Unit 2 Lecture 5

Bacterial Genetics

DNA Code

The gospel message that you must learn out of this unit is that DNA makes RNA which makes proteins. An organism is what it is because of the proteins that its cells make. If the organism cannot make the proteins it needs, it cannot survive. The total amount of DNA in a cell is its genome.

Let's go back to the basics of DNA. DNA is the blueprint that tells the cell through RNA what proteins to make. It is usually composed of two strands of nucleotides (remember some viruses can be single stranded). A nucleotide is made from nitrogenous bases, purines (adenine & guanine) and pyrimidines (cytosine & thymine). They will pair up according to base pair rules, adenine pairs with thymine and guanine pairs with cytosine. DNA length is measured in base pairs (bp). The DNA of E. coli, for example is 4.6×10^6 bp long. DNA is stored on one or two chromosomes in the bacterial cell in a region of the cell called the nucleoid. DNA is also stored on plasmids, but DNA stored on plasmids is not necessary for cell replication or survival. The genomic sequence (nucleotide pattern) of many microorganisms has already been determined. Prokaryotic organisms are haploid, i.e. they have 1*n* number of chromosomes. There are three categories of genes that are coded for in the DNA sequence: genes that code for proteins (structural genes), genes that code for RNA, and genes that code for gene expression (regulatory genes).

A nucleotide has a sugar (deoxyribose) and a phosphate group (HPO^{4-}). DNA forms a double alpha helix (a twisted ladder formation). One nitrogenous base by itself is a nucleotide. The combination forms a base pair. For example:

Adenine	A	Guanine	G
Thymine	T	Cytosine	C

Sections of DNA are the genes (a certain segment of DNA that contains the necessary code to make a protein or RNA molecule). One of the purposes of DNA is to maintain the genetic code during reproduction and to provide variability. Replication is the production of identical strands of DNA. This must occur prior to cell reproduction. Organisms use semi-conservative replication. Each "old strand" of DNA serves as a template upon which the "new strand" is synthesized. The double strands separate to form two templates. DNA nucleotides paired according to base pairing rules. Thus a segment of DNA will look as follows:

leading strand	AGCTCCGGTAAC
lagging strand	TCGAGGCCATTG

The black line above can be represented as a hydrogen bond that splits during replication. The result of replication is that now there are two strands. Below shows where they came from:

old leading strand	AGCTCCGGTAAC	new strand	AGCTCCGGTAAC
new strand	TCGAGGCCATTG	old lagging strand	TCGAGGCCATTG

The second function of DNA is in its relationship to proteins. DNA determines the protein's primary structure, the order and type of amino acids in the chain, which in turn determine its shape and function. DNA is the blueprint that tells the cell how to and which kinds of proteins to make. The expression of a gene is known as its phenotype. More will be presented later when RNA is discussed.

Palindromes, an inverted repeat, are sequences of DNA that read the same forward and backward, i.e. GAATTC› (reading forward) and CTTAAGG ‹ (reading backward). Notice that the bp are complimentary to each other. They function as a starting point for DNA replication, binding site for enzymes, and permit loops to form in supercoiling DNA. Restriction endonucleases are enzymes that hydrolyze DNA at palindromic sites and are used in recombinant DNA technology.

RNA Code

RNA is usually composed of single strands of nucleotides (some viruses are double stranded. Their nitrogenous bases are purines (adenine & guanine) and pyrimidines (cytosine and uracil). When making RNA, they will pair up according to base pair rules, adenine pairs with uracil and guanine pairs with cytosine. The sugar is ribose and there is a phosphate group present.

Types of RNA

Messenger RNA (mRNA) is produced from DNA patterns during transcription (master DNA code is first copied onto mRNA through transcription) and carries the DNA master code to the ribosomes. RNA polymerase joins RNA nucleotides paired according to base pairing rules. Each gene produces a unique mRNA that consists of chains of codons (nucleotide triplets) that code for sequences of amino acids in proteins.

A codon is a nucleotide triplet. Each specific codon represents a particular amino acid. For example, CAU is histidine and GUA is valine. Proteins are made from a combination of amino acids.

Transfer RNA (tRNA) is also a copy of DNA code. It contains sequences of bases that form hydrogen bonds with complimentary sequences of the same tRNA strand forming hairpin loops in a cloverleaf configuration that brings amino acids to the ribosome and helps in translation. There are 64 codon patterns possible from the four nucleotides. Each tRNA can bind to one of

these sixty-four different anti-codons. 61/64 codons represent one of twenty different amino acids. Three of the sixty-four types are start or stop codons. The sixty-one possible codons code for only twenty different amino acids which means that one amino acid may be encoded by more than one triplet codon. This degeneracy of the genetic code may function to protect the cell from minor mutations.

