**DNA: The Genetic Material**

**Heredity transformation:**

* **Griffith** (1928) studied streptococcus pneumonia and “influenza”
* Two strains of S. Pneumonia: A (virulent) and R (non-virulent)

**DNA is the transforming material:**

* **Avery et al.** (1944) repeated Griffith’s experiment; particularly #4
* Removing all protein from the “transforming material” did not destroy its ability to transform R strain cells. (Concludes that protein was not the virulent factor)
* DNA-digesting enzymes destroyed all transforming ability

**Virus Genes are DNA:**

* **Hershey and Chase** (1952) investigated bacteriophages (Bacteriophages: Viruses that only infect bacteria)
* Bacteriophages are only composed of DNA and a protein coat
* They the first trial, they used 35S because they know it will go into the protein coat of the bacteriophage
* For the 2nd trial, they used 32P because it will go into the DNA of the bacteriophage
* Experiment concludes that the DNA of the bacteriophages was transferred to the bacteria (Review slide 3)

**DNA structure: (A nucleotide in slide 4)**

* Phosphate group (attached to the C in the 5’ position)
* Sugar (@ the 2’ position, we have a H group in DNA/ OH in RNA)
* OH at the 3’ position

**Nitrogenous Base:** Connected at the 1’ position

**Purines:**

* Adenine
* Guanine

**Pyrimidines:**

* Cytosine (DNA and RNA)
* Thymine (DNA)
* Uracil (RNA)

Nucleoside: the building blocks that are added to the growing chain of DNA

A & T form 2 hydrogen bonds

C & G form 3 hydrogen bonds

Phosphodiester bond (with a Phosphate) connecting two sugar bases in DNA.

3’ hydroxyl with form a phosphodiester bond with the 5’ phosphate of adjacent nucleotides (in DNA/RNA)

5’ – 3’ polarity or directionality: DNA grows from 5’ to 3’

**Chargaff’s Rules:**

1. A/T=1 and G/C = 1 (Pairing ratio is 1 to 1)
2. Ratios vary wildly among species (The percentages of A/T and C/G vary wildly in different species)

**Franklin and Wilkins (1952):**

* Determined DNA was helical via X-ray diffraction studies
* DNA is 2 nm in diameter; complete turn of the helix every 3.4 nm
* They did not deduce that the structure was a double helix

**Watson and Crick (1953):**

* Proposed that DNA had a double helix structure
* Two sugar-phosphate (phosphodiester) backbones
* Complementary base pairing facilitated by hydrogen bonding
* Nucleotides strands are antiparallel (Opposite in direction) to each other (5’ – 3’ & 3’ – 5’)
* Deduced the structure of DNA using evidence from others’ work

**Slide 10:**

* Major Groove: The area were proteins access the DNA, so it can be expressed
* Minor Groove

**DNA replication: Semiconservative Process:**

* **Meselson and Stahl** (1958) studied the process of DNA replication
* **How did meslon and Stahl deduced that it was the semiconservative process?** We know that E. coli has a circular DNA that is double stranded. They grow the E. coli in a medium with 15N (a heavy Nitrogen isotope) in it. They then transferred the E. coli into a different medium containing 14N (lighter than 15N). We know that N will be incorporated into the DNA. Then they took samples of the E. coli that is in 14N that were previously grown in 15N. They looked at the samples after 0,1,2 rounds of replication. They centrifuged the samples then looked at the sample. After 0 rounds, the DNA was blue (was full of 15N). After 1 round, the band at the bottom disappeared, and a new band appeared which was a little bit higher up, meaning it was slightly less dense (That means it contained 14N). After 2 rounds of replication, the band from round 1 stayed there but we have a new band that was higher than band from round 1. This new DNA only had 14N in it.

If wewent beyond 3 rounds, the blue band (15N) will never disappear. It will always be there, hybridized with a yellow band (14N).

