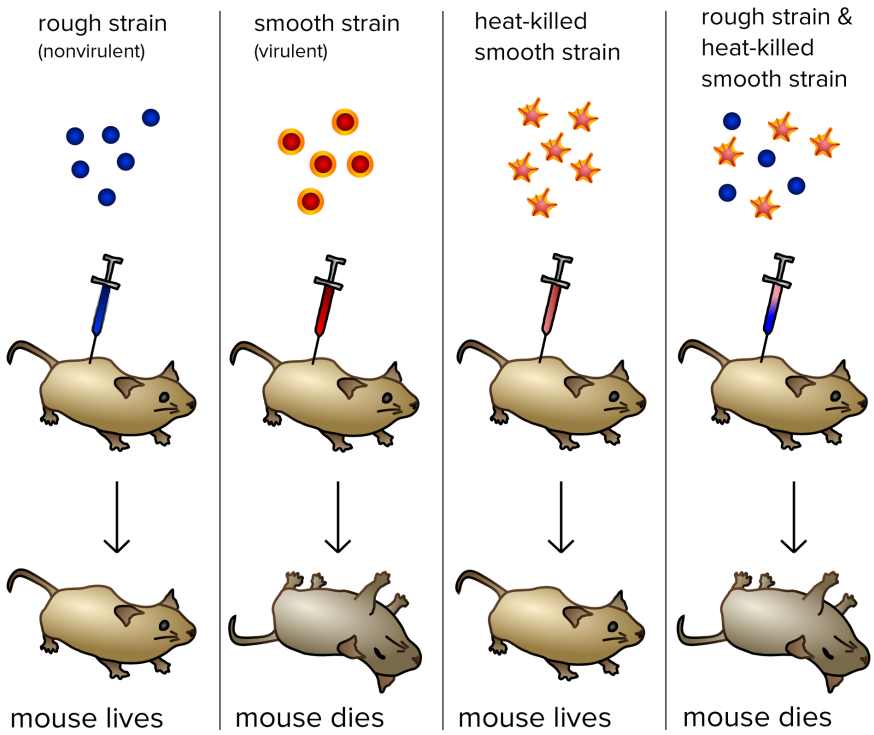
Name_____ Grade 10
Date_____ Study Guide

In 1928, British bacteriologist Frederick Griffith conducted a series of experiments using *Streptococcus pneumoniae* bacteria and mice. Griffith wasn't trying to identify the genetic material, but rather, trying to develop a vaccine against pneumonia. In his experiments, Griffith used two related strains of bacteria, known as R and S.

- **R strain.** When grown in a petri dish, the R bacteria formed colonies, or clumps of related bacteria, that had well-defined edges and a rough appearance (hence the abbreviation "R"). The R bacteria were nonvirulent, meaning that they did not cause sickness when injected into a mouse.
- **S strain.** S bacteria formed colonies that were rounded and smooth (hence the abbreviation "S"). The smooth appearance was due to a polysaccharide, or sugar-based, coat produced by the bacteria. This coat protected the S bacteria from the mouse immune system, making them virulent (capable of causing disease). Mice injected with live S bacteria developed pneumonia and died

Griffith concluded that the R-strain bacteria must have taken up what he called a "transforming principle" from the heat-killed S bacteria, which allowed them to "transform" into smooth-coated bacteria and become virulent.



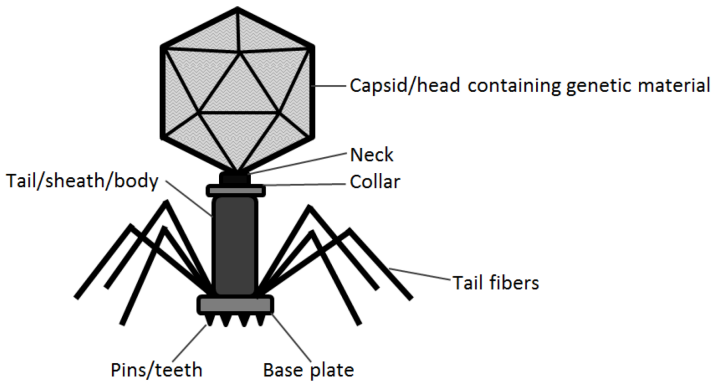
While Griffith's experiment had provided a surprising result, it wasn't clear as to what component of the dead S strain bacteria were responsible for the transformation. 16 years later, in 1944, Oswald Avery, Colin Macleod and MacLynn McCarty solved this puzzle.

They worked with a batch of heat-killed S strain bacteria. They divided it into 5 batches. In the first batch, they destroyed the polysaccharide coat of the bacteria; in the second batch they destroyed its lipid content; they destroyed the RNA of the bacteria in the third batch; with the fourth batch, they destroyed the proteins; and in the last batch, they destroyed the DNA. Each of these batches was individually mixed with live R strain bacteria and injected into individual mice.

From all 5 mice, all of them died except the last mouse. From all the dead mice, live S strain bacteria was retrieved. ***This experiment clearly proved that when the DNA of the S strain bacteria were destroyed, they lost the ability to transform the R strain bacteria into live S strain ones.*** When other components, such as the polysaccharide coat, lipid, RNA or protein were destroyed, transformation still took place. Although the polysaccharide coat was a virulent factor, it wasn't responsible for the transfer of the genetic matter.