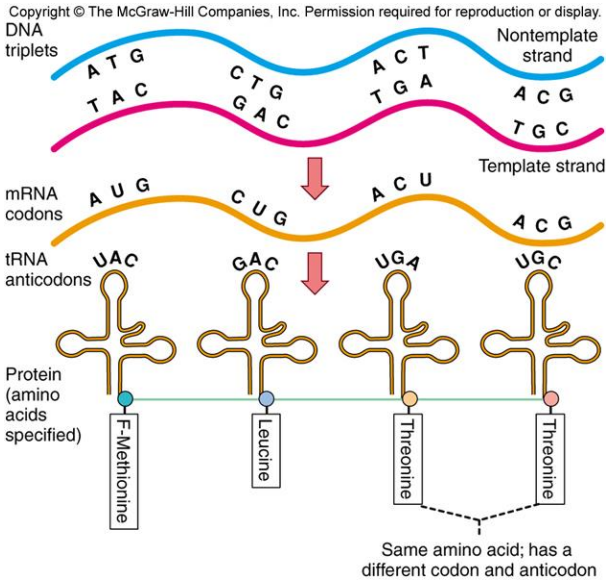
Ribosomal RNA (rRNA) forms the major part of the ribosome and participates in protein synthesis.

Protein Synthesis Sequence

DNA unzips. Transcription produces mRNA using the DNA code by complimentary base pairing rules. Specific tRNA picks up a specific amino acid. The tRNA codon attaches to complimentary mRNA codon. mRNA attaches to ribosome. tRNA anticodons attach to complimentary codons of mRNA amino acids join to produce protein. Translation is the production of a protein from an mRNA strand. All elements needed to synthesize a protein are brought together on the ribosome.

If you know the mRNA of an organism you can easily figure out the protein that is made as well as the DNA code that dictates which protein is to be made. Let's put all this together with the following example.

Template strand of DNA	TAC GAC TGA TGC
Coding strand of DNA	ATG CTG ACT ACG
mRNA (coded from the coding strand of DNA)	AUG CUG ACU ACG
tRNA (transfers the correct polypeptide)	UAC GAC UGA UGC
amino acid codon makes	AUG CUG ACU ACG



Amino acids made: f-methionine (always the start codon), leucine, threonine, and threonine.

The process for Prokaryotes versus eukaryotes is similar but not identical. Bacteria transcribe and translate simultaneously because there is no nucleus. Bacterial polyribosomes attach themselves directly to mRNA as it is released by its DNA template. Viruses use the host cellular mechanisms to make their proteins. Eukaryotic cells, especially animal cells, have introns (noncoding sections) that are interspersed between exons (coding regions).

There are Antibiotics that affect transcription and translation. Remember, if you prevent an organism from producing the proteins it needs, the organism will usually not survive. Therefore, blocking their protein synthesizing mechanism without disrupting the cell synthesis of the host will inhibit growth of infectious agents. The antibiotics usually attack ribosomes because ribosomes are different in bacteria than in a eukaryotic cell. Examples of antibiotic that block protein synthesis are chloramphenicol, tetracycline, and clindamycin.

Mutation Mechanisms

A Mutation is defined as permanent changes in the DNA that may be passed along from generation to generation. A mutation is usually a rare event. A Wild type (strain) is a microorganism that exhibits non-mutated characteristics. The mutant strain can show variance in morphology, nutritional characteristics, genetic control mechanisms, and resistance to chemicals, temperature preference, and any type of enzymatic function.

Causes of mutation can be spontaneous such as a random change in DNA or they may arise from mistakes in DNA replication. Mutations may also be induced due to chemical or physical factors. Many chemical mutations are also carcinogenic. Usually a mutation results in cell death. DNA does have the ability to repair itself under certain conditions during a proofreading sequence.

Categories of mutations are based on the alterations of base sequences of DNA or they are based on overall effects of the mutation. Point mutations change the nature of one gene. A **Frameshift mutation** is a point mutation that deletes or inserts a single nucleotide in the sequence. **Substitution** occurs when a wrong base pair is put in place of correct base pair producing an error in base pairing, thus a change in the codon. Inversion is change in one or two codons (adjacent base pairs change position). A **Silent mutation** occurs when base pairs change but there is no change in amino acid produced. **Missense** is change in the base that causes an alteration in one codon. With consequences that range from none to severe. The result could be a faulty, nonfunctional protein, a different, but functional protein, or there may be no significant alteration in protein function. Nonsense (STOP codon) are large mutations in which whole chromosomes are lost or large genetic sequences are inserted, and are usually fatal. Major mutations alter the number of genes present in bacteria.

