T)
DNA Replication – 6 steps

1. A portion of the DNA unwinds and opens up (unzips).
2. An RNA primer is added to the parent strand.
3. An enzyme **DNA polymerase** binds to one side of the open DNA (leading strand) and moves **towards the fork** fitting in new complementary nucleotides (A-T and C-G).
4. On the second side of the open DNA (lagging strand), the RNA primer is again followed by **DNA polymerase** which again binds to the open DNA and moves **away from the fork** fitting in new complementary nucleotides. However in this direction only smaller fragments called "Okazaki fragments" are formed.
5. Next the RNA primers are replaced with DNA nucleotides.
6. Finally the Okazaki fragments are stitched together by the enzyme **DNA ligase**.
DNA Replication

Leading Strand
RNA Primer
Parental Strand
Replication Fork
Lagging Strand

Newly Synthesized DNA Strand
Direction of Replication Fork Movement

Newly Synthesized DNA Strand
Okazaki Fragments
Direction of Replication Fork Movement

Okazaki Fragments
Protein Synthesis
• Literally "making protein".
• The process is divided into two parts:
  • 1) Transcription
  • 2) Translation
Transcription

• the process wherein a molecule of mRNA (messenger RNA) is made using a template strand of DNA.

• **Step 1 Initiation**
  a section of DNA (called a gene) opens up and a promoter sequence allows an enzyme **RNA Polymerase II** to attach to 1/2 the parent DNA.

• **Step 2 Elongation**
  pre-mRNA forms using open DNA as template **RNA Polymerase II** assembles the RNA nucleotides complementary to the DNA template strand.
Transcription

- **Step 3 Termination**
  when RNA Polymerase II reaches a terminator sequence of base pairs along the DNA template, transcription halts.

- before it leaves the nucleus, the pre-mRNA is processed by:
  1) having its ends caped to protect it.
  2) having introns (non coding sections) removed while leaving exons (coding sections) in place.

- mRNA moves to cytoplasm to find a ribosome.
Step 1 Initiation

- A section of DNA (called a gene) opens up and a promoter sequence allows an enzyme RNA Polymerase II to attach to 1/2 the parent DNA.
Transcription

Step 2

• Step 2 Elongation pre-mRNA forms using open DNA as template RNA Polymerase II assembles the RNA nucleotides complementary to the DNA template strand.
Transcription

Step 3

- Step 3 Termination when RNA Polymerase II reaches a terminator sequence of base pairs along the DNA template, transcription halts.
Transcription - mRNA Processing

- before it leaves the nucleus, the pre-mRNA is processed by:
  - 1) having its ends caped to protect it.
  - 2) having introns (non-coding sections) removed while leaving exons (coding sections) in place.
Translation - Overview

- the process of creating a polypeptide (protein) using the genetic information present in the mRNA molecule.
- occurs in cytoplasm at a ribosome.
Translation – The Steps

• **Step 1 Initiation**
  when the mRNA attaches itself to both the ribosome and the tRNA at the “AUG” initiator sequence.

• **Step 2 Elongation**
  Every 3 nucleotides of mRNA called a "**codon**" codes for a particular amino acid.
  Transfer RNA (tRNA) carrying an amino acid, binds its "**anticodon**" to the complementary mRNA codon.
Translation – The Steps

• A peptide bond forms between adjacent amino acids and the “empty” tRNA is released to find another amino acid. This continues as the mRNA slides along the ribosome.

• **Step 3 Termination**
  Translation is terminated when a “stop codon” is reached in the mRNA strand. The completed polypeptide (now called a protein) is released.
Translation Step 1

• **Step 1 Initiation** when the mRNA attaches itself to both the ribosome and the tRNA at the “AUG” initiator sequence.
Translation Step 2

• **Step 2 Elongation**
  Every 3 nucleotides of mRNA called a "codon" codes for a particular amino acid. Transfer RNA (tRNA) carrying an amino acid, binds its "anticodon" to the complementary mRNA codon.
A peptide bond forms between adjacent amino acids and the “empty” tRNA is released to find another amino acid. This continues as the mRNA slides along the ribosome.
Translation Step 3

• **Step 3 Termination**
  Translation is terminated when a “stop codon” is reached in the mRNA strand. The completed polypeptide (now called a protein) is released.
Transcription to Translation

DNA molecule

Gene 1

Gene 2

Gene 3

DNA strand (template)

ACCAAAACCGAGT

3’

5’

transcription

mRNA

5’

UGGUGUGUCUCAC

3’

translation

Protein

Trp

Phe

Gly

Ser

Amino acid

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Protein Synthesis in detail

TRANSCRIPTION

- DNA
- RNA transcript
- RNA polymerase
- Poly-A

RNA PROCESSING

- Exon
- Intron
- RNA transcript (pre-mRNA)

NUCLEUS

AMINO ACID ACTIVATION

- Aminoacyl-tRNA synthetase
- Amino acid
- tRNA

CYTOPLASM

- mRNA
- Growing polypeptide
- Ribosomal subunits
- Cap
- Poly-A

- Ribosome
- Anticodon
- Activated amino acid

TRANSLATION
THE ROLE OF THE RIBOSOME IN TRANSLATION

Amino acid binds to growing protein chain
mRNA binds to codon
Ribosome
mRNA binds to codon
Completed protein
Codon
Arginine
Leucine
Isoleucine
Ala
Pro
Pro
Arg
Leu
Ile
Recombinant DNA

• Also known as genetic engineering.
• What is Recombinant DNA?
• Genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species.

