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DNA

- The G-C bond has three hydrogen bonds
  - This is more strong than the A-T bond, which only has two
- **Reannealing/Rehybridization** = the reformation of hydrogen bonds after heating DNA

5' to 3'

- We *always* write single strained DNA/RNA in 3' -> 5' notation
  - Meaning, when you write complementary strands, you need to flip it

Use of Alternate Isotopes

- In biochemistry, alternate isotopes of elements can be used to track how and where substances are used throughout the cells
  - *e.g.*
    - ${}^{1}H$(normal), ${}^{2}H$(heavy), and ${}^{3}H$(radioactive)
    - Carbon-12, Carbon-14
- Examples
  - Distinguishing between nucliec acid and protein
    - DNA has Phospohorus, while protein does not
    - Proteins have Sulfur, while DNA does not
  - Figuring out whether DNA replication was semiconservative, conservative, etc
    - Heavy nitrogen used in initial nucleic acids
    - After replication, examine content of new double helices

Specifics of replication

- Eukaryotic cells have linear chromosomes
- Prokaryotic cells have a circular chromosome
- **Origin of replication(ori)** = the place at which replication begins
- Since Prokaryotic cells have smaller genomes and faster replication, it can simply copy it all in one go
  - In other words, there is only one ori
- Since Eukaryotic cells have much, much larger genomes and slower replication, it must copy at many places in parallel
  - In other words, there are *many* ori's
- **Histone** = a protein structure that DNA wraps around to increase density
- **Template-driven polymerization** = using the complement strand

  - Add in dATP, dTTP, dGTP, and dCTP
    * Whichever base pairs with the existing template, **DNA polymerase** will eject a **pyrophosphate** and forms the backbone

Additional Detail

1. **DNA Helicase** creates a replication fork by splitting the double strand
   - Since DNA replication happens from 3' to 5'(or, equivalently, the complement is synthesized from 5' to 3'), the two single strands cannot be replicated the same way
     - **Leading strand** = the strand where DNA-polymerase can continue to synthesize the complement as the replication fork moves
     - **Lagging strand** = the strand where DNA-polymerase is working in the opposite direction of the growth of the replication fork
2. **Single-strand binding protein(SSBP)** binds to each strand to prevent reformation of hydrogen bond
3. **DNA polymerase** needs a **primer** in order to bind to the strand
   - **Primer** = a short stretch of RNA/DNA that allows DNA polymerase to bind to the strand
   - In leading strand, the replication can take place with just one primer
   - In lagging strand, the replication has to take place with many primers
     - **Ribonuclease/RNAse** goes over the strand and removes the primers
     - Then, DNA polymerase uses DNA in between the empty primers as primers and fills in the gaps
       * But, DNA polymerase cannot fill in the last base pair; **DNA Ligase** does that
4. As DNA Helicase unzips, it twists remainder of strand so that it gets harder and harder to unzip
   - **Topoisomerase** comes and "cuts" the DNA to relieve that tension, and then reattaches the DNA so that Helicase can continue its job

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Proofreading in DNA Replication

- DNA Polymerase has an exonuclease that can correct the prior-inserted base
  - But can *only* correct previously placed base
- **Base excision repair**
  - Base pair is broken, nucleotide is "pivoted" away from double helix, and base is replaced

- **Nucleotide excision repair**
  - When UV light hits adjacent Thymines, a covalent bond happens, forming a "thymind dimer"
  - The surrounding bases are removed, and new DNA is laid down
  - **Xeroderma pigmentosum** = an autosomal recessive genetic disease that is caused by a defect in the genes that code for proteins involved in nucleotide excision repair

Telomeres

- **Telomeres** = the tips of chromosomes that fail to get replicated typically
  - This is a consequence of the fact that DNA polymerase needs a primer in order to start adding DNA
    - On 3' end, primer is laid down at very end, so that primer will never be overwritten with DNA
      - Because you cannot put down a primer after the strand ends
    - **Telomerase** = an enzyme that can actually fill in RNA primers
      - Important in stem cells and gametes

Transcription

- Faithful transcription of DNA is *less* important than in replicating DNA
  - This is because many, many mRNA transcripts are produced, so it's likely that enough will get it correct
- RNA actually have the freedom to form more interesting shapes
  - Unlike DNA
- In eukaryotes, transcription takes place in nucleus, in prokaryotes, in nucleolus
- Steps
  1. Instead of RNA polymerization starting at a primer, RNA Polymerase binds to a **promoter**
     - **Promoter** = a non-coding section of DNA that is used as a origin for transcriptiont
     - RNA polymerase actually has a helicase built in as well
     - Only the "template" strand of the double-stranded DNA is ever transcribed
     - Only about 1.5% of DNA actually codes for proteins; the rest is control information
     - A lot of DNA sequences are involved giving information as to where transcription should happen
       - *e.g.* TATA box
  2. RNA polymerase continues to add nucleotides and backbone is synthesized

3. Eventually, RNA polymerase breaks hydrogen bond between itself and the DNA

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Transcription

Before transcription

- **Transcriptome** = the set of all RNA molecules in a cell
- Prior to transcription, the DNA is wound up in the chromosome
  - Histones are very positive proteins, and DNA phosphates are negative, which is why DNA wraps around them
  - **Chromatin** = the histones and DNA in one bundled matrix
  - Two ways to modify chromatin
    1. **Acetylation** = add an acetyl group onto Lysine
       * **Upregulation** = increases activity of transcription
       * Forms **Heterochromatin**
    2. **Methylation** = add a methyl group onto certain bases
       * **Downregulation** = decreases activity of transcription
       * Forms **euchromatin** ### After transcription
- **5' capping** = a modification to the 5' end
  - The 5' terminal nucleotide actually maintains it's three phosphates
    * Because RNA polymerase doesn't attach first nucleotide to a primer, the triphosphate stays intact
  - This process happens soon after transcription begins
    * Because the 5' end will be left in cytoplasm as the rest of the mRNA is synthesized
- **3' polyadenylation** = a modification to the 3' end
  - First, mRNA is cleaved at a poly(A) site
  - Then, a few hundred adenines are added to the end of the 3' end
    * Called a **Poly-A Tail**
    * This is because degradation of the 3' end will happen naturally over time
      · This process will make it so the degredation will only damage non-coding regions for a while
    * The poly-A tail is used as a marker for nuclear export
- **Splicing** = the removal of segments(called **introns**) from pre-mRNA
  - **Intron** = non-coding region of pre-mRNA
  - **Exon** = coding region of pre-mRNA