The Hershey-Chase experiments

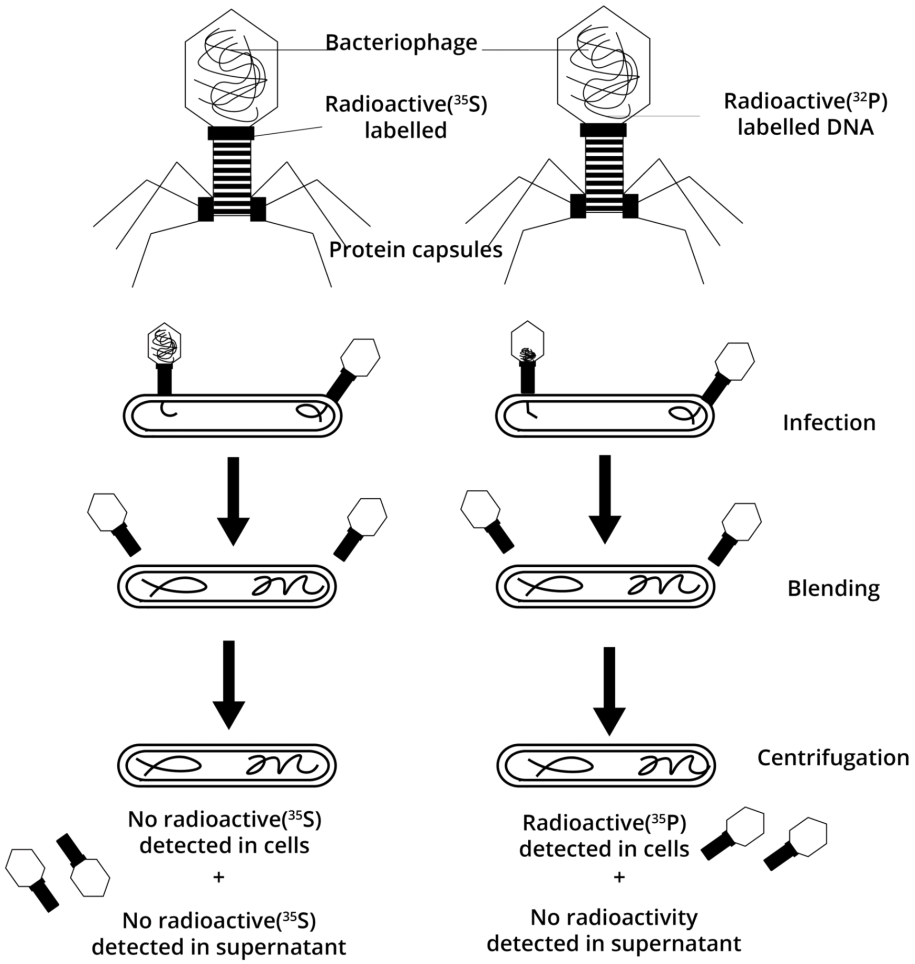
In their now-legendary experiments, Hershey and Chase studied **bacteriophage**, or viruses that attack bacteria. The phages they used were simple particles composed of protein and DNA, with the outer structures made of protein and the inner core consisting of DNA.

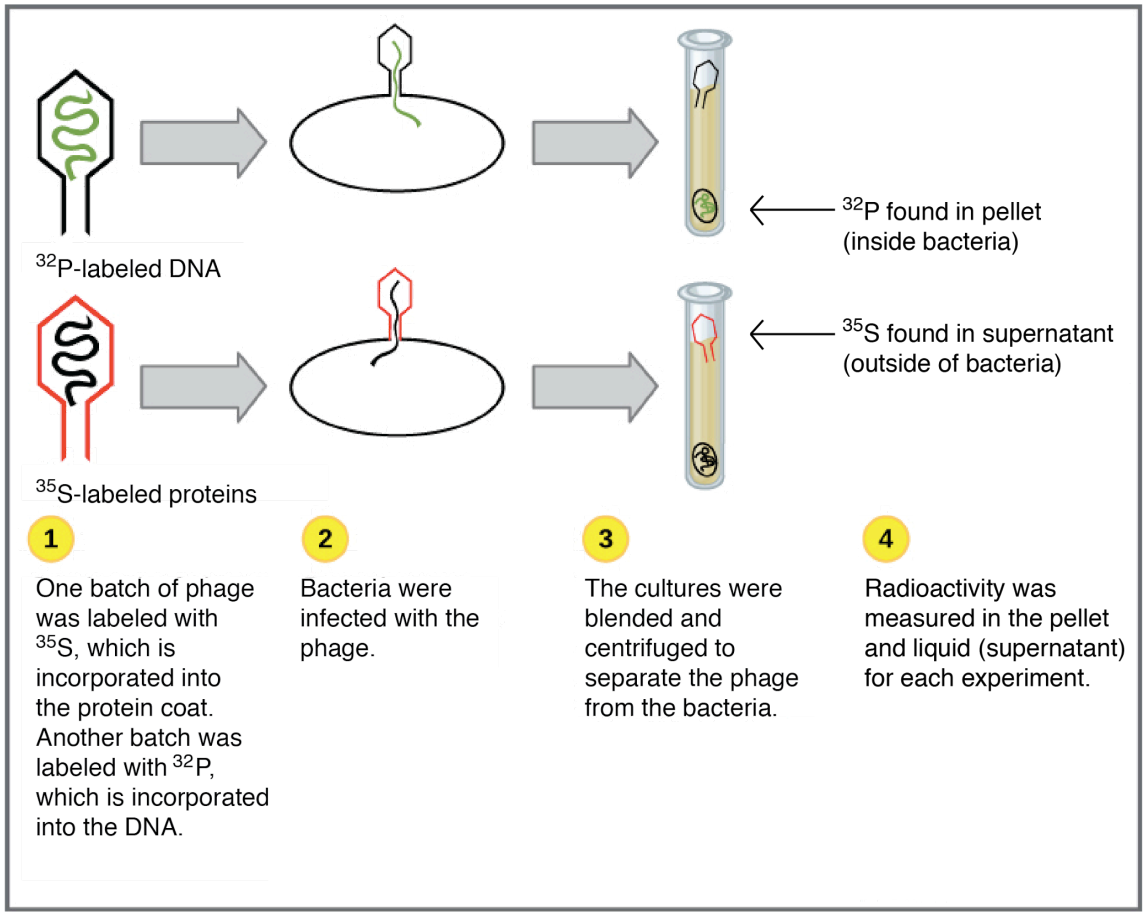


Hershey and Chase knew that the phages attached to the surface of a host bacterial cell and injected some substance (either DNA or protein) into the host. This substance gave "instructions" that caused the host bacterium to start making

lots and lots of phages—in other words, it was the phage's genetic material. Before the experiment, Hershey thought that the genetic material would prove to be protein

To establish whether the phage injected DNA or protein into host bacteria, Hershey and Chase prepared two different batches of phage. In each batch, the phage was produced in the presence of a specific radioactive element, which was incorporated into the macromolecules (DNA and protein) that made up the phage.

