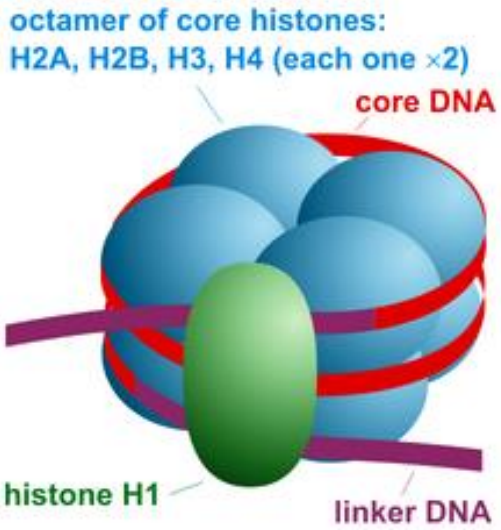
Understandings:

1. Discuss how discovery of DNA structure suggested mechanism for DNA replication.
- Together with the X-ray evidence from Rosalind Franklin and Erwin Chargaff's study of base composition enabled Watson and Crick to build a suitable model by trial and error. Their discovery basically led to the semi-conservative replication.

2. Explain how nucleosomes aid in supercoiling DNA.
- One core difference between prokaryotic DNA and eukaryotic DNA is that eukaryotic DNA has proteins called histones, while most prokaryotic DNA don't. Indeed, that is why we call them "naked strands".

But what does histones have to do with nucleosomes?

Histones wrap around the DNA strands into small balls called nucleosomes, simply to pack the DNA. The nucleosome consists of two copies of 4 histones (total 8), then an additional histone holds the 8 histones and the DNA strand intact.



The next question is how it contributes to supercoiling. This supercoiling is just a natural result of the presence of nucleosomes. Have you ever tried to twist a tie and it will eventually twist by itself into a more compact form? That is exactly why. The supercoiled DNA is what we know as chromosomes.

3. State what leading and lagging strand is, and how they differ during replication.

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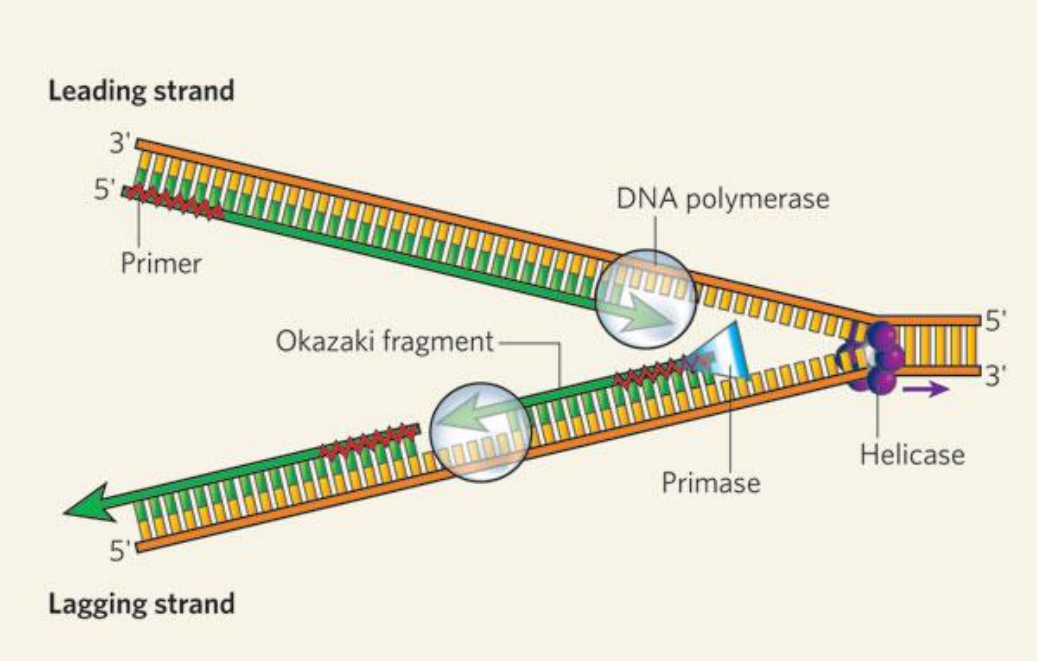
The terms might seem daunting, but everything is logical.



When the helicase (a.k.a. replication fork) splits the DNA, We will have two strands anti-parallel to each other.

The DNA polymerase in the leading strand (strand that gets exposed from 3' to 5') can move in the direction 5'3' without interruption since helicase keeps splitting in the same direction.

