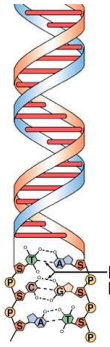
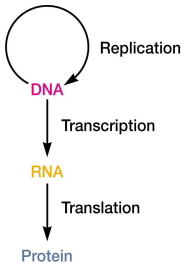
Microbial Genetics



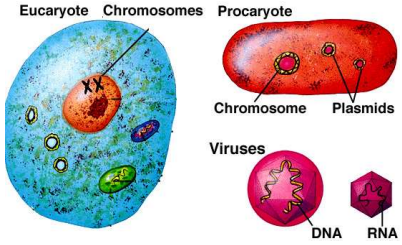
Genes and Genomes
The Central Dogma
DNA Replication
Transcription
Translation
Gene Expression



Genes and Genome:

* The genome is all genetic instructions (genes) for development of cellular structures, metabolic functions, and their regulation (organization of which, when, where, and how much).

* Genes are located within chromosomes (linear or circular) or plasmids, as specific regions of DNA (or RNA for some viruses).



Genes: "The Basic Unit of Heredity"

• Genes represent the instructions for making a particular product, which are passed on to each new daughter cell when a parent cell divides.

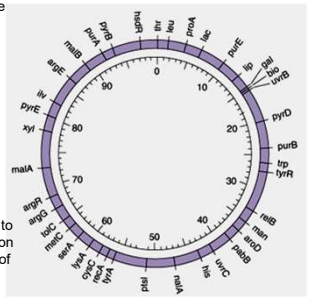
• Gene product refers to the actual structures, enzymes or regulators.

• Most gene products will be a polypeptide, which fold-up into functions proteins.

• The instructions are manifest as a unique sequence of nucleotide base pairs within a larger DNA molecule.

• A universal genetic code is followed to convert base pair sequence information into the proper amino acid sequence of the protein product.

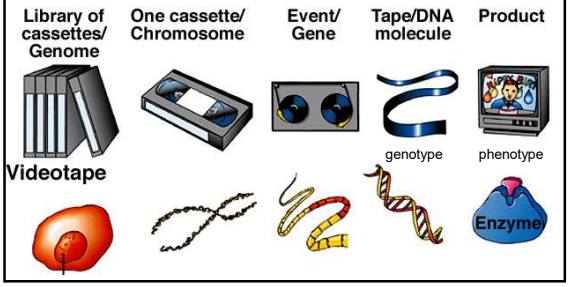
• We can "map" the location of genes on chromosomes, called loci.



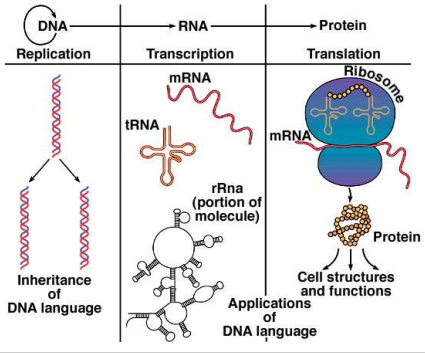
Escherichia coli chromosome map.

• The genotype of an individual refers to its particular genetic make-up. It's the potential for all characteristics we can observe. A particular gene may have some variation in the sequence between individuals, called alleles.

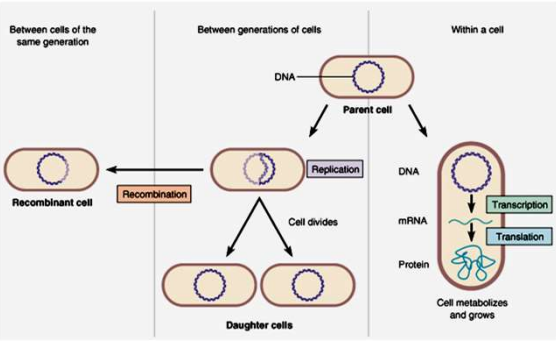
• The phenotype is the actual characteristics observed for an individual, or manifestation of genotype. Based on which allele(s) one individual has for a given gene will cause variation in phenotype within a species (e.g. skin color).