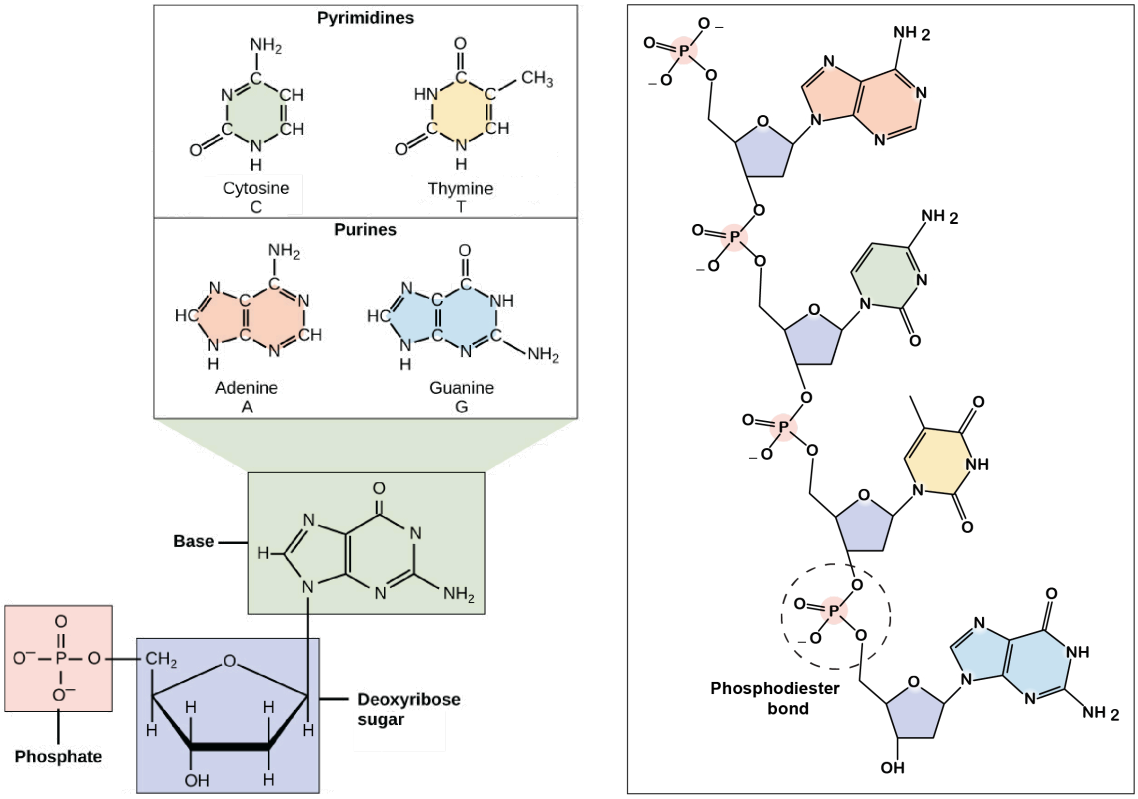




The components of DNA

From the work of biochemist Phoebus Levene and others, scientists in Watson and Crick's time knew that DNA was composed of subunits called **nucleotides.** A nucleotide is made up of a sugar (deoxyribose), a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), guanine (G) or cytosine (C).

C and T bases, which have just one ring, are called **pyrimidines**, while A and G bases, which have two rings, are called **purines**.



DNA nucleotides assemble in chains linked by covalent bonds, which form between the deoxyribose sugar of one nucleotide and the phosphate group of the next. This arrangement makes an alternating chain of deoxyribose sugar and phosphate groups in the DNA polymer, a structure known as the **sugar-phosphate backbone.**

Chargaff's rules

One other key piece of information related to the structure of DNA came from Austrian biochemist Erwin Chargaff. Chargaff analyzed the DNA of different species, determining its composition of A, T, C, and G bases. He made several key observations:

- A, T, C, and G were not found in equal quantities (as some models at the time would have predicted)
- The amounts of the bases varied among species, but not between individuals of the same species
- The amount of A always equalled the amount of T, and the amount of C always equalled the amount of G (A = T and G = C)

These findings, called **Chargaff's rules**, turned out to be crucial to Watson and Crick's model of the DNA double helix.

The structure of DNA, as represented in Watson and Crick's model, which was discovered using **photo 51** taken by Rosalind Franklin using x-ray crystallography, is a double-stranded, antiparallel, right-handed helix. The sugar-phosphate backbones of the DNA strands make up the outside of the helix, while the nitrogenous bases are found on the inside and form hydrogen-bonded pairs that hold the DNA strands together.
Double-stranded DNA is an **antiparallel** molecule, meaning that it's composed of two strands that run alongside each other but point in opposite directions. In a double-stranded DNA molecule, the 5' end (phosphate-bearing end) of one strand aligns with the 3' end (hydroxyl-bearing end) of its partner, and vice versa.

