Molecular biology lecture #2

In the previous lecture, we got to chapter 12. We talked about DNA structure and reached the Watson and Crick model and the double helix. Today, we are going to finish chapter 12 and start with 13.

The first slide (slide #22) shows the 2 DNA strands, which we already mentioned. It shows the 2 anti-parallel strands. Being parallel means they're complementary (each A is paired with a T and each G with a C) anti each other means that one of them is 5'-3' while the other is the opposite; 3'-5'.

Hydrogen bonding between the bases differs; between T & A there’re 3 bonds, between G & C there're 2 bonds.

The DNA strands don’t stay straight, the double stranded will take a helical shape; and just like a spring, they can take a right-handed (clockwise) or a left-handed (anti-clockwise) rotation. Most of our DNA, as humans, is **right-handed in the B form**.

The DNA being in the B, Z or A form depends on how compact the double helix is, meaning, just like a spring its 'rings' can be far from each other or compacted together. Z is the most compact, then A, and B is a bit loose, this is measured by the distance between one turn and the next. For example, in B form, the distance is 3.4 Angstrom between each turn and the next.

All these forms are found in nature, but our DNA is in the B form.

DNA molecule characteristics:

Denaturation of DNA:

- Denaturation means the splitting of the 2 DNA strands; breaking the hydrogen bonds between them.

- It differs form protein denaturation, which means the loss of tertiary structure; unfolding of the globular protein. Protein denaturation is irreversible and the frages of polypeptides will mix and precipitate in the solution and **never** reanture.

- Compared with protein denaturation, DNA denaturation doesn’t affect the tertiary structure at all, it only breaks the hydrogen bonds between the two strands which is very **reversible.**

- It's done by heating the DNA to split the 2 strands (heating increases vibrations between the 2 strands till they break) and reversed by cooling them. This can be done millions of times and will always work.

- Reversing the denaturation is known as hybridization. It is the reassociation of the 2 complementary strands. Not only the 2 sister complementary strands, but can be applied also to any two different DNA fragments that share a complementary portion to bind to each other, even a complementary RNA can bind to the DNA.

- Another way of denaturation is by putting the DNA in an alkaline solution (or to raise the PH of the solution). When PH is raised, the phosphate group (the acidic group in DNA) will tend to lose its protons into the solution (to act as a buffer, just like any acidic group that's put into an alkaline solution). This will lead to increase in the phosphate group negative charge ($PO4^{-2}$). When these negative charges appear strongly on the 2 strands, they'll rebel each other.

- If a double stranded is made of a hybrid DNA-RNA is put into an alkaline solution, after the splitting of the 2 strands, the RNA will be broken down for some reason (breaking its phosphodiester bonds along with the hydrogen bonds between the 2 strands). DNA won't be affected.

- If it wasn't for this character of DNA, genetic engineering (which we'll talk about in chapter 17) wouldn't have been possible.

- DNA is a very robust molecule, it can endure heating and many other things, in response it'll only split and this is very useful in genetic engineering.

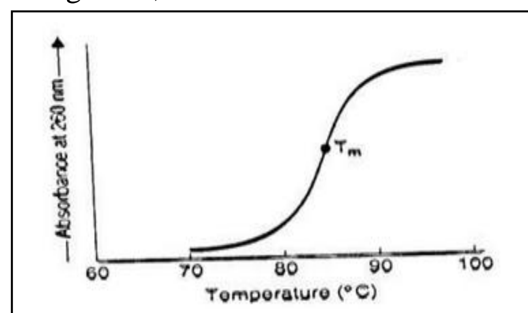
- Denaturation is sometimes called **melting**. If we say the 2 DNA strands melted, it means they split (Hydrogen bonds were broken).

- Each DNA has a certain temperature that it splits at, for example some can split at 80, 85 or 90. **Melting temperature** or denaturation temperature is the temperature 50% of the DNA splits at.