The Central Dogma of Biology

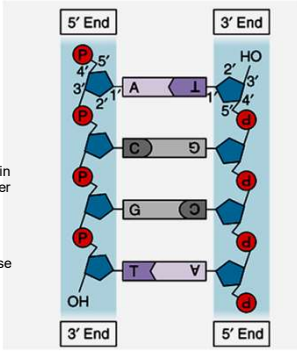


Genetic Information Flow in Bacteria

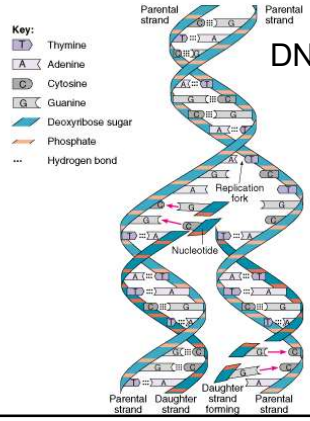


DNA Overview

- Deoxyribonucleic Acid (DNA)
- Two polymers of deoxynucleotides (called strands) twisted into a helix structure.
- Deoxynucleotides have 1 of 4 nitrogen bases (thymine, cytosine, adenine, guanine).
- DNA strands run antiparallel (oriented in opposite directions. Stands held together by H-bonds between complementary base pairs.
- Base pair complementation between antiparallel strands follows universal base pairing rules:
  - purine to pyrimidine:
    - A to T (2 H-bonds)
    - G to C (3 H-bonds)



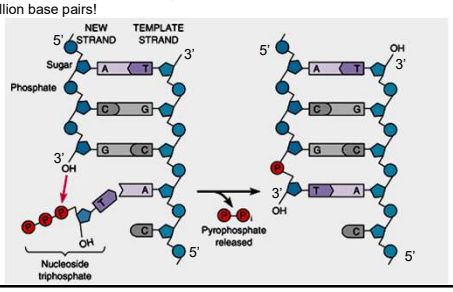
DNA Replication



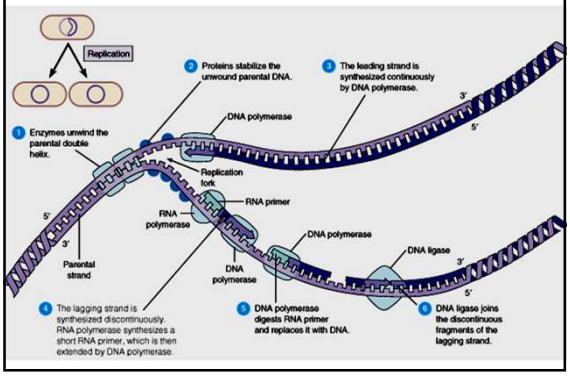
* New strands of DNA are made by polymerizing deoxynucleotides by an enzyme, DNA Polymerase.
- DNA Polymerase requires a parent DNA strand to serve as a template to direct synthesis of the new complementary antiparallel daughter strand.
- In this regard, DNA replication is semiconservative.
- Notice how the parent double strands must break H-bonds between complementary bases at the replication fork so to provide a single parent strand for use as template.

Action of DNA Polymerase:

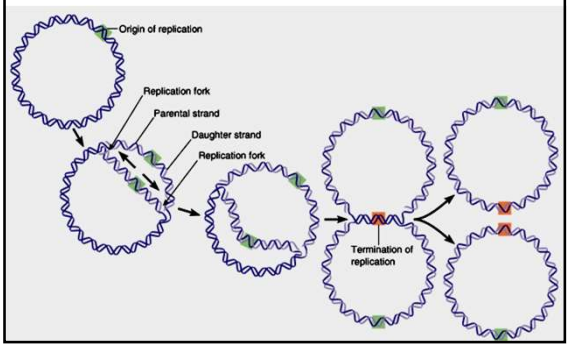
- DNA Polymerase only adds deoxynucleotides to the 3' end of a forming daughter (new) strand; thereby, the two daughter strands form in opposite directions due to the antiparallel orientation of the two parent (template) strands.
- DNA Polymerase has the ability to "proof reading" its work and to correct any mismatched base pairs. It is so good that errors only happen once in about every 10 billion base pairs!



DNA Replication in Detail:



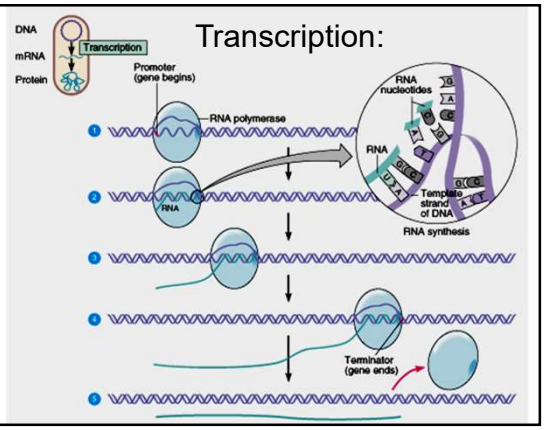
Circular DNA will have two replication forks moving in opposite directions from the Origin of Replication.



