SUMMARY OF REPLICATION, TRANSCRIPTION & TRANSLATION

DNA replication is the process cells use to make new DNA, and is **semi-conservative** in that each new DNA double-helix formed contains half of the DNA strand replicated. Replication as it occurs within cells requires a DNA template, energy provided by **nucleoside triphosphate** molecules (dNTPs and rNTPs), and multiple different types of enzymes. **DNA-dependent DNA-polymerase** is the primary enzyme required to build DNA (and the only one used in the PCR). This enzyme catalyzes the formation of **phosphodiester bonds** at the free 3' ends of existing nucleotide strands (builds 5' to 3'). It does this by removing a **pyrophosphate** (PO_4~PO_4) from each new NTP, and using the energy released to bind the remaining phosphate group of the nucleotide to the 3' carbon of deoxyribose (a water molecule is released in the process).
Prokaryotic cells have five DNA polymerase enzymes designated as DNA polymerase I, II, III, IV and V. DNA polymerase III is an enzyme complex made up of several proteins, and is the primary builder, i.e., is responsible for the replication of chromosomal DNA, plasmid DNA, phage DNA, etc. It can also correct replication errors by removing nucleotides from the 3' ends of nucleotide strands. It is a double-stranded DNA binding protein, so requires the activity of DNA polymerase I. It forms parts of a body called the **replisome** that is located at each replication fork during DNA replication, and can catalyze base pair formation at the rate of about 1000 nucleotides per second.
DNA polymerase I is the most abundant. It begins replication by adding nucleotides to RNA primers, and then removes these primers (can catalyze reactions both at 3' and 5' ends of nucleotide strands). It is also involved in DNA repair. DNA polymerase II is involved in repair and as a back-up to DNA polymerase III (has similar building ability). DNA polymerase IV works to stall replication by DNA polymerase III, thus allowing more time for repair mechanisms. DNA polymerase V is involved in the repair of damaged DNA.

DNA polymerase enzymes in eukaryotic cells are also multiple and are designated as DNA polymerase β, γ, σ and μ (beta, lambda, sigma and mu).

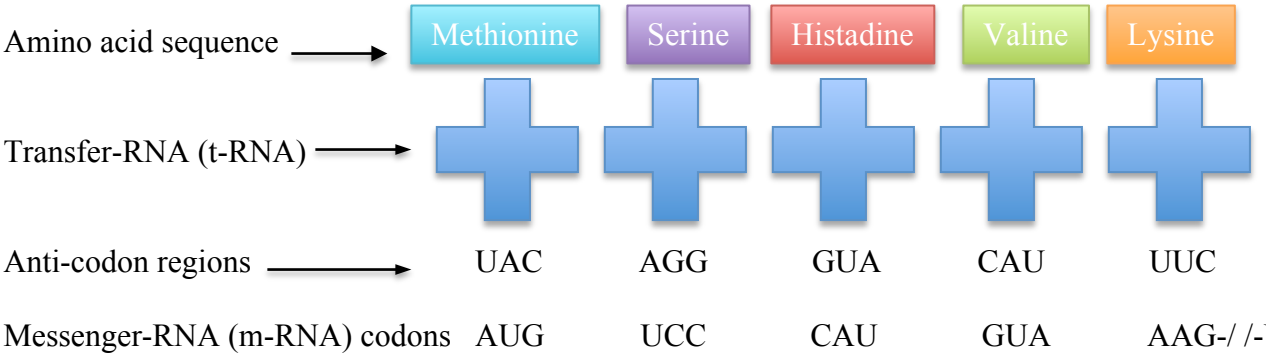
DNA replication begins at the **origin of replication** (one point on a ccc-DNA molecule, multiple points on linear DNA molecules), and proceeds in both directions from that point. **Helicase enzymes** separate the two DNA strands (break hydrogen bonds) and **topoisomerase enzymes** (gyrase enzymes) prevent the DNA from becoming tangled. **DNA primase** (a DNA dependent RNA polymerase) binds with and is activated by DNA helicase (within a complex called a primosome) and then begins building a **short RNA primer** using rNTPs as the energy source. The arrangement of rNTPs is determined by the arrangement of bases in the template DNA strand. DNA polymerase I enzymes eventually bind and begin adding dNTPs to the free 3' ends of each growing nucleotide strand. Then DNA polymerase III attaches and builds the new DNA of each **leading strand** as a continuous sequence.
Because DNA polymerase enzymes can only build from 5' to 3' (can only add nucleotides to the free 3' ends of growing nucleotide strands) the **lagging strand** is synthesized as a series of short DNA segments called **Okazaki fragments**. Each of these contains a short RNA primer synthesized by DNA primase enzymes (as described above) and is built in a direction opposite that of the leading strand because the two strands of the DNA double helix are antiparallel. Eventually DNA polymerase I enzymes remove the RNA primers, and **DNA ligase enzymes** bind the Okazaki fragments into a continuous DNA sequence.
In bacteria such as *Escherichia coli*, replication of the entire chromosome requires approximately 60 minutes, but bacteria can undergo binary fission every 20 minutes because they run multiple replication cycles at once. Replication cycles begin at the origin of replication (*ori*) and proceed around the covalently-closed, circular chromosome (ccc-DNA) in both directions. Before the first replication cycle is completed, a second and third cycle are begun.

