Cycle 3: Inheritance of Sameness

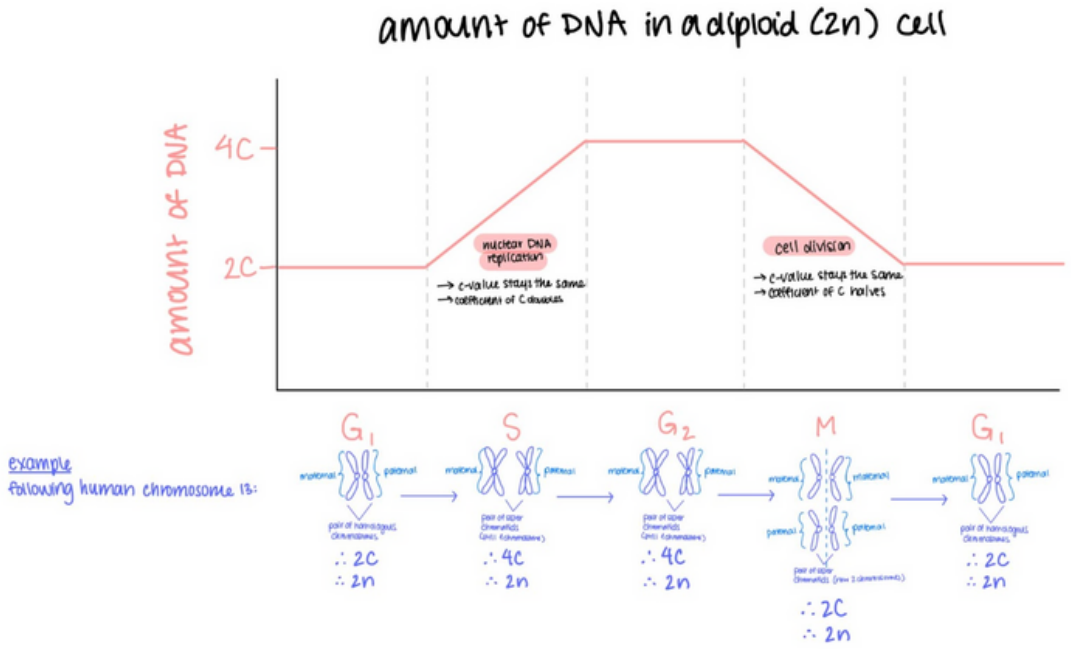
Introduction to the Genome

What is a genome? A genome is essentially defined as all of the DNA contained in ONE COPY of an organism's chromosomes, including **mitochondrial DNA, nuclear DNA, and chloroplast DNA.**
Tip: # of chromosomes = # of centromeres... this means 1 chromosome that duplicates into 2 sister chromatids during the S phase of the cell cycle STILL counts as 1 chromosome right after duplication. Then, when the sister chromatids separate in anaphase, each sister chromatid counts as 1 chromosome for a total of 2. The reason why this happens is because when sister chromatids split apart, the centromere they shared also splits into 2 centromeres. This is important for the next concept: **ploidy** and **c-value**.

Term	Definition	Example
n-Value	the number of UNIQUE nuclear chromosomes present in an organism, i.e. the number of chromosomes in one complete monoploid set Note: is constant throughout cell cycle, even through mitosis and meiosis	Humans have 23 unique chromosomes therefore n = 23
Coefficient of n AKA ploidy	The number of UNIQUE copies of the monoploid set (the number of sets of homologous chromosomes) Note: haploid (ploidy/2) and monoploid (always equal to 1n) are the same in humans. In cases where ploidy is not 2, such as in tetraploid organisms (4n), haploid refers to 2n while monoploid still refers to 1n. Therefore, the terms haploid and monoploid have distinct definitions Note: is constant throughout the cell cycle (a 2n organism will remain 2n through interphase and mitosis...it only drops down to 1n during meiosis)...it technically does double briefly during anaphase (a 2n organism will be 4n during anaphase of mitosis, 4n during anaphase of meiosis 1, and 2n during anaphase of meiosis 2, and down to 1n by the end of meiosis 2) but this course will not ask you about ploidy during anaphase. The reason why ploidy doubles during anaphase is because the sister chromatids split apart, meaning the number of centromeres doubles AKA the number of chromosomes doubles	Humans have two unique copies for each of the 23 chromosomes, for a total of 46 chromosomes, therefore ploidy = 2 (diploid) The human genome can be considered as 2n