  It depends on:

  1. The type of bases that DNA has; the more G-C content in it, the higher its melting temperature is.
  2. The length of the DNA; the longer the DNA the higher its melting temperature.

- To get the DNA to be 100% denaturated, we only need to get above the melting temperature by 1-2 degrees. Ex.: DNA starts melting at 85, when it reaches 86 50% has melted, at 87 it's 100%.

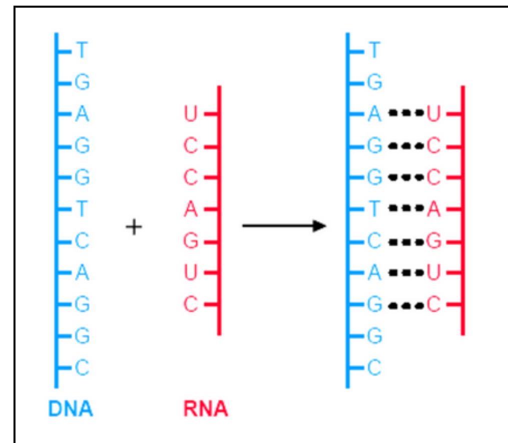


- The figure showing temperature on the X axis and absorbance on Y axis is for an experiment, it's done as follows:

  1. A DNA is placed within a test tube, that tube is heated
  2. A light is passed through the DNA solution. If the light is passing through a homologous solution of the double stranded DNA, it'll give a certain absorbance.

3. When the tube is heated, the strands are broken and then the light absorbance will suddenly change.
4. At the moment of change, the absorption is measured and then it's known when the DNA split.

- In the slide showing the DNA-RNA hybrid, the DNA is way longer than the RNA, and that is because the RNA is only complementary for a portion of the DNA. (U binds A better than T)



Genomes:

- A genome means all DNA content in the cell.
- E. Coli genome vs. human Genome:

E. Coli	Humans
Single chromosome	46 chromosomes
Circular genome	Linear (has a beginning and an end)
Exists on its own. It doesn't have any associated proteins 'cause its DNA is small (2 mm).	Associated with histons. It needs to be wrapped around them for packing (it can reach 2 m without them.)
3,000 genes	~30,000 genes
A single copy for each gene (A haploid cell).	2 copies for each gene (We have 23 pairs of chromosomes; diploid cells)
All its genome is coding	Long distances between each coding gene and the next

- Coding regions in our genome are the minority; 70% of our DNA is non-coding; junk DNA.

- The coding regions in our DNA make 30%. The non-coding regions can exist within the coding regions (introns), they make 25-30%, which are even higher in number than the coding ones (exons) themselves 5%.

- This junk DNA function is unknown. Some say it's the result of accumulation by our evolution, but knowing how efficient our cells are in maintaining everything (the cell doesn't spend as much as 1 ATP without a proper reason) it's not likely that they'll keep as much as 95% of the DNA for no reason. Most likely, these non-coding regions have another huge function that's unknown to us yet. As far as we know, the DNA is a template that is translated to RNA to make protein, so another function can exist without our knowledge.

- Our genome is still a mystery. Our genes (coding regions) make 5% of our DNA (30%, if you count the introns) and most of their functions are unknown. We still don't even know much of their resulting proteins functions.

DNA replication (chapter 13)

- A cell copies its DNA when it divides, so that both daughter cells are identical to each other and to their mother cell.

- We all started from a zygote. From that moment on, and through every cell division, all our resulting cells have the same DNA as that zygote, which means its copying is greatly accurate.

- DNA polymerase that copies the DNA is so accurate that it makes a mistake each 10-100 billion nucleotide (our DNA has 6 billion nucleotide).
  Imagine if we write the genetic code on broadsheets (which is used for print newspapers) you will need 70,000 sheet!

- We have a lot of DNA polymerases to copy the DNA, each gets 10-20 thousands nucleotide to copy.