On the other hand, lagging strand (exposed from 5'3') the polymerase cannot go nonstop because it can only work in 5'3' direction. A RNA primase, a type of RNA polymerase, will create a RNA primer. RNA primer is a small sequence of RNA strands that act as starting point for the DNA polymerase III to work on because DNA polymerase cannot start by itself. This means that RNA is involved during replication. Therefore, it will "lag" and start anew every time a new part of the strand is exposed. The new fragments created are called Okazaki fragments. Then DNA polymerase I will remove RNA primer and replace with DNA.

4. Explain process of DNA replication, mentioning all relevant enzymes.

- Briefly mentioned in 2.7, there are 4 numerous enzymes during replication.

Splitting enzymes:

Helicase – this one splits the DNA.

Topoisomerase/gyrase – this enzyme travels ahead of the helicase and loosens the tension of the DNA. Basically, it eases the job for the helicase. But be aware that it is much more complicated than just this.

Regenerating enzymes in leading strand:

Primase – this forms the RNA primer, which is essentially the starting point for DNA polymerase.

DNA polymerase – these bring the nucleotides and hydrogen and covalently bond them.

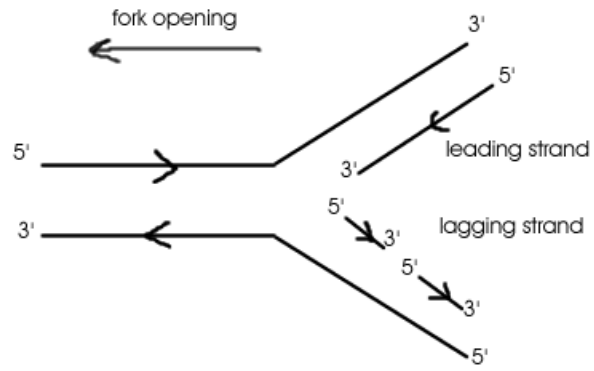
Regenerating enzymes in lagging strand:

Primase – this forms many RNA primers since more is needed every time new parts of DNA are exposed.

DNA polymerase – these bring the nucleotides and hydrogen and covalently bond them.

DNA ligase – these connect the gaps between Okazaki fragments.

5. Explain the direction of replication.
- Remember that the direction of DNA polymerases is from 5' to 3'.



6. Discuss the uses for non-coding regions.
- We call areas that code for amino acids *gene*. However, not all parts in our DNA codes for amino acids. What do the rest do then? Some produce tRNA and mRNA, and some act as enhancing/silencing a gene expression.

We eukaryotes are mostly composed of non-coding regions, maybe up to 95% in humans. Within these non-coding regions, there might be repeating sequences. And further, there are two types of repeating sequences: moderately repetitive sequence and highly repetitive sequence (satellite DNA).

An example of repetitive sequence is telomeres. It essentially is located in the end of the chromosome. Its function is to not have a function. Huh? Well, I mean that whenever DNA replicates, it cannot copy all the DNA sequence form start to end. Some in the beginning and end gets lost. Telomeres are the ones that get sacrificed, hence preserving the important part of DNA.

Extra notes

- When we say highly repetitive sequences, we are basically comparing it with unique sequences and these are the ones we are more familiar with. Just to clarify the difference, let's compare them because many seem to be confused between these two types.

Unique sequences	**Highly repetitive sequences**
It is unique, meaning that it appears only once in a gene.	It appears many times in a gene.
Usually long sequences.	Usually short sequences.
Often a gene, therefore coded into a protein.	Not a gene that never gets coded.
Identical in same species, except for the mutated genes where few bases have changed.	Varies extremely much between individuals, thus often used in forensics.
Exons	Introns
Constitutes about 5% or more.	Constitutes 95% or less. This is crazy I know. I suddenly feel I have an enormous potential to become Spiderman if all of my DNA were used.

Note that prokaryotes don't have introns; therefore they don't have repetitive sequences. Only very rare ones may have.

Applications and skills:

1. Understand the Heresy and Chase experiment.
- Let's organize our minds first.

Initially, Crick and Watson discovered the structure of DNA. Then, Meselson and Stahl proved semi-conservatism.

Now, during Heresy and Chase, people did not know what genetic material was actually made of. They knew that chromosomes contained both DNA and proteins (histones), but not the actual genetic material. So they conducted a very brilliant experiment.

This experiment also involves isotopes, but this time, they are radioactive! Also, they knew that the characteristic for DNA was phosphorous and for proteins it was sulphur. In addition, they knew that viruses inject genetic material and that the coating was made of proteins.

How did they use that? Well, follow along because this is quite simple, but elegant.

1. They grew a virus in two different environments: one with S-35 and another with P-32, both very radioactive.

2. This means that the proteins in the S-35 will be radioactive and the DNA in the other is radioactive with P-32, since they must use molecules to build up.

3. So let's take the virus exposed in S-35 first. It was put together with bacteria (host cells). After some time, we would expect that genetic material is injected and the protein coating outside, hence separated.