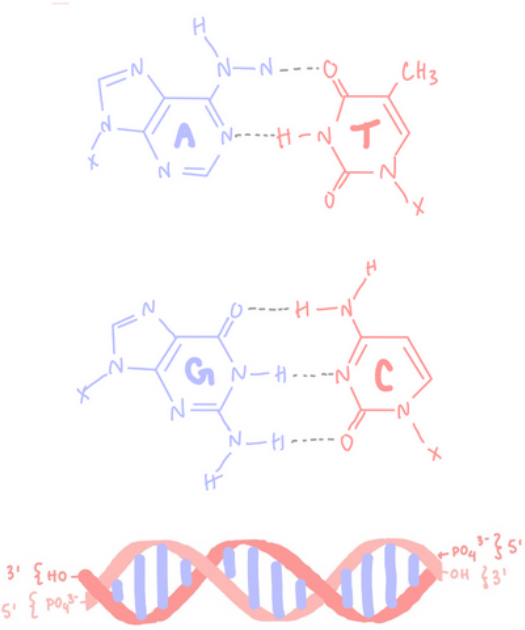
Term	Definition	Example
Chromosome number	The total number of chromosomes in all sets combined	Humans have 46 chromosomes total (2n where n=23)
C-value	The amount of DNA in ONE SET of an organism's NUCLEAR chromosomes AKA genome size Note: measured in # of base pairs OR mass Note: is constant throughout the life cycle for each organism and NEVER CHANGES Note: no relationship with n (a greater C-value doesn't necessarily mean a greater n and vice versa)	The amount of DNA in ONE SET of a human's nuclear chromosomes = the amount of DNA in one haploid set (1n) = about 3 picograms
Coefficient of C	The number of times the genome is present in a cell of the organism Note: is NOT constant throughout the life cycle Note: is equal to the number of sister chromatids in homologous chromosomes Note: basically what you multiply the C-value by to get the TOTAL DNA IN AN ORGANISM	*C-value of human gamete: 3 picograms* *Coefficient of C of human gamete: 1 Mass/total amount of DNA of human gamete: 1×3 = 3 picograms* *C-value of human zygote: 3 picograms* *Coefficient of C of human zygote: 2* *Mass/total amount of DNA of human zygote: 2×3 = 6*

C-value paradox: your genome size (C-value) has no direct correlation with how complex of an organism you are...flowering plants (less complex) have a larger genome size than mammals (more complex). The reason for this is that larger genomes tend to contain more non-coding DNA (does not code for any genes) such as transposable elements.



The Structure of DNA

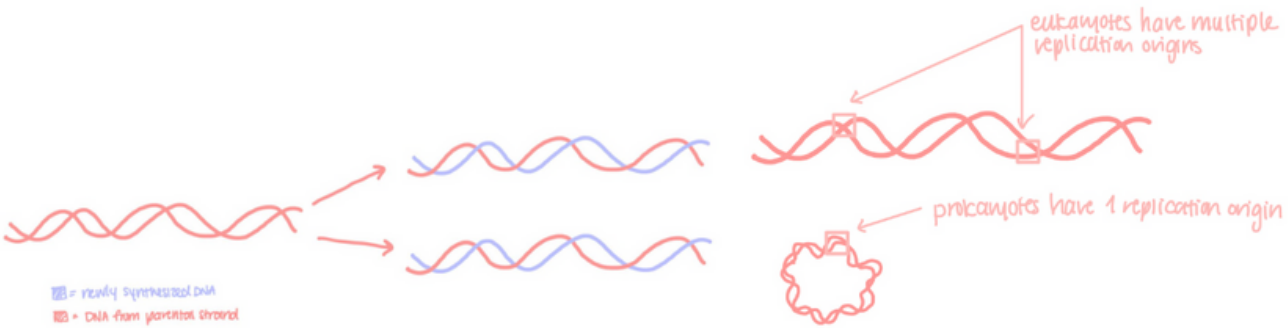
- DNA is a double helix consisting of two **antiparallel** strands of nucleotides.
- Each strand is made of nucleotides connected by a **phosphodiester bond**
- Each nucleotide consists of a 5-carbon sugar (**deoxyribose**), a nitrogenous base **(cytosine (C), adenine (A), guanine (G), thymine (T))**, and a **phosphate group**
- Adenine and guanine are **purines**, cytosine and thymine are **pyrimidines**
- Purines have two rings, pyrimidines have one ring
- A pairs with T, C pairs with G (Apple in the Tree, Car in the Garage)
- 3 hydrogen bonds between G and C, 2 hydrogen bonds between A and T
- The 5' ends have a **free phosphate group**
- The 3' ends have a **free OH group** which is used as the starting material for DNA polymerization, catalyzed by DNA polymerase i.e. DNA polymerase cannot polymerize if there is no 3' OH to work with