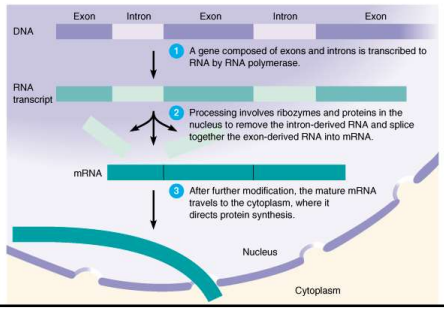
Transcription:

- Getting genetic information from DNA into a gene product requires synthesis of RNA.
- RNA is synthesized by RNA polymerse, which added ribonucleotides to the 3' end of the growing RNA polymer.
- RNA polymerase requires a DNA strand as a template for adding complementary ribonucleotides (recall T is replaced by U).
- Transcription begins at a site on the DNA, called a promotor, located immediately before a gene or a set of genes (operon). This is where the RNA Polymerase first binds to the DNA.
- Transcription of RNA proceeds until RNA Polymerase reaches the terminator DNA sequence at the end of the gene or operon. Both mRNA and RNA Polymeraser are released.
- RNA used to direct protein synthesis by the ribosome (translation) is called a message RNA (mRNA).
- Other RNA function as gene products themselves, as is the case for ribosomal RNA (rRNA), transfer RNA (tRNA) and small catalytic RNA.

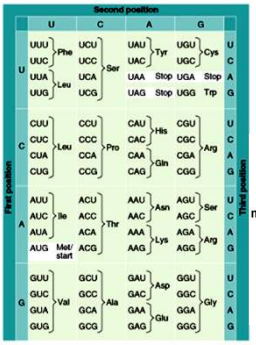
Transcription:



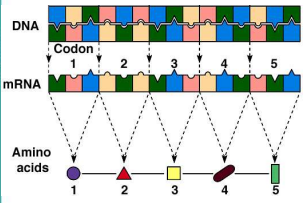
Eukaryote transcription is more complex than prokaryote. The RNA produced in transcription needs to be further processed prior to its function as a mRNA in translation to a polypeptide.



The Genetic Code

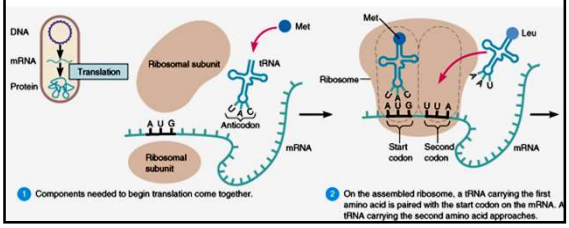


- Every three mRNA nucleotides represent a codon to be translated into a particular amino acid based on the Genetic Code.
- Translation begins at the Start Codon (AUG) and proceeds codon by codon there after until a Stop Codon (one of three) is reached.

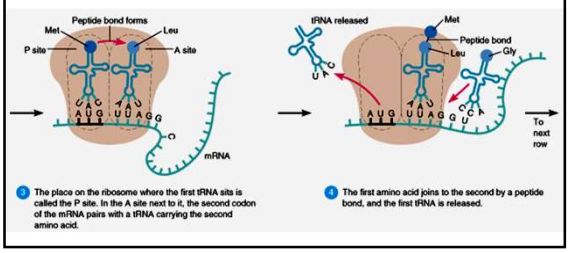


Translation (Protein Synthesis):

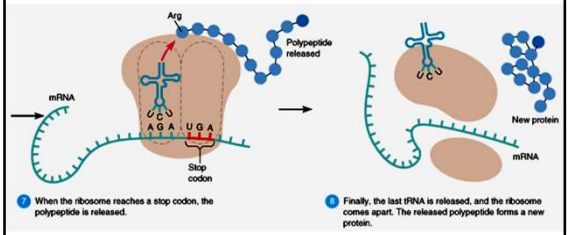
- Codons are complementary to a region of three nucleotides, called anticodons, on transfer RNA (tRNA) molecules.
- Start codon is first recognized by ribosome subunits, which sandwiches the mRNA and allows the first tRNA to bind by base pair complementation between mRNA codon and tRNA anticodon.
- tRNAs only bind if "charged" with its correct amino acid (methionine for start codon).