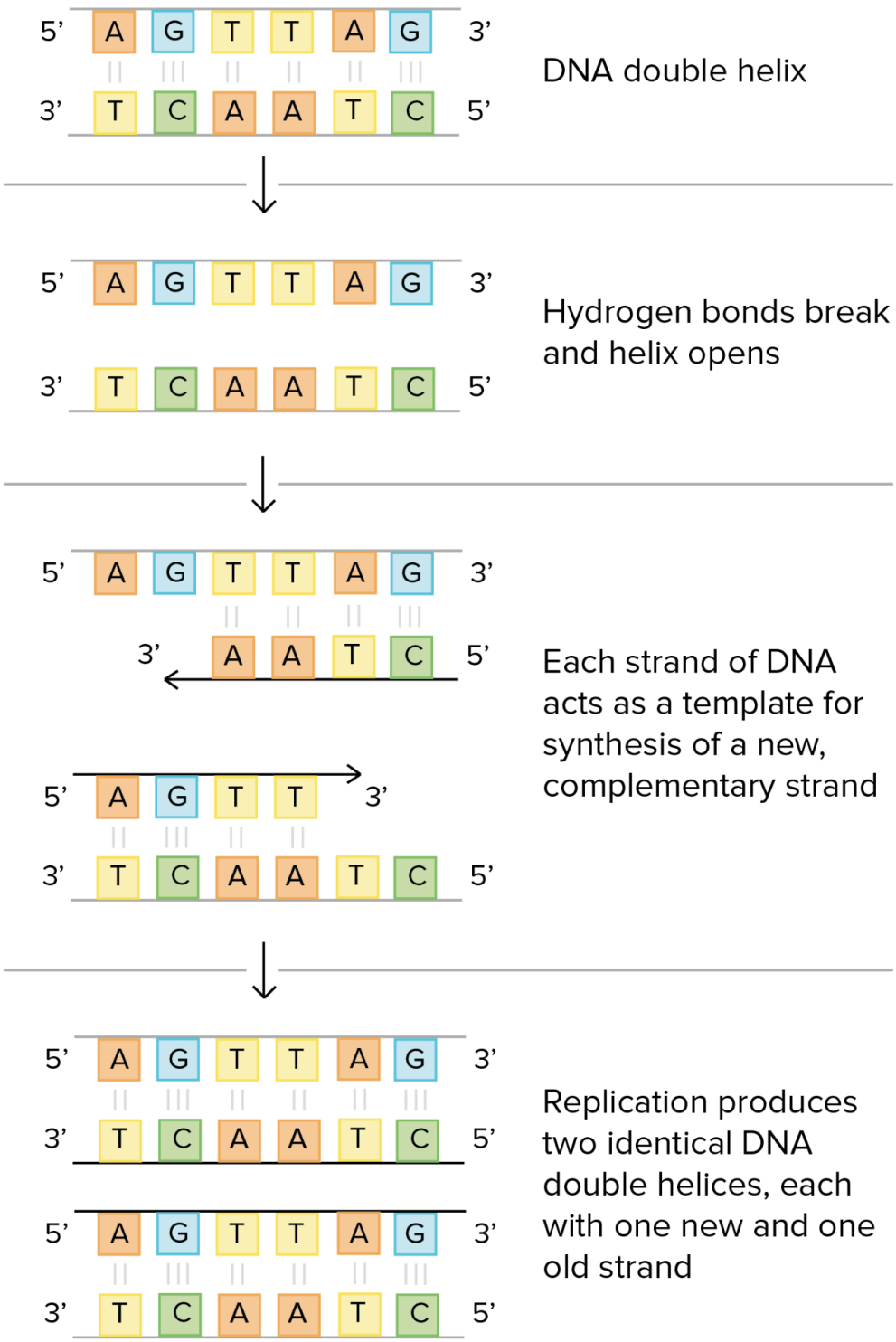
Base pairing explains Chargaff's rules, that is, why the composition of A always equals that of T, and the composition of C equals that of G. Where there is an A in one strand, there must be a T in the other, and the same is true for G and C. Because a large purine (A or G) is always paired with a small pyrimidine (T or C).

Although Watson and Crick's original model proposed that there were two hydrogen bonds between the bases of each pair, we know today that G and C form an additional bond (such that A-T pairs form two hydrogen bonds total, while G-C pairs form three).

DNA replication is semi-conservative. This means that each of the two strands in double-stranded DNA acts as a template to produce two new strands.

Replication relies on complementary **base pairing**, that is the principle explained by Chargaff's rules: adenine (A) always bonds with thymine (T) and cytosine (C) always bonds with guanine (G).

Process of replication:

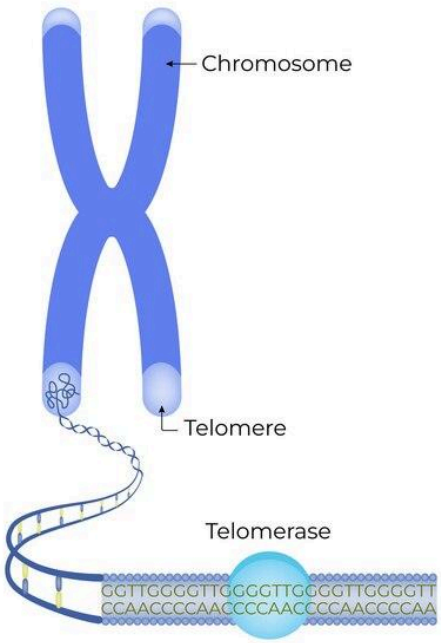


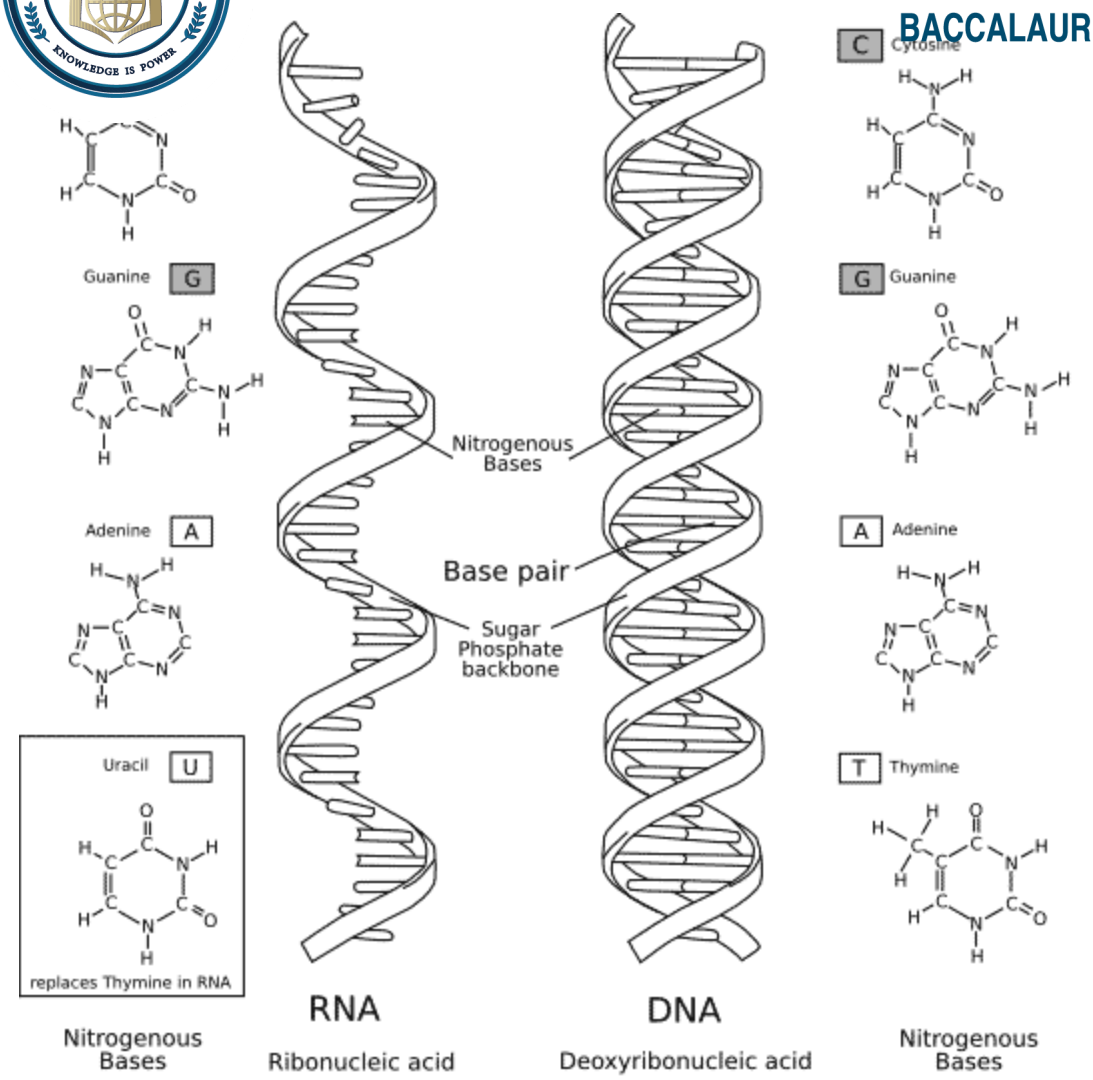
The primary enzyme involved in this is ***DNA polymerase*** which joins nucleotides to synthesize the new complementary strand. DNA polymerase also proofreads each new DNA strand to make sure that there are no errors.

Leading and lagging strands

DNA is made differently on the two strands at a replication fork. One new strand, the *leading strand*, runs 5' to 3' towards the fork and is made continuously. The other, the *lagging strand*, runs 5' to 3' away from the fork and is made in small pieces called *Okazaki fragments*.

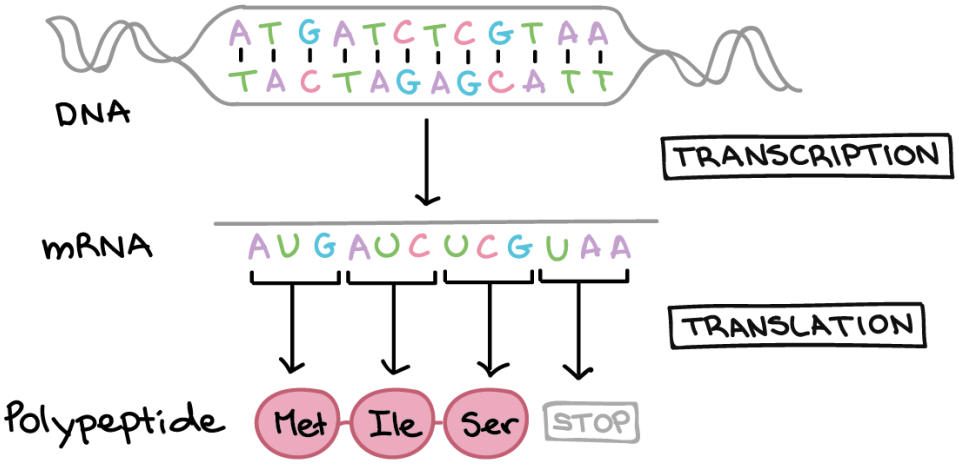
Repetitive regions at the very ends of chromosomes are called **telomeres**, and they're found in a wide range of eukaryotic species, from human beings to unicellular protists. Telomeres act as caps that protect the internal regions of the chromosomes, and they're worn down a small amount in each round of DNA replication.





Central Dogma of Molecular Biology: Doctrine that genetic instructions in DNA are copied by RNA, which carries them to a ribosome where they are used to synthesize a protein.

THE CENTRAL DOGMA



Protein Synthesis: Process in which cells make proteins that includes transcription of DNA and translation of mRNA.

Genetic Code: Universal code of three-base codons that encodes the genetic instructions for the amino acid sequence of proteins.

Codon: Group of three nitrogen bases in nucleic acids that makes up a code "word" of the genetic code and stands for an amino acid, start, or stop.

Transcription: Process in which genetic instructions in DNA are copied to form a complementary strand of mRNA.

RNA Polymerase: An enzyme that helps produce RNA during transcription.

Promoter Site: Region of a gene where a RNA polymerase binds to initiate transcription of the gene.

Introns: Non-coding regions of mRNA that are removed by splicing.

Extrons: Coding regions.

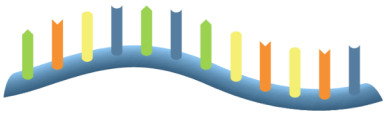
Translation: Process in which genetic instructions in mRNA are "read" to synthesize a protein.

Types of RNA

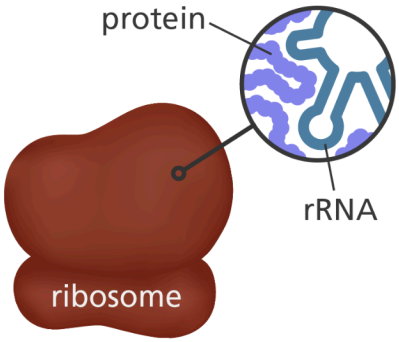
Messenger RNA (mRNA): Type of RNA that copies genetic instructions from DNA in the nucleus and carries them to ribosomes in the cytoplasm.