* **Conservative Process:** the 2 DNA strands opened up, you copied each strand, but those 2 parent strands that were copied came back together, and the new copies, they came back together too and formed hydrogen bonds.
* **Semiconservative Process:** the 2 DNA strands opened up, each strand is copied, but then those copies and the original stay together
* **Dispersive Process:** the 2 DNA strands opened up, and fragmented into pieces, and the pieces were copied.

**Replication Requirements:**

* A template of the DNA (leading/lagging strands) to be copied
* An enzyme (**DNA polymerase**) to make a copy of the template strands
* An RNA primer (**DNA primase**) to initiate DNA replication. *This is considered the initiation step for DNA replication.* You then add Deoxyribose nucleotides if you are making DNA copies or Ribose nucleotides if you are making RNA copies
* Building blocks (nucleotides, but they are technically **Nucleosides**) to assemble the polymers (**nucleic acids**)

**How the replication happens (Slide 13):**

The hydrogen bonds between the 2 strands of DNA are broken and the replication fork is formed (Remember: One end is 5’-3’ and the other is 3’-5’). The RNA primase (Pink sphere) is laying down RNA primers on the leading strand. The DNA polymerase starts adding nucleotides in the 5’-3’ direction (The DNA polymerase is running toward the opening of the fork, and adding nucleotides).

**The lagging strand**: It’s called the lagging strand because the process of adding RNA primers and nucleotides is not as continues as the leading strand. We need to lay a RNA primer, then synthesize DNA, add another primer, then synthesize DNA, so on and so forth. In other words, you need a series of RNA primers on the lagging strand, when you only need 1 on the leading strand. DNA polymerase on the lagging strand is running toward the replication fork.

Visual DNA Replication: (<https://www.youtube.com/watch?v=-mtLXpgjHL0>)

**Prokaryotic DNA replication:**

* Bacteria have a single, double-stranded, circular chromosome
* DNA replication is initiated at the origin of replication (oriC)
* DNA elongation (of both strands of parental DNA) ensues bi-directionality (Both strands are going to be copied simultaneously, hence the 2 replication forks in slide 14)
* DNA replication ends at a termination site

**The Replisome:**

* The replication “organelle”

**Enzyme and Protein functions in DNA replication process:**

* **DNA Helicase:** Unwinds the double helix, breaking the hydrogen bonds
* **DNA Primase:** Synthesizes RNA primers
* **Primosome:** DNA Helicase and DNA Primase
* **Single-Strand binding protein:** Stabilizes single-strand regions (**Another definition**: Proteins responsible for keeping the fork open by preventing the reformation of hydrogen bonds between the 2 strands)
* **DNA gyrase:** Relieves torque in DNA that is still in the double helix confirmation. Because If you unwind the DNA from one side, then it supercoils on the other side, and we don’t want that.
* **Definition of DNA Polymerase**: a protein complex responsible for bringing in nucleoside triphosphates such that they form complementary base pairs with the template strand, and then form phosphodiester linkages.
* **DNA polymerase lll (Short: Pol lll):** Synthesizes DNA
* **DNA polymerase l**: Erases RNA primer and fills gaps with deoxyribose nucleotides (in the 5’ – 3’ direction. For DNA replication)
* **DNA ligase:** Joins the ends of DNA segments (forming phosphodiester bonds); DNA repair

**DNA polymerase:**

* **DNA Pol l:**
* Requires a primer
* Adds nucleotides in the 5’ – 3’ direction
* Has 3’ – 5’ exonuclease activity (proofreading)
* Has 5’ – 3’ exonuclease activity (**Primer removal)**
* **DNA Poll ll:**
* Requires a primer
* Adds nucleotides in the 5’ – 3’ direction
* has 3’ – 5’ exonuclease activity (proofreading)
* **DNA Pol lll:**
* Requires a primer
* Adds nucleotides in the 5’ - 3’ direction (Major polymerase)
* Has 3’ – 5’ exonuclease activity (Proofreading)

**Similarities between DNA pols:**

1. Can layout DNA
2. Requires RNA Primers
3. Add nucleotides in the 5’ – 3’ direction (Another wording: Add nucleotides on the 3’ Hydroxyl of a previous nucleotide
4. **Has 3’ – 5’ exonuclease activity (Proofreading of DNA)**

Prokaryotes don’t have the proof reading capacity, which is why they produce exponentially and there is a lot of mutations happening (Think bacteria)

Re: When the phosphodiester bond is formed, the 2 phosphate that leave the nucleoside triphosphate is called Piral phosphate (PPi).