• The new DNA becomes part of the host's genetic makeup and is replicated.
• New proteins are made as a result.
Recombinant DNA – For the treatment of diseases

• 1) Insulin - Recombinant insulin has almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes.

• 2) Human Growth Hormone - GH is administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice led to some patients developing serious diseases.
3) Clotting Factors for hemophiliacs - Recombinant blood-clotting proteins are administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce clotting factors in quantities sufficient to support normal blood coagulation.

4) Hepatitis B Vaccine Production - Prevention of HB infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.
Recombinant DNA – For the production of genetically modified foods

1) Herbicide Resistance Plants - Agricultural crops (including soy, corn, sorghum, canola, alfalfa, wheat and cotton) have been developed which incorporate a recombinant gene that results in resistance to herbicides.

Work on Round-Up resistant Canola was done at the U of M in Winnipeg.

2) Insect Resistant Crops - adopted in agriculture and gardening, plants have been developed which express a recombinant form of the bacterial protein, which may effectively control some insect predators.
Recombinant DNA – For the production of genetically modified foods

• 3) Biosteel - a high-strength fiber material made of the recombinant spider silk protein extracted from the milk of recombinant goats.

• Biosteel is a lightweight, strong, and versatile materials being considered for a variety of medical and industrial applications (medical sutures and replacing Kevlar for bulletproof vests).

• 4) High Protein Wheat – after isolating a gene in wild wheat that increases the grain protein content by 15%, researchers at U of Cal Davis have successfully spliced the gene into domestic wheat.
Recombinant DNA – For the production of genetically modified foods

• 5) Better Cheese – scientists have engineered a cheese making bacteria that has a gene that produces an enzyme (a protein) that eliminates the bitter taste created during ripening of certain cheeses.

• 6) Genetically modified tomatoes are 15% sweeter than regular tomatoes.

• 7) Rodent Altered Lettuce - by splicing rat genes into lettuce, Virginia Tech scientists have increased the vitamin C content of lettuce. They have yet to work out a name as “Rat Lettuce” seems somewhat unappetizing.
Recombinant DNA – How it Works

• The basic technique of recombinant DNA involves digesting a section of DNA with a restriction enzyme (a molecular scissors)
• The restriction enzyme cuts DNA at specific sites.
• A DNA molecule from the organism of interest is also digested, in a separate tube, with the same restriction enzyme.
• The two DNAs are then mixed together and joined, using an enzyme called DNA ligase (linker)
Recombinant DNA – How it Works

[Diagram showing the process of recombinant DNA construction]

**Molecule A**

5’ --- G-G-A-T-C-C --- 3’

3’ --- C-C-T-A-G-G --- 5’

**Molecule B**

5’ --- G-G-A-T-C-C --- 3’

3’ --- C-C-T-A-G-G --- 5’

Digest each with same restriction endonuclease, **BamHI**

Sticky ends

Mix

Seal with DNA ligase (pink)

Recombinant DNA
Bacteria used to Produce Human Growth Hormone
Genetically Modified Plants – can produce a protein insecticide
What is it about fingerprint analysis that allows it to function as a means of identifying criminals?

What is it about DNA Typing (DNA analysis) that allows it to function as a means of identifying criminals?

- Begins with a sample of an individual's DNA
- Usually a throat swab – but can be obtained from any cellular material - blood, saliva, semen, or other appropriate fluid or tissue from personal items (e.g. toothbrush, razor, etc.).
DNA Typing – Why it’s Used.

1) To identify rapists and other criminals.
2) To determine paternity.
3) To determine if a hopeful immigrant is really a son or daughter of an already established resident.
DNA Typing – How it Works

• The sample is then analyzed to create the individual's DNA profile.

• The DNA profile is then compared against another sample to determine whether there is a genetic match.
DNA Typing – How it Works

• Although other techniques are used today, the first methods for finding out genetics used for DNA profiling involved restriction enzyme digestion.

• A restriction enzyme (molecular scissors) is added to the sample of DNA.

• The DNA is cut into various fragments.

• The cut up DNA is separated using a technique called gel electrophoresis.
Gel Electrophoresis

1. Restriction enzymes cleave DNA into smaller segments of various sizes.

2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.

3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.