4. We blend with a mixer to separate the viruses stuck on bacteria membrane.

5. Now, we centrifuge it to separate the bacteria, and the "rest"/non-genetic part. The solid part is the pellet and the floating part supernatant.

6. We collect the supernatant (liquid) and see its radioactivity.

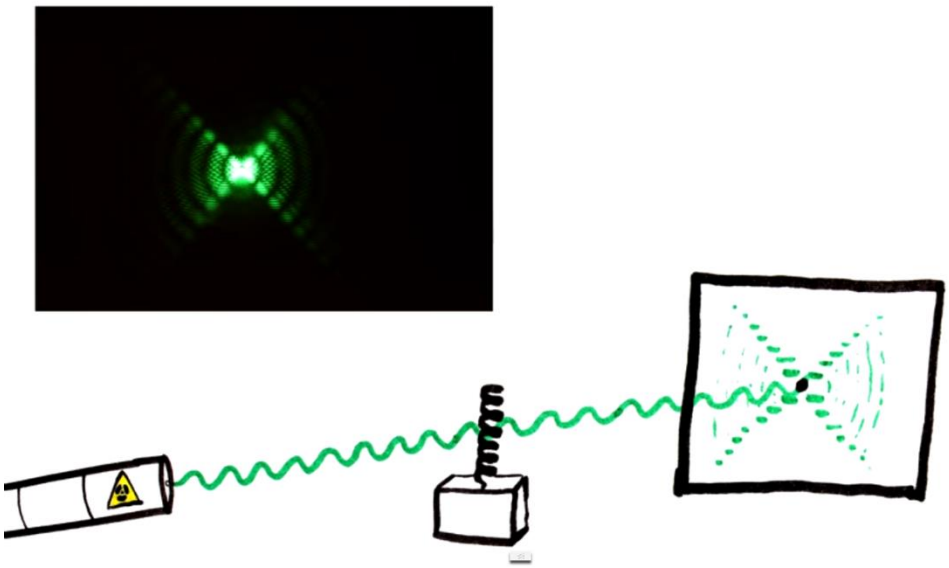
7. Now repeat from 3-6 with P-32.

What result did they get? They got that for supernatant with S-35, radioactivity was very active. For supernatant with P-32, radioactivity was very low.

What does that mean? It must mean that the majority of P-32 was in pellet (solid), hence inside the bacteria as genetic material. Now, we know that phosphorous was the marker for DNA, thus it is logical to say that genetic material that the virus injected contained DNA.

2. Discuss the evidence provided by Rosalind Franklin and her X-ray experiment.

- The basic principle used is diffraction. Rosalind shot an x-ray on a material (biological things with DNA obviously) and gathered the imaged that showed scattering. The image is recorded by x-ray film. A little bit like the picture below.

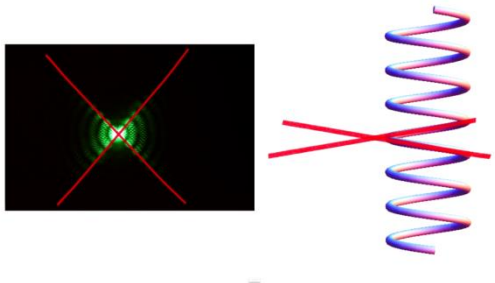


That image was extremely valuable and told very many things.

1. The cross meant that it is helical.

2. The angle showed how thin/steep the helix was.

3. The distance of different places showed the compression, width of wire, etc.



3. Explain what tandem repeats are, and be able to interpret the data.

- We mentioned that we had repetitive sequences in our non-coding DNA. Variable number tandem repeat (VNTR) is essentially short sequences that we can use to identify the individual, since it is unique for everybody.

Ex, if we have a repetitive sequence GGCT, we can cut those using restriction enzymes and put them in gel electrophoresis and identify how many it has. Then that data could be used to compare with others or evidence!

4. Be able to use molecule visualization software (Jmol probably) to analyse the relationship between protein and DNA within a nucleosome.
- OK

5. Explain the use of nucleotides that contains dideoxyribonucleic acid.
- How on earth do we determine the sequence of bases in a strand?

What we do is that we put the DNA strand we want to determine into a test tube with enzymes and nucleotides to enable replications. Among those nucleotides, we have something called dideonucleotides that has been marked with fluorescent. These will act as stoppers of the replications because they don't have an oxygen molecule in 3'.

Then, what happens is that we get all the positions of A, T, G, C independently. Here we assume that all ddA/ddT/ddG/ddC bind at least once to the existing position. Then we have ordered according to increasing size with gel electrophoresis!

TOK:

1. Highly repetitive sequences were once classified as "junk DNA" showing a degree of confidence that it had no role. To what extent do the labels and categories used in the pursuit of knowledge affect the knowledge we obtain?