**Semi-discontinuous Process:**

* Poll lll can only add nucleotides to the 3’ end of strands (Means that DNA is built from the 5’ – 3’)
* DNA strands are antiparallel to each other
* Leading strand is synthesized continuously
* Lagging strand is synthesized discontinuously. DNA fragments built on the lagging strand are called Okazaki fragments

(View Semi-discontinuous process in slides)

**Slides 20-24:**

* Clamp Loader (In the Replisome): Produces Beta-Clamps and puts it on the leading and lagging strands
* **Beta-Clamps:** Holds the Pol lll on each DNA strand

1. A DNA polymerase lll enzyme is active on each strand. Primase synthesizes new primers for the lagging strand
2. The loop in the lagging strand template allows replication to occur 5’ to 3’ on both strands, with the complex moving to the left.
3. When the polymerase lll on the lagging strand hits the previously synthesized fragment, it releases the Beta-Clamp and the template strand. DNA polymerase l attaches to remove the primer
4. The clamp loader attaches the Beta-Clamp and transfers this to polymerase lll, creating a new loop in the lagging strand template. DNA ligase joins the fragments after DNA polymerase l removes the primers.
5. After the Beta-Clamp is loaded, the DNA polymerase lll on the lagging strand adds bases to the next Okazaki fragment

Visual DNA Replication: (<https://www.youtube.com/watch?v=TNKWgcFPHqw>)

**Eukaryotic DNA Replication:**

* Eukaryotic chromosomes have multiple origins of replication
* More complex DNA replication enzymes than prokaryotes
* Synthesizing the ends of the chromosomes (Ends of chromosomes names: **Telomeres**) is difficult because of the lack of a primer (you can’t add a primer at the end of a base pair); linear chromosomes (***NOT circular***) shorten with each round of replication
* **Slide 25**: When Pol l takes out the RNA primer, it cannot replace with nucleotides. (**Re**: all Polymerases need an RNA primer to synthesize DNA). This results in a missing nucleotide that was supposed to be complimentary to the original strand. With multiple replication rounds, this leads to shorter DNA.
* Why does a cell age (Called **cell senescence**: Diploid cells stop dividing)? One reason is the decline in enzyme responsible for maintaining the telomeres at the end of our chromosomes. Having telomeres prevent DNA from shortening. When you go through multiple rounds of DNA replication, the telomeres could be lost, resulting in the loss of genes. You are technically losing genes that code for protein that are responsible for the viability of a cell. One reason why the cell age, is because the enzyme responsible for keeping the ends of DNA long (**Telomerase**), so that you don’t tap onto what’s good, like true genes, the activity of that enzyme declines as you age.

In some cancer cells, the enzyme telomerase is up-regulated, so the genes for telomerase are up-regulated and more of the enzyme is produced, so the likelihood of the chromosome shortening with DNA replication goes closer to 0%, and because of that, these cells can virtually live forever.

The problem of the possibility of DNA shortening is a problem arising from the lagging strand, not the leading strand.

We have long, repeat regions of non-sense, DNA at the end of our chromosomes, they are called **Telomeres**. They are essentially there so when we lose the ends of our DNA, we are not tapping into our true genes.

**How telomerase works (Slide 26):** Telomerase is a protein but has an RNA template in it. Telomerase sticks with the ends of DNA, and they have a sequence that can for complementary base pairs with the strand missing the RNA template. This adds up extra base pairs that literally code for nothing, but when the DNA divides, you lose a bit of that extra base pairs so you don’t tap onto your true genes.

(check the 2 end slides about the process in the pdf)