Transcription is the process cells use to synthesize RNA and occurs within the nuclei of eukaryotic cells, the nucleoids of prokaryotic cells, within mitochondria and chloroplasts and in association with plasmids. Like replication, transcription requires a DNA template, energy (provided by rNTPs) and enzymes. Transcription requires a type of enzyme called **DNA-dependent RNA polymerase** that in prokaryotic cells is a complex composed of six subunits. The core enzyme complex (α,α,β, β' and ω) is the primary builder, but a protein called **sigma factor** is essential for the initiation of transcription (bacteria typically have multiple different types of sigma factor proteins). The sigma factors of RNA polymerase bind to specific regions of DNA called **promoter sites** and thereby determine where transcription will begin. The DNA strand being transcribed will unwind just ahead of the polymerase enzyme as it proceeds along, and wind back up behind it.

Nucleotide sequence of DNA strand being transcribed is – TACAGGGTACATTTC-/ /-ATC
Nucleotide sequence of RNA strand being synthesized is – AUGUCCCAUGUAAAG-/ /-UAG

The nucleotide sequence of the template DNA determines the nucleotide sequence of the RNA molecule being synthesized as shown above (note that RNA contains uracil in place of thymine). In prokaryotic cells transcription is typically **polycistronic**, i.e., each m-RNA molecule synthesized is a copy of multiple structural genes. In eukaryotic cells transcription is monocistronic, and each m-RNA made undergoes **post-transcriptional modification** before it is ready for use. This process is accomplished by **spliceosomes** (s-RNA molecules are ribozymes) and involves the removal of **introns**, the splicing of **exons** and the addition of a cap and poly-adenine tail to each m-RNA made.

Translation is the process cells use to synthesize proteins and occurs at **ribosomes**. In prokaryotic cells the larger ribosomal subunit contains protein and **23S ribosomal-RNA**, and it is the r-RNA that catalyzes the formation of peptide bonds between amino acids. This **ribozyme** is called **peptidyl transferase**. **Transfer-RNA** molecules carry individual amino acids to the ribosomes, and a set of proteins called **aminoacyl-t-RNA-synthase** enzymes catalyze reactions insuring that each t-RNA is carrying the correct amino acid. The primary structure of each protein (sequence in which amino acids are arranged) is determined by the nucleotide sequences found in **messenger-RNA** molecules (m-RNA). When **aminoacyl-t-RNA** molecules reach the ribosomes, their **anti-codon** regions form hydrogen bonds with complementary **codons** on the m-RNA being translated (as coordinated by 16S r-RNA molecules).

