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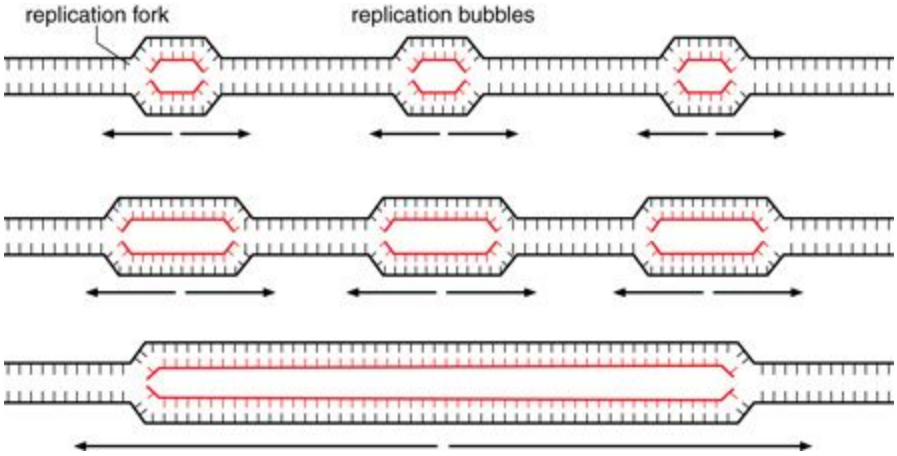
H. Bio. per. 3
6 / 7 / 15

DNA Replication and Protein Synthesis Steps

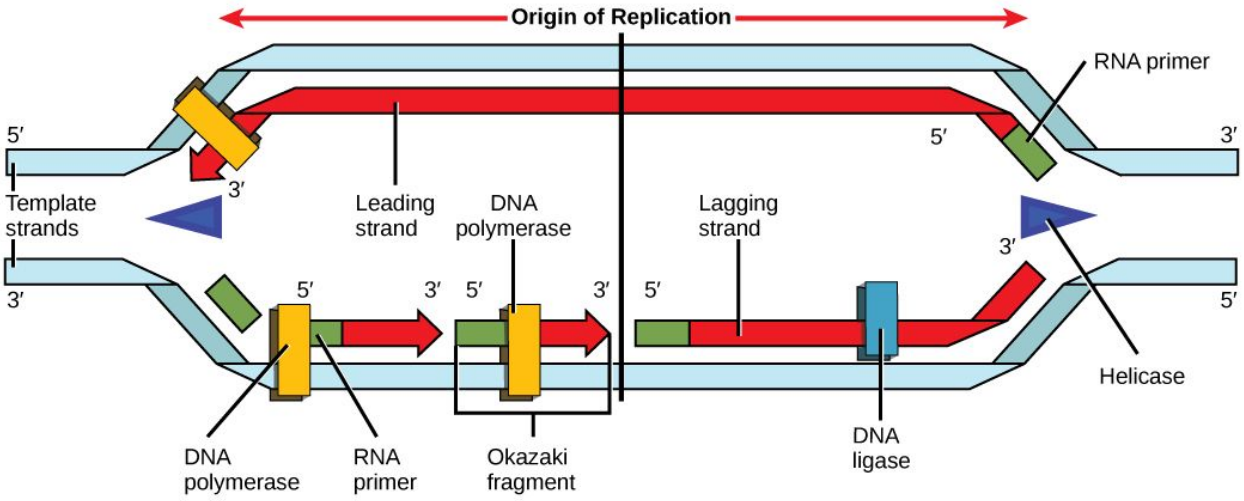
DNA Replication

1. DNA is uncoiled as chromatin in the nucleus during the S stage of interphase.
2. At certain "replication origins," DNA helicase can attach to the DNA strand and start unwinding (breaking the H-bonds) and opening it up (in both directions). This creates a "bubble" with two "replication forks" on each end.