- Bacterial polymerase, on the other hand, doesn't have this accuracy; it makes mistakes leading to the evolution of bacteria to be able, for example, to resist Ampicillin.
  Another example on revolution within the same species, green butterflies live in a forest, then the climate changed, less rain, less vegetation --> Desertification --> green butterflies are apparent for the predators --> less green butterflies --> then a mutation happened in the DNA of a butterfly on the gene of the green color to become yellow which more suitable to the environment --> after few generations all the butterflies are yellow (This an example of nature selection, too)

- DNA is double stranded, so in order to replicate it we need to split the 2 strands. This is done by heating in labs, in our cells heating is not an option, an enzyme; the helicase helix is used for that purpose.

- **<u>Origin of replication:</u>** certain sequences that the helicase read to open the 2 DNA strands from. Appear as a bubble (or a <u>replicon</u>). It has two angles called replication forks.

- After the helicase opens the strands the polymerases come and each take a fork to start the replication in both directions; meaning the replication is <u>bidirectional</u>.

- Each strand in the mother cell is used to make a new strand. So when we end up with two daughter cells, each cell half DNA comes from the mother cell, meaning each cell reserved half the DNA of the mother cell. That's why DNA is called **<u>semi-conservative</u>.**

- Our DNA is huge, so one origin of replication is not enough; we have multiple origins to accelerate the process and save time. At the end, the bubbles fuse together. Bacteria has a single origin of replication because its DNA is small.

- In a single replication fork, the two resulting strands, just like any DNA molecule, need to be anti-parallel. The DNA polymerase works from 5'-3', so the movement of the fork (caused by the helicase as it opens the 2 strands) is favored by the direction of the movement of the polymerase. One of the strands is made **continuously**; is called the **<u>leading or continuous strand</u>** (its 5'-3' direction is the same as that of the polymerase).

- A problem occurs in the second strand made at the fork of replication; this strand will be synthesized 5'-3' in a direction *<u>away</u>* from the helicase and fork movement. In this case, as the fork opens the 2 strands, the DNA polymerase gets the chance to copy the DNA in little fragments (200-300 bases) **uncontinuously**, making a strand called the **<u>lagging strand or Okazaki fragments</u>** (the Japanese scientists who discovered it).

- Okazaki fragments are linked together by an enzyme called **<u>DNA ligase.</u>** This enzyme is needed because the DNA polymerase synthesizes the fragments 5'-3' but it can't link them 3'-5'.

- **<u>Primase</u>**: it's an enzyme that helps the DNA polymerase start its job by placing a double stranded RNA for the polymerase to start from; a **<u>primer</u>**. The primer is needed because as the polymerase work from 5'-3', it needs at least one 3' OH group to start from, that's why the primer is not very long; 6-10.

- Surprisingly, the primer is made of RNA nucleotides, it's not known why. It is removed by an RNase, then another polymerase comes and fills the primer's place and finally the ligase connects it.

- Each Okazaki fragment needs a single primer. The leading strand needs only one.

- **<u>Single strand binding proteins:</u>** proteins that bind the single strands so that they would not snap back to each other (fast renaturation or hybridization). This can happen because when the 2 DNA strands were denatured, the temperature within the cell was 37 C which is very suitable for them to renature again, these binding proteins prevent that from happening; they bind to the strands just after the helicase had split then open.

- **<u>Topoisoemrase:</u>** this enzyme works to stop the strands from breaking as the helicase is rotating them against their usual direction while splitting them. It works ahead of the

helicase; it identifies the stress points, cuts the twisted strands relieving them from stress and then rejoins them afterwards.

- People with a mutation in the topoisomerase that led to insufficiency in its function will surely get cancer. That’s because as their cells are multiplying, their DNA will be broken multiple times. With time, one of these broken points can hit an oncogene causing cancer.

- There're more than one type of topoisomerase, 1, 2, 3...etc. they make a family.

"Faith is the art of holding on to things your reason once accepted spite of your changing moods."
-C. S Lewis

Sorry for any mistake. Good luck and don’t forget to be awesome ^^