Ribosomal RNA (rRNA): Type of RNA that helps form ribosomes and assemble proteins.

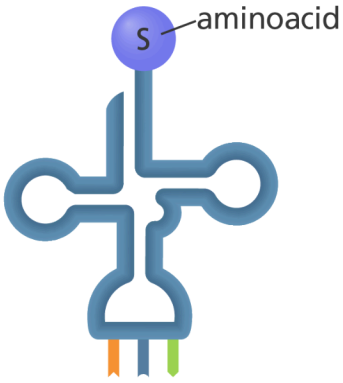
Transfer RNA (tRNA): Type of RNA that brings amino acids to ribosomes where they are joined together to form proteins.



messenger RNA (mRNA)



ribosomal RNA (rRNA)



transfer RNA (tRNA)

Protein molecules play a huge role in the body, as many of our structures are made of protein. The genetic instructions in DNA are carried by RNA to the ribosomes where the proteins are made. The relationship between DNA→RNA→protein is known as the **central dogma of molecular biology.**

The Genetic Code

During **protein synthesis,** the protein is built up one amino acid at a time. DNA contains the information that determines which amino acid comes next. DNA is made up of four different nitrogen bases: ***adenosine (A), thymine (T), cytosine (C), and guanine (G).***

These bases make up the **genetic code.** All living things have the same genetic code. Groups of three of these bases form a **codon** that stands for an amino acid or codes for a start or stop signal.

Role of RNA

DNA always stays in the nucleus, yet the actual process of protein synthesis occurs in the ribosomes of the rough endoplasmic reticulum. Instructions coding for a specific protein from the DNA are transferred to the ribosomes in the form of RNA, a small molecule that can leave the nucleus. The codons in RNA are complementary to the codons in DNA, so the thymine (T) in DNA is replaced with uracil (U) in RNA.

There are three main types of RNA:

messenger RNA (mRNA): copies the genetic instructions from DNA in the nucleus and carries them to ribosomes in the cytoplasm

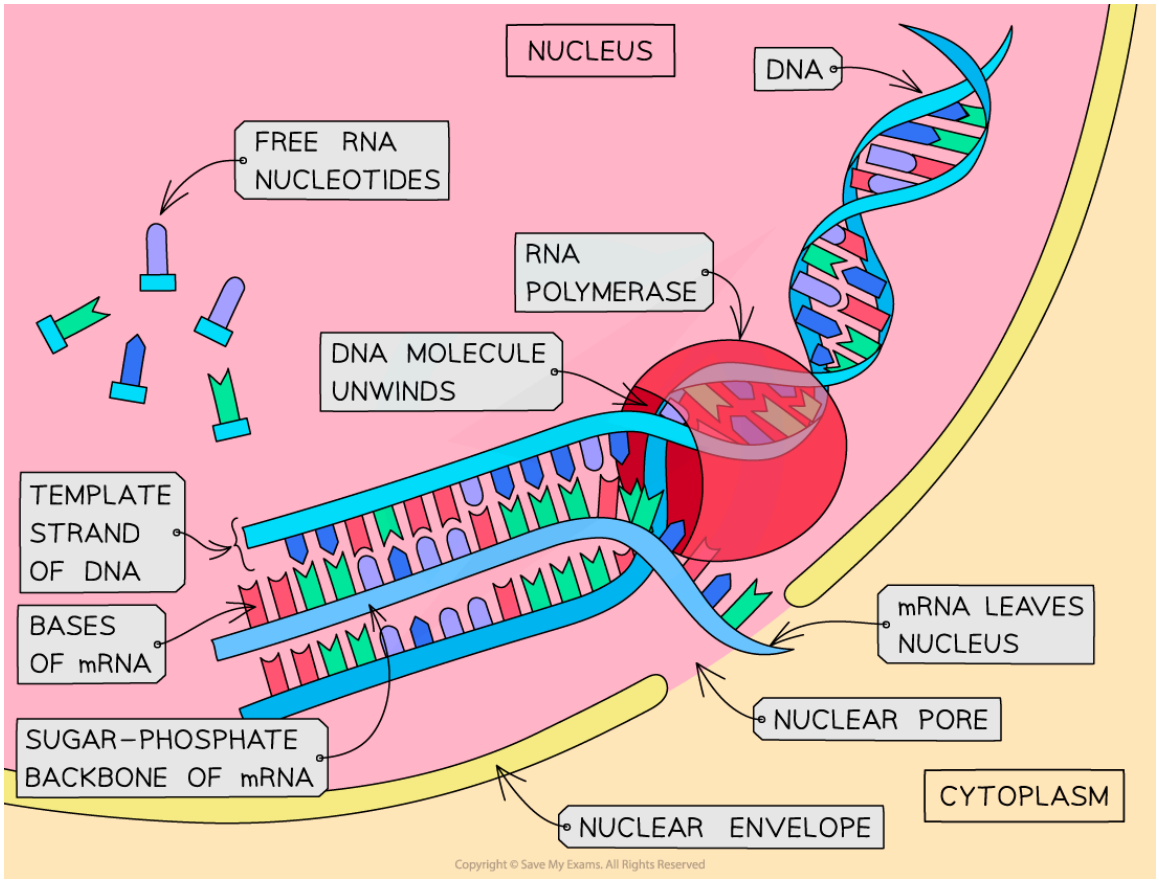
ribosomal RNA (rRNA): helps form ribosomes

transfer RNA(tRNA): brings amino acids to ribosomes

The process of transcription

- Transcription occurs in the nucleus of the cell
- A section of the DNA molecule unwinds; this section contains the gene from which a particular polypeptide (protein) will be produced
- Unwinding occurs due to the breaking of hydrogen bonds between the complementary base pairs; the DNA is said to be 'unzipped'
    - This reaction is catalysed by the enzyme helicase, as in DNA replication
- The gene to be transcribed is now exposed
- A complementary copy of the code from the gene is made by creating a molecule of mRNA
    - Free activated RNA nucleotides pair up (via hydrogen bonds) with their complementary DNA bases on the 'unzipped' DNA molecule; this DNA strand is called the template strand
        - The strand of the DNA molecule that is not transcribed is called the non-template strand or the non-transcribed strand
        - The base sequence of the non-transcribed strand will be the same as the base sequence of the mRNA transcript, but with uracil replacing thymine