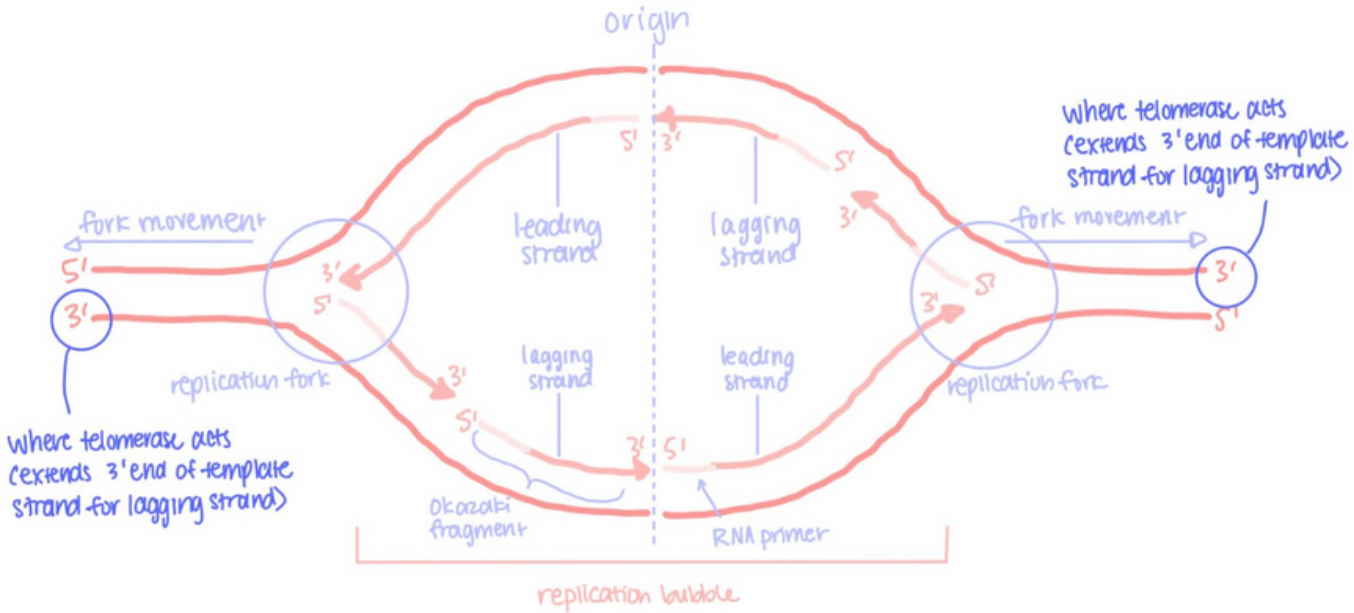


DNA Replication

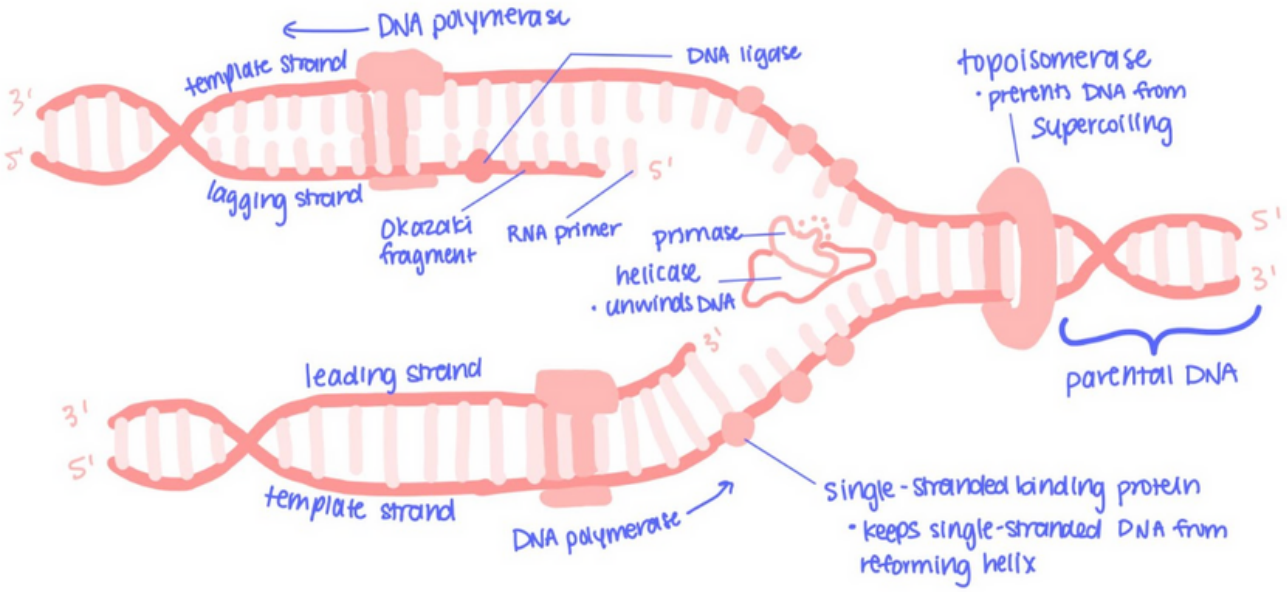
DNA replication is semi-conservative, meaning each daughter helix will have one strand from the original helix and one new strand synthesized by DNA polymerase (this is proven by the Meselson-Stahl experiment). DNA replication starts at an **origin of replication** (called an ori) where a replication bubble gets initiated (which is basically just two replication forks that get extended as replication continues). The replication bubble is created when helicase unwinds the double helix and separates the strands, creating two single-stranded segments. Each replication fork consists of a **leading strand** (which DNA polymerase synthesized continuously) and a **lagging strand** (which DNA polymerase cannot synthesize continuously and must instead synthesize in short bursts).