3. When the fork is large enough (for a protein to get in), a DNA polymerase III enzyme attaches to the DNA strand that is growing from the 3' to 5' end. It starts using base pairing the original strand (the "template") to match the nucleotides and attach them to the growing strand backbone (using dehydration synthesis), creating the second polynucleotide strand from 5' to 3' (antiparallel to original strand) (red in above diagram), thus forming a complete DNA strand. On this strand, the "leading strand," the polymerase works continuously, because it can continuously attach new nucleotides to the 3' end of the growing strand.
4. On the "lagging strand", the DNA replication happens slower and is more interrupted. Because the lagging strand is opening up from 5' to 3', the growing strand opens up 3' to 5', in the direction that the polymerase cannot work. Therefore, there has to be a RNA primase, which creates a short (~10 nucleotide) piece of RNA that attaches to a piece of DNA close to the fork of replication, from which the 3' end is exposed. Then, the DNA polymerase III can come in and start base pairing and synthesizing DNA because it has a 3' end anchor. When it reaches the next RNA primer, DNA polymerase III leaves and DNA polymerase I enters, and it replaces the old RNA primer (used as an anchor and not needed anymore) with DNA nucleotides. These fragments, called Okazaki fragments, are still not bonded by their backbone, however,

and DNA ligase has to come in and bond the two Okazaki fragments together. This happens repeatedly.



5. Each of these bubbles opens up wider and wider until they run into each other, diverging and becoming two separate strands of DNA (see first diagram).

Notes:

- DNA replication is "semi-conservative," meaning that some of the some (half) of the new DNA is from the old DNA.
- After the base pairing by DNA polymerase, nuclease can recognize errors in the replication. DNA polymerase and ligase can redo the base pairing and mending to fix it. This process is called "excision repair."

Protein Synthesis

1. This can happen any time during interphase. It starts in the nucleus.

Transcription (putting a gene from DNA into mRNA)

2. Initiation
    a. At a "promoter" region in DNA (usually a TATA box), RNA polymerase attaches to the DNA.
    b. RNA polymerase starts unwinding and unzipping (breaking H-bonds in) DNA.
3. Elongation
    a. At the end of the promoter region, the polymerase starts to transcribe the nucleotide sequence into RNA nucleotides— it uses a template strand of DNA (the other strand is unused) to base-pair-match the RNA nucleotides, forming a complementary strand to the template strand.
        i. Like in DNA replication, the addition of a nucleotide comes from a nucleoside trophosphate, which releases a pyrophosphate, which gives off energy.
    b. The growing mRNA strand breaks off as it grows, and the DNA re-zips and rewinds itself, from the energy created from the release of the pyrophosphate.
4. Termination
    a. The RNA polymerase reaches a stop sequence, which causes it to break off, as well as the RNA, from the DNA.
    b. The DNA fully re-zips and rewinds itself back into its regular, double-helix shape.

mRNA preparation

5. Still in the nucleus, the mRNA has to be prepared so that it is ready to enter the ribosome.
6. On the end that is going to feed into the ribosome, there is a single guanine added (the 5' cap). On the other end there is a repetition of 50-250 adenosines (the (3') poly-A tail). This is to have the mRNA feed the right way into the ribosome, and so that it is not attacked by enzymes in the cytoplasm.
7. Introns (non-coding nucleotide sequences) are removed from the mRNA, so that only the exons are left.
8. The mRNA now floats out into the cytoplasm, and to a ribosome.

Translation (turning an mRNA gene into a amino acid sequence (protein primary structure))

9. Initiation
    a. The 5' end of mRNA attaches to a smaller subunit of a ribosome (made of rRNA and made in the nucleolus). Then, a larger ribosomal subunit attaches.
    b. In the P site of the larger subunit, there is a initiator tRNA molecule carrying methionine, whose anticodon matches with the AUG start codon on the mRNA, from which the mRNA starts being read. Because the only start codon is AUG, all protein primary structures begin with a methionine amino acid (which may be trimmed off later).
10. Elongation
    a. Another tRNA molecule with the matching anticodon for the next codon (three nucleotide bases on the RNA, which codes for a specific amino acid; in DNA, the groups of three are called "triplets") comes into the A site (adjacent to the P site). It carries with it an enzyme and an amino acid.
    b. The amino acid chain from the tRNA in the P site bonds to the amino acid in the tRNA in the A site (with a peptide bond).
    c. The ribosome shifts down one codon, so that the tRNA in the P site moves to the E site, and the one from the A site (with the polypeptide chain) moves to the P site. The one in the E site exits the ribosome to pick up more amino acids, and the A site is now empty.
    d. Elongation repeats.
11. Termination
    a. When one of several stop codons is reached, then the tRNA, rRNA, mRNA, and polypeptide chain will all dissociate. The protein can now continue to form further structures (i.e. secondary structure, tertiary structure, potentially quaternary structure).
        i. If the ribosome was connected to an ER, then the protein goes straight into the ER.
        ii. If the ribosome was free-floating, the protein would go into the cytoplasm to get assembled.