All mutations are not bad. The genetic transfer of genes that allow an organism to adapt to changes in a new environment ensure a better chance of survival of that organism. Some environmental factors that influence the genes are physical factors or chemical factors.

A number of repair mechanisms have evolved in bacterial cells to minimize the damage to DNA. They are direct DNA repair, excision repair, post-replication repair, SOS repairs and error prone repairs.

Gene transfer

Horizontal gene transfers are the transfer of genes from one species of organisms to another (broad host range) or between two closely related species (narrow host rage). These have been 'bad' such as the gene that created *E. coli* O157:H7 and in transferring resistance mechanisms between species. There have also been 'good' gene transfers such as the gene transfer among soil organisms that created bacteria capable of degrading polluted sites. The mechanisms of gene transfer are conjugation, transformation, and transduction.

Conjugation is the exchange or addition of large portions of DNA. It requires F+ and F- strains of bacteria (F = fertility plasmid). The donor and recipient are both alive and transfer is made via pili. DNA is copied first, and then sent to another cell. This may allow extreme variation and may occur between different genera. Plasmids are transferred. The importance of these phenomena is in the transfer of drug resistance genes from one species to another.

Transformation occurs when DNA from dead organisms is absorbed into a live competent recipient. New DNA replaces innate DNA with new characteristics from the dead organism.

Transduction (lysogeny) is genetic transfer by means of a bacteriophage (donor is lysed bacterial cell). The virus injects its own nucleic acid + DNA from a previous host into a new host. The new DNA replaces innate DNA with new characteristics.

Another type of gene transfer is the transposon. Transposons (jumping genes) are mobile genetic elements that can transfer DNA within a cell from one position to another in the genome or between different molecules of DNA. Sometimes this insertion inactivates genes and the cell dies. Other times, unique properties are expressed.

Genetic Engineering

Tools and Techniques of Genetic Engineering

- Restriction endonucleases clip polynucleotide strands of DNA at selected positions.
- Oligonucleotides are shorter segments of base pairs.
- Polymerase Chain Reactions (PCR) amplifies small amounts of DNA into larger quantities for further analysis. Real-time PCR instrumentation

amplifies DNA and interprets the results as the test is running thus shortening the turn-around-time.
- Gel electrophoresis is used to isolate fragments of DNA that can be inserted into vectors, multiplied by PCR, or preserved in a gene library.
- Nucleic acid hybridization and probes can detect specific nucleotide sequences in unknown samples. These are often used in the clinical setting to diagnose the cause of an infection from a patient or to identify a culture of an unknown organism.

Recombinant DNA technology

- Deliberately removes genetic material from one organism and combines it with another to form genetic clones.

Recombinant Products

- Hormones and Drugs: Erythropoietin, tissue plasminogen activating factor (dissolves blood clots), Hemoglobin A, Factor VIII, relaxin, proleukin (treat kidney cancer)
- Enzymes and hormones
- Pesticides
- Probes
- Vaccines: Hepatitis B, H. influenza type b, experimental malaria and AIDS

Transgenic Organisms are foreign genes that are introduced into organisms (process is called transfection). The process has been used with microbes ("Designer Organisms") to produce frost-free bacteria, bacteria that are also an insecticide, viral vaccines, and engineered organisms that clean up oil spills, pesticides, and other toxic substances. It has also been used with plants to produce plants that are pesticide resistant, disease resistance, as well as those that have increased yields and are improved fruits and vegetables. In animals, transgenic organisms have been produced to study human genetic disease, improve animal husbandry by engineering for better protein production and other products.

Genetic Treatments

Gene Therapy replaces a faulty gene with a normal gene in people with a fatal or extremely debilitating disease. There have been some problems but also some tremendous accomplishments-notably severe combined immunodeficiency syndrome. The theory is to use a retro viral vector to insert gene. UW – Madison has patented stem cell lines for replacement genes.

Antisense and Triplex DNA Technology are genetic medicines that bypass gene problems and protein altogether.

Genetic Maps

Mapping of the genes on the chromosomes is the process of delineating the relative order and position of genes and nucleotides. It allows genetic fingerprinting (who you are). Microbe genome maps provide information about an organism's metabolism, growth characteristics, and relatedness to other microbes. It will and is being used in genetic screening and genetic therapy in humans as well as being a useful tool in forensic science. It is also being used to determine virulence factors in organisms. Many organisms, as well as viruses, have been mapped already.

The determination of gene or lack of a gene in certain genetic diseases brings up question of ethics. Is it right to manipulate the genome of an organism, be it small as a virus or as large as a human? What will the consequences be?