- DNA polymerase reads the template strand in the 3' to 5' direction and synthesizes the new strand in the 5' to 3' direction
- Replisome: the complex of proteins at each replication fork carrying out DNA replication; includes DNA polymerase, helicase, topoisomerase, single-strand binding proteins, primase, DNA ligase
- Prokaryotes have ONE replication bubble, eukaryotes have MULTIPLE replication bubbles (larger genome = need more bubbles for faster replication)
- The lagging strand consists of Okazaki fragments, which are then joined together by DNA ligase
- Since DNA polymerase needs a free 3' OH to start polymerizing a strand, primase must first come in to polymerize the first few nucleotides, called an RNA primer (primase does NOT need the 3' OH). once the first few nucleotides have been polymerized, DNA polymerase comes back in to continue elongation of the strand; each Okazaki fragment needs its own RNA primer
- After elongation finishes, RNA primers are removed, DNA polymerase comes in once again to fill in the gaps where the RNA primers were initially, and then DNA ligase seals the disconnects between the nucleotides polymerized during regular elongation and the nucleotides polymerized where the RNA primers were
- Leading strand: ONE RNA primer
- Lagging strand: MANY RNA primers


eukaryotes have multiple replication origins
prokaryotes have 1 replication origin
= newly synthesized DNA
= DNA from parental strand


origin
where telomerase acts (extends 3' end of template strand for lagging strand)
fork movement
leading strand
lagging strand
fork movement
where telomerase acts (extends 3' end of template strand for lagging strand)
replication fork
lagging strand
leading strand
replication fork
Okazaki fragment
RNA primer
replication bubble

replisome


DNA polymerase
template strand
DNA ligase
topoisomerase
• prevents DNA from supercoiling
lagging strand
Okazaki fragment
RNA primer
primase
helicase
• unwinds DNA
parental DNA
leading strand
template strand
DNA polymerase
single-stranded binding protein
• keeps single-stranded DNA from reforming helix

Mechanisms to Ensure Inheritance of Sameness

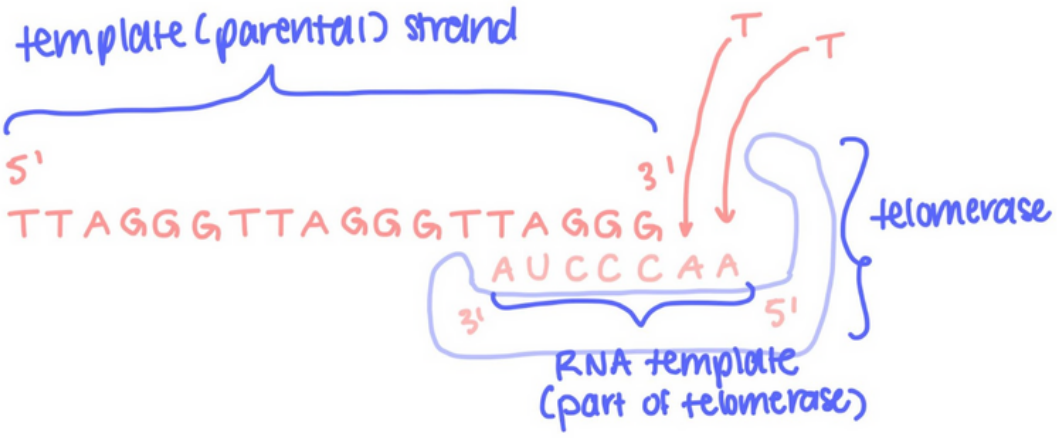
1. Semi-conservative replication
2. Complementary base-pairing
3. Proofreading by DNA polymerase