- The ribosome moves down the mRNA by one codon (3 nucleotides) to make room for addition of the next charged tRNA in what is called the ribosome A-site.
- Once a second charged tRNA binds at the A-site, a peptide bond will form between the amino acid in the P-site and that on the tRNA in the A-site.
- The "uncharged" tRNA will release from the P-site and, again, the ribosome will move down the mRNA by one codon.



- The process continues, codon by codon, adding amino acids to a growing polypeptide, until the ribosome reaches a Stop Codon.
- No new amino acid is added by the stop codon, rather it facilitates the release of the last amino acid from its tRNA; thereby releasing the polypeptide.
- Again the polypeptide may now fold into a functional protein.



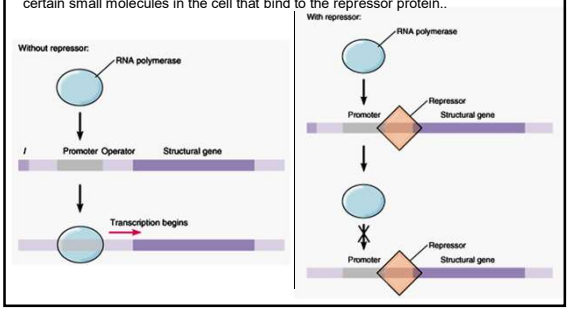
Regulation of Gene Expression:

- Gene expression refers to the combined process of transcription and translation of genetic information to a functional protein.
- Not all genes are expressed at any one time, nor are they always expressed at the same level.
- Gene expression is tightly regulated, or controlled, so that the cell only makes the gene products that it needs for efficient growth under its current environmental conditions.
- What's an operon? Regulatory protein binding region and downstream gene(s).
- Regulatory proteins called repressors or activators act as off and on switches for transcription, respectively.
- Negative regulation involves repressor proteins that respond to cell conditions so to actively repress (prevent) RNA Polymerase from beginning transcription of the gene (or operon) by binding onto the DNA at the operator site.
- Positive regulation of transcription also can occur. Here, environmental conditions in the cell causes an activator protein to bind to the promoter site for a gene (or operon), which enables RNA Polymerase to begin transcription.

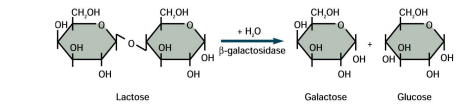
Negative Regulation:

• Binding of a repressor to the operator site prevents RNA Polymerase from transcribing the gene(s).

• A repressors ability to bind to DNA is determined by the presence or absence of certain small molecules in the cell that bind to the repressor protein..



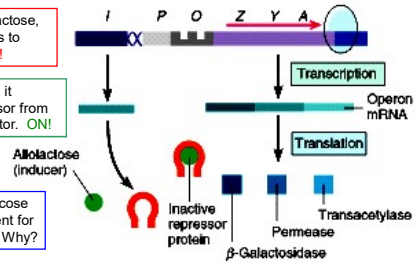
E.g. *E. coli* Lactose Catabolism (*lac*) Operon



When **no** allolactose, repressor binds to operator. OFF!

With allolactose, it prevents repressor from binding to operator. ON!

However, glucose must be absent for transcription! Why?



Positive Regulation:

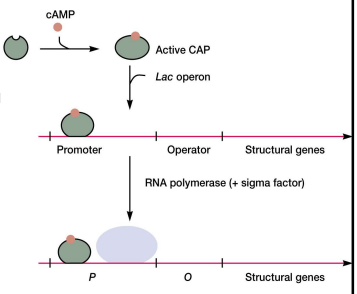
E.g. *E. coli* Lactose Catabolism (*lac*) Operon

• *E. coli* growth using glucose is more efficient than growth on lactose.

• How is wasteful expression of *lac* operon genes prevented when glucose is present?

• *lac* operon needs an activator bound to promoter site for any chance of transcription, regardless of the repressor.

• The catabolic activator protein (CAP) only binds in the absence of glucose.



The *lac* Operon in Action:

• *E. coli* growth is slower on lactose than on glucose.

• Glucose will be used before lactose given a choice. Here the repressor does not stop transcription; however the CAP activator does by not binding to the promoter.)

• Once glucose runs out the CAP activator binds, and the *lac* operon is expressed in the presence of lactose.

• Why is there a lag in growth when glucose runs out? This is called diauxic growth.