    - The sugar-phosphate groups of these RNA nucleotides are then bonded together by the enzyme RNA polymerase to form the sugar-phosphate backbone of the mRNA molecule
- When the gene has been transcribed, the mRNA molecule is complete, the hydrogen bonds between the mRNA and DNA strands break, and the DNA molecule re-forms into its double helix structure
- The mRNA molecule then leaves the nucleus via a pore in the nuclear envelope



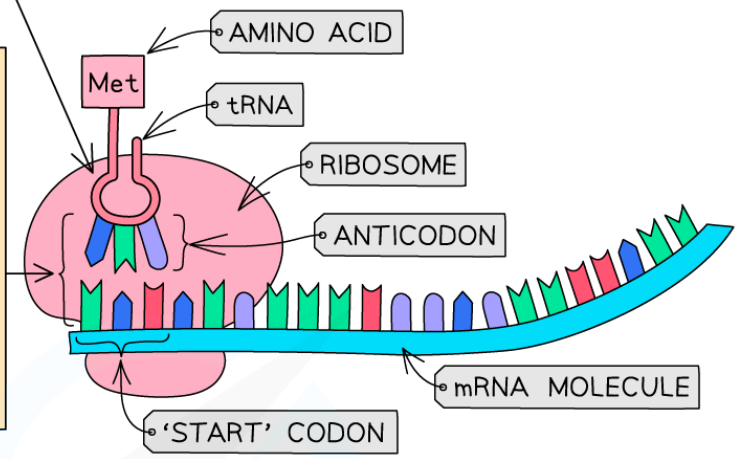
The process of translation

- This stage of protein synthesis occurs in the cytoplasm of the cell
- After a transcribed mRNA molecule leaves the nucleus, it attaches to a ribosome
- Within the cytoplasm, there are free molecules of tRNA (transfer RNA)
- tRNA has an anticodon (a triplet of unpaired bases) at one end and a site for a specific amino acid at the other
    - There are at least 20 types, each with a unique anticodon and corresponding amino acid
- The tRNA molecules bind with their specific amino acids (found within the cytoplasm) and bring them to the mRNA molecule on the ribosome
- The anticodon on each tRNA molecule pairs with a complementary triplet (codon) on the mRNA molecule
- Two tRNA molecules fit onto the ribosome at any one time, bringing the amino acid they are each carrying, side by side
- A peptide bond is then formed between the two amino acids
    - The formation of a peptide bond between amino acids requires energy, in the form of ATP
    - The ATP needed for translation is provided by the mitochondria within the cell
- This process continues until a 'stop' codon on the mRNA molecule is reached – this acts as a signal for translation to stop; the amino acid chain coded for by the mRNA molecule is complete
- This amino acid chain then forms the final polypeptide

1 IN THE CYTOPLASM THE mRNA ATTACHES TO A RIBOSOME

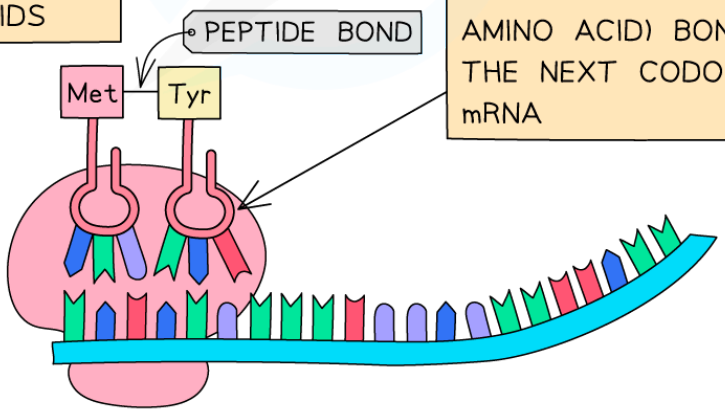
2 EACH tRNA HAS THE COMPLEMENTARY **ANTICODON** TO THE CODON ON THE mRNA

3 THE FIRST tRNA (WHICH ALWAYS CARRIES THE METHIONINE AMINO ACID) FORMS HYDROGEN BONDS WITH THE FIRST OR **'START' CODON** (AUG) ON THE mRNA.



5 A PEPTIDE BOND FORMS BETWEEN THE AMINO ACIDS

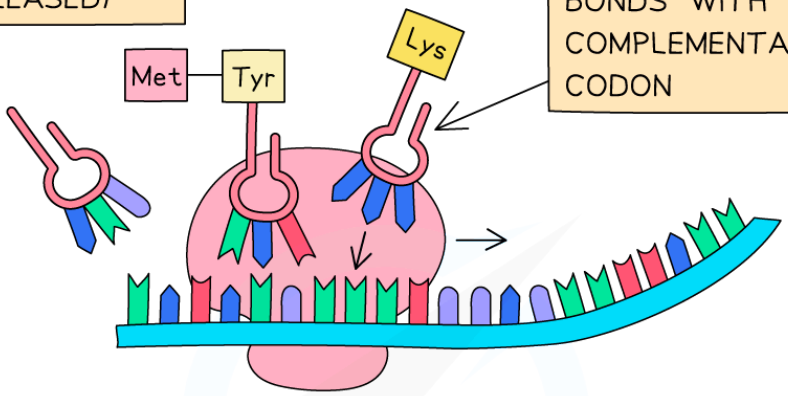
4 THE SECOND tRNA (BRINGING THE SECOND AMINO ACID) BONDS WITH THE NEXT CODON ON THE mRNA