End-replication Problem

Due to the nature of lagging strand and the fact that RNA primers need to be continuously set down on the lagging strand, the replication bubble on the very end of the chromosome creates a problem: it is impossible for DNA polymerase to polymerize the end of the lagging strand (there are no more nucleotides further upstream on the template for primase to set a primer down). The solution to this are telomeres: extraneous repetitive segments of DNA (TTAGGG) at the ends of chromosomes that don't actually contain any important genetic information. Telomeres act as a buffer, they offer extra nucleotides on the template strand for the lagging strand (i.e. on the 3' ends of the parental strand) for primase to set down primers, allowing DNA polymerase to do its job all the way to the end.

However, telomeres are not infinite. Though they do prevent important DNA from being lost from the ends of chromosomes, they do themselves get shortened with every replication cycle (the end-replication problem still exists since the issue with RNA primers does not get eliminated with telomeres). The Hayflick limit is the number of replications a cell can undergo before its telomeres have been shortened to the point where important DNA will start to get lost with more replications. For a typical cell, this is about 60-70 divisions. Once the Hayflick limit is reached, the cell will become senescent, meaning that it will enter irreversible cell cycle arrest and will no longer continue to replicate. If a cell somehow manages to bypass the senescence signal and continue dividing, it will reach a crisis state, where it will undergo apoptosis (planned cell death).

The nature of telomeres is that they will become shorter and shorter with every cell division. However, there is an enzyme that can extend telomeres, hypothetically allowing for infinite cell divisions as the telomeres don't ever become dangerously short. This enzyme is called telomerase, and it is constitutively active in embryonic stem cells (this is how zygotes grow into fetuses...if telomerase wasn't active, the zygote would reach its Hayflick limit long before it grows into a baby) and male germline cells (spermatocytes). Telomerase is also active in adult stem cells, but at much lower levels, which only SLOWS DOWN telomere shortening and increases the Hayflick limits. This means that in adult stem cells, telomerase only extends lifespan of the cells but does not prevent the cells from reaching senescence (i.e. they will eventually enter cell cycle arrest, unlike embryonic stem cells). Telomerase is NOT expressed in somatic cells, unless they are cancerous tumor cells which usually have reactivated telomerase allowing for the characteristic uncontrolled proliferation found in cancer cells.



Practice Question: Analyze a tetraploid organism with 16 unique nuclear chromosomes and 4 mitochondrial chromosomes in a complete monoploid set. The total number of base pairs in one set of the 16 nuclear chromosomes is 2500.

Figure out the following values:

1. n-value during G1, S, G2, and mitosis
2. Coefficient of n during G1, S, G2, and mitosis
3. Chromosome number during G1, S, G2, and mitosis
4. Genome size/C-value during G1, S, G2, and mitosis
5. Coefficient of C during G1, S, G2, and mitosis
6. n-value during meiosis 1
7. Coefficient of n during meiosis 1
8. Chromosome number during meiosis 1
9. Genome size/C-value during meiosis 1
10. Coefficient of C during meiosis 1
11. n-value during meiosis 2
12. Coefficient of n during meiosis 2
13. Chromosome number during meiosis 2
14. Genome size/C-value during meiosis 2
15. Coefficient of C during meiosis 2

Answers:

1. 16
2. always 4n (briefly 8n during anaphase but quickly drops back down to 4n)
3. always 64 (briefly 128 during anaphase but quickly drops back down to 64)
4. 2500
5. 4C during G1, 8C during S/G2/prophase/metaphase/anaphase, 4C during telophase
6. 16
7. 4n at the start, 8n during anaphase 1, 2n at the end of meiosis
8. 64 at the start, 128 during anaphase 1, 32 at the end
9. 2500
10. 4C at the start, 8C during S/G2/prophase/metaphase/anaphase, 4C during telophase
11. 16
12. 2n at the start, 4n during anaphase 2, 2n at the end of meiosis
13. 32 at the start, 64 during anaphase 2, 32 at the end
14. 2500
15. 4C during prophase/metaphase/anaphase, 2C during telophase

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