Mutations

- classifications:
    - DNA structure mutations
        - point mutations: when a single nucleotide is incorrect, inserted, or deleted, causing an amino acid to be different
        - frame-shift mutations: when there exists an extra or missing nucleotide, causing an amino acid and all the ones downstream (all the nucleotides down in the 5' → 3' direction) to be different
            - usually more serious than point mutations, because it affects multiple amino acids
    - protein structure mutations
        - nonsense mutations: a point mutation that causes a codon to become a stop codon
            - can greatly shorten proteins, therefore having an enormous effect on the protein
        - missense mutations: a point mutation that changes an amino acid from one to another

        - silent mutations: mutations that don't actually change the amino acid (some amino acids are coded by multiple codons)
        - conservative mutations: mutations that change amino acid to another of same type
            - less serious than non-conservative, worse than silent
        - non-conservative mutations: mutations that change amino acid to another of a different type
- mutations originate from DNA but show on proteins
- mutations can be classified by their effects on DNA or protein
- "mutagenesis" is production of mutations, and is caused by
    - DNA replication and recombination (aging)
    - mutagens
        - radiation
        - chemicals

Notes:

- process called "central dogma of molecular biology," and discovered by Francis Crick, Nobel Prize winner who found out the structure of DNA
- "mRNA" = "messenger RNA"
- "tRNA" = "transfer RNA"
- "rRNA" = "ribosomal RNA"

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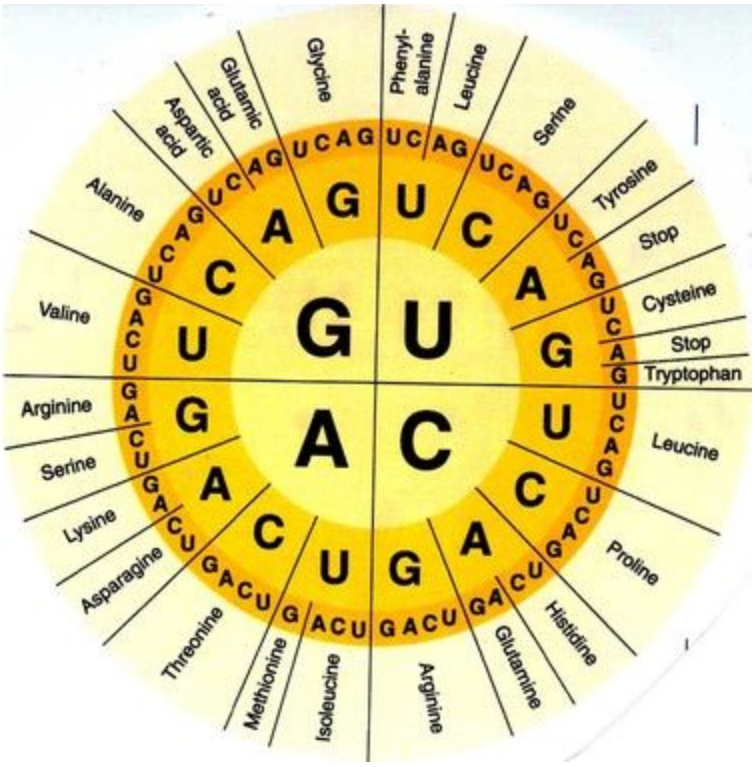
- codons can be found out (by a human) with a chart



	U	C	A	G
U	UUU = phe UUC = phe UUA = leu UUG = leu	UCU = ser UCC = ser UCA = ser UCG = ser	UAU = tyr UAC = tyr UAA = stop UAG = stop	UGU = cys UGC = cys UGA = stop UGG = trp
C	CUU = leu CUC = leu CUA = leu CUG = leu	CCU = pro CCC = pro CCA = pro CCG = pro	CAU = his CAC = his CAA = gln CAG = gln	CGU = arg CGC = arg CGA = arg CGG = arg
A	AUU = ile AUC = ile AUA = ile AUG = met	ACU = thr ACC = thr ACA = thr ACG = thr	AAU = asn AAC = asn AAA = lys AAG = lys	AGU = ser AGC = ser AGA = arg AGG = arg
G	GUU = val GUC = val GUA = val GUG = val	GCU = ala GCC = ala GCA = ala GCG = ala	GAU = asp GAC = asp GAA = glu GAG = glu	GGU = gly GGC = gly GGA = gly GGG = gly