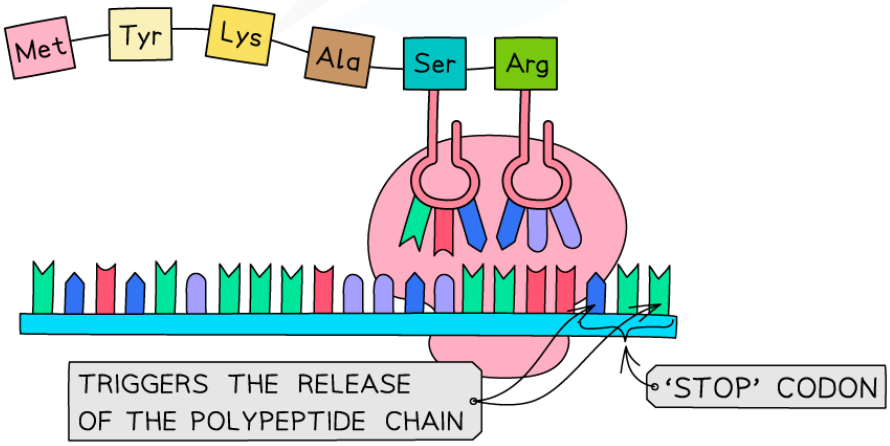
6 THE RIBOSOME MOVES ALONG THE mRNA (IN A 5' TO 3' DIRECTION) 'READING' THE NEXT CODON

8 THE FIRST tRNA (NOW WITHOUT AN AMINO ACID IS RELEASED)

7 THE THIRD tRNA (CARRYING THE THIRD AMINO ACID) BONDS WITH THE COMPLEMENTARY CODON

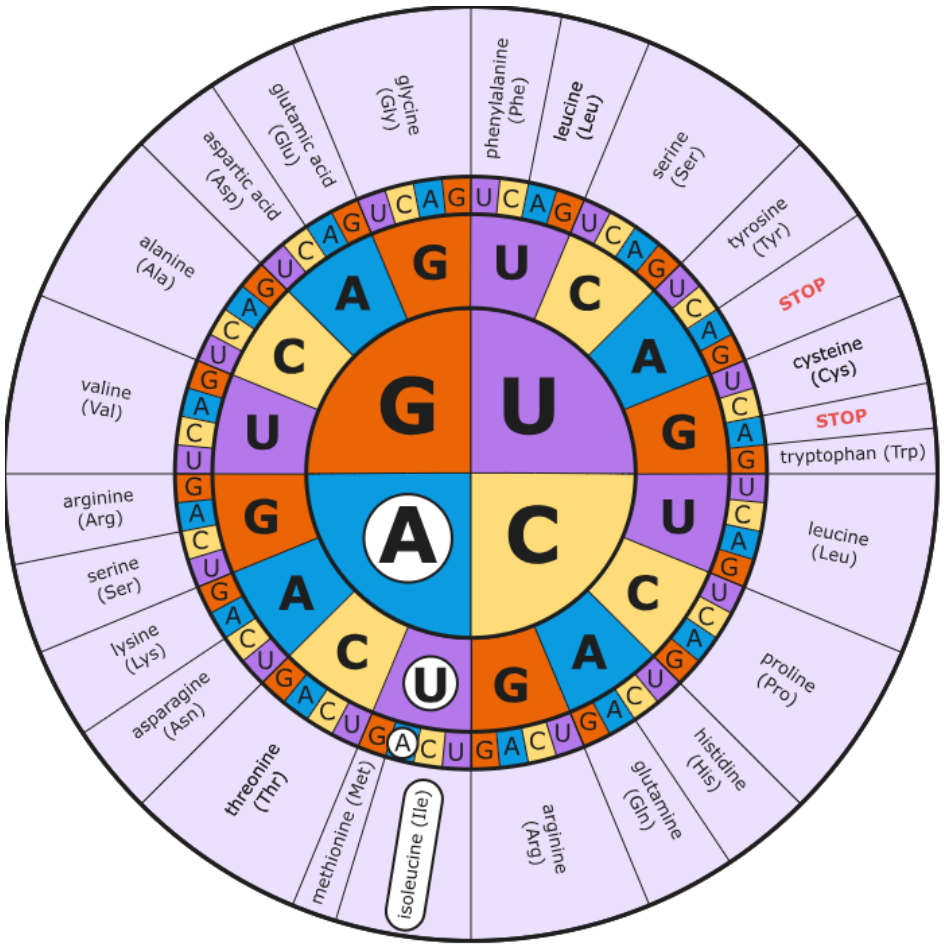


9 THE RIBOSOME CONTINUES TO 'READ' THE mRNA MOLECULE BUILDING A POLYPEPTIDE CHAIN UNTIL IT REACHES A 'STOP' CODON



To figure out the amino acids that make up protein, search for the start codon (AUG).

- There is only one start codon-protein synthesis must always start from AUG.
  From AUG, group three letters at a time. Use a table to find what amino acid each codon codes for.
- Continue until you reach a stop codon (UAG, UGA ,and UAA).
- The start and stop codons do not code for any amino acids-they only signal for protein synthesis to begin or to stop.



Consider the following nucleotide sequence, which represents an mRNA molecule: 5' -ACUGAUGUUUGUUCCCUGCUGA- 3'

Use the following codon circle to translate the mRNA molecule into its corresponding polypeptide sequence, beginning at the first available start codon (AUG).

Worked Example

Use the rules of base pairing and the genetic code chart above to deduce the amino acid sequence coded for by the following DNA sense strand sequence:

TTC GAG CAT TAC GCC

Answer:

Step 1: convert the sense strand into the template strand

- We first need to convert the DNA sense strand into the DNA antisense strand, which is the same as the template strand
- This is done using base pairing rules: A-T and C-G
- The sense strand = TTC GAG CAT TAC GCC
- The antisense, or template, strand = AAG CTC GTA ATG CGG

Step 2: convert the DNA template strand into mRNA codons

- Base pairing can be used again to work out the mRNA sequence that will form during transcription
- Remember that mRNA has U instead of T
- DNA template strand = AAG CTC GTA ATG CGG
- mRNA strand = UUC GAG CAU UAC GCC

Step 3: use the genetic code chart for the first amino acid

- First base in codon = U, second base = U, third base = C
- So we're looking in the top-left box of the table; this amino acid is Phe

Step 4: repeat for the remaining 4 codons

- GAG = Glu
- CAU = His
- UAC = Tyr
- GCC = Ala
- So the sequence = **Phe-Glu-His-Tyr-Ala**