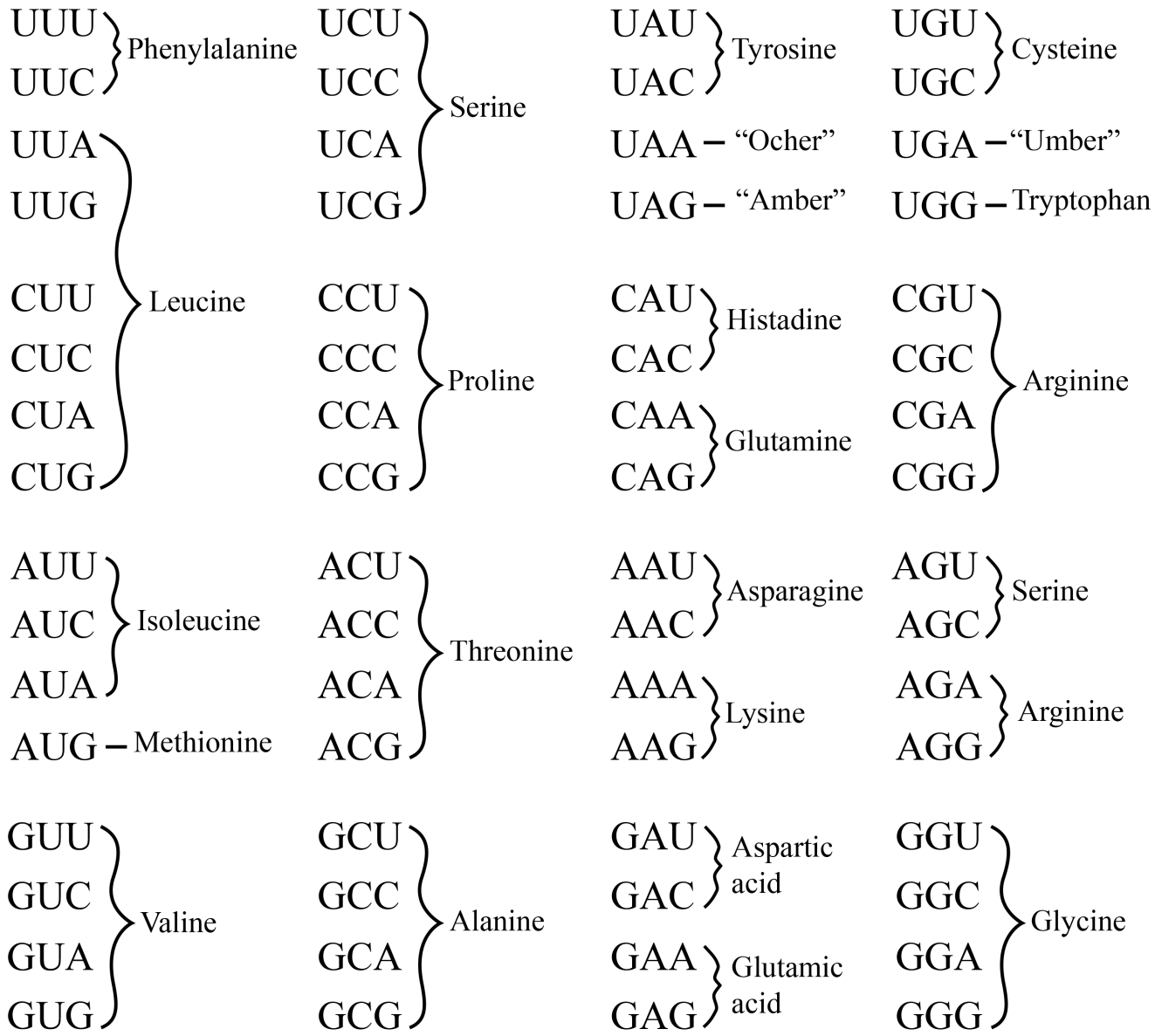


In the example above, the m-RNA contains a gap (indicated by base-/ /-base) and the last codon does not code for any anti-codon region because it is a **terminator codon** (stop codon) and ends translation. The codon AUG is an **initiator codon** (start codon) but also encodes the amino acid methionine (formylmethionine in bacteria).

Important note - The genetic code is made from codons, not anti-codons, so do not use anti-codon sequences when translating m-RNA sequences into polypeptide sequences on paper.

The Genetic Code

Although there are variations found within some cells and mitochondria, the most commonly represented genetic code is like the one shown below. Because multiple codons encode the same amino acid the code is said to be redundant.



Note: "Ocher", "Amber" and "Umber" are not amino acids. The three codons, UAA, UAG and UGA are terminator codons and do not encode any amino acids.