4. Smaller DNA segments move faster and farther than larger DNA segments.
Results - An Actual Rape Case

• EVIDENCE #1 - semen stain left on the victim's clothing
• EVIDENCE #2 - semen removed from the vagina of the rape victim
• DNA from suspects #1 and #2
• DNA of the victim herself.
• Why is the victim’s DNA always included?
Results - An Actual Rape Case

• To further help remove all reasonable doubt, a statistical statement can be made regarding the probability that another random person would share that same DNA fingerprint.
• Each allele (band of DNA) has its own frequency in the population.
• These individual frequencies are multiplied together to fine the probability of having the same exact DNA fingerprint.
DNA Typing – Paternity Testing

• With paternity testing – would we expect the same type of exact DNA fingerprint matching as is the case with criminal investigations?

• NO. You get ½ your DNA from each parent.
Mutations and Cancer

• A mutation is any change in the genetic code (a failure of DNA repair).
• There are 3 general types of mutations:
  • 1) Single Base Substitutions
  • 2) Insertions and Deletions
  • 3) Translocations
Single Base Substitutions

• A single nucleotide (base) is replaced by another. Example: Adenine (A) replaced with Guanine (G)
• There are 3 types of single base substitutions.
  • 1) Missense Mutation - alters the codon to produce different protein.
  • 2) Nonsense Mutation - alters codon to one of the STOP codons resulting in a shortened protein.
  • 3) Silent Mutation - the altered codon happens to code for the same amino acid as the original therefore no change in the protein produced.
Missense Mutation

Original DNA code for an amino acid sequence.

DNA bases

Original: CATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCATCAT

Amino acid: His His His His His His His

Replacement of a single nucleotide.

CATCATCATCATCATCATCTCATCATCATCATCATCATCATCATCATCATCAT

Incorrect amino acid, which may produce a malfunctioning protein.

U.S. National Library of Medicine
Nonsense Mutation

Original DNA code for an amino acid sequence.

DNA bases

C A G C A G C A G C A G C A G C A G C A G C A G

Gln Gln Gln Gln Gln Gln Gln

Amino acid

Replacement of a single nucleotide.

C A G C A G C A G T A G C A G C A G C A G

Gln Gln Gln Stop

Protein

Incorrect sequence causes shortening of protein.
Silent Mutation

ATG  GAA  GCA  CGT
Met  Glu  Ala  Gly

ATG  GAG  GCA  CGT
Met  Glu  Ala  Gly

Codons of the genetic code

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Cys

Arg

Stop

Trp
Insertions and Deletions

• Extra nucleotides (base pairs) are added or deleted from the DNA of a gene.

• If you had to choose between the following deletion mutations which would you choose?
  a) 1 nucleotide is deleted
  b) 3 nucleotides are deleted

• Insertions and deletions of 1 or 2 base pairs result in a "frameshift" and are particularly devastating.

• Insertions and deletions in multiples of 3 do not create a frameshift.
Insertion Mutation

Original DNA code for an amino acid sequence.

DNA bases

Amino acid

Insertion of a single nucleotide.

Incorrect amino acid sequence, which may produce a malfunctioning protein.

U.S. National Library of Medicine
Deletion Mutation

Deletion mutation

Original DNA code for an amino acid sequence.

DNA bases

His His His His His His

Amino acid

Deletion of a single nucleotide.

His His His Leu Ile Ile Ile

Incorrect amino acid sequence, which may produce a malfunctioning protein.

U.S. National Library of Medicine
Frameshift Mutation

Frameshift mutation

Original DNA code for an amino acid sequence.

DNA bases

C A T T C A C A C C A G T A C T C A T G C T A T

Amino acid

His Ser His Val Leu Met Leu

Frameshift of one DNA base results in abnormal amino acid sequence.

U.S. National Library of Medicine
Translocation Mutation

- Transfers of an entire piece of one chromosome to a non-homologous chromosome.
Mutations and Cancer

• Anything that damages the DNA and causes a mutation can cause cancer.
• If the mutation is such that it "codes" for the cell to divide uncontrollably, then cancer has begun.
• Therefore chemicals that damage the DNA increasing the risk of cancer are called carcinogens.
• Other factors such as exposure to radiation can also damage the DNA and lead to cancer causing mutations.
The Ames Test

• An Ames test can be used to determine if a chemical is carcinogenic or not.
• The test uses a mutant strain of bacteria which lacks the ability to produce a certain amino acid.
• If, after exposure to the "test chemical", colonies of bacteria begin to grow again, it is assumed that the "test chemical" caused a high number of mutations in the bacteria allowing it to again produce the amino acid it was lacking.
• Thus the "test chemical" is deemed a mutagen and classified as "carcinogenic".
The Ames Test

- A and B are the control plates.
- C and D are the experimental chemicals.
- Does C pass the Ames test?
- Is C a carcinogen?
- Does D pass the Ames test?
- Is D a carcinogen?