Name:______________

Chemistry 462 Biochemistry
Second Hour Exam

1. (10 points) Compare and contrast the structure of a prokaryotic and a eukaryotic chromosome. Include in this discussion comments on size, complexity, and information content of the DNA.

Prokaryotic	Eukaryotic
about 1 million bases pairs	25-600 million base pairs
Single chromosome	Multiple Chromosomes/cell
No defined superstructure, although localized to nucleous	Localized to membrane bound nucleus Condensed into compact Chromosome structure at certain points in cell cycle
A single double stranded circular piece of DNA	Each Chromosome is a linear double strand of DNA
Single origin of replication	Several origins in each chromosome
High information content most DNA codes for protein or regulatory site	Low level of information. Much DNA Is highly repetitive and does not code for either protein or regulatory sequences
Little or no introns	Most messages sequences contain introns and exons

2. (10 points) A closed circular DNA molecule in its relaxed form has a *Lk* of 500. Approximately how many base pairs are in this DNA? How is the linking number altered (increase, decrease, remains the same, becomes undefined) when (a) a protein complex is bound to form a nucleosome, (b) a protein complex is bound to form a nucleosome AND the DNA is reacted with *E coli* topoisomerase I (c) one strand of DNA is broken, (d) DNA gyrase (*E coli* topoisomerase II) and ATP are added to the solution (e) the double helix is denatured by heat?

Problem 5 from chapter 24

If relaxed DNA then Wr is 0 ot Lk= Tw + Wr, and Lk = Tw = 500

B form DNA with 10.5 bp/turn then 500/10.5 = 5,250 bp

a. No change = covalent structure hasn't changed so LK is constant

b. Lk will decrease. When nucleosome bind DNA in introduced a negative supertwist in the region it binds and, and there is an offsetting + supertwist in the unbound DNA. The topoisomerase will relax the + supertwist, so the DNA will be left with a - supertwist where the nucleosome is bonding.

c. when one strand is broken the Lk becomes undefined

d. Lk will decrease. Gyrase used ATP energy to put negative superturns nto the DNA

e. No change. The DNA will lose its base pairing, but the covalent structure does not change so Lk must remain unchanged.

3. (10 points) Describe the structure of a histone in as much detail as possible.

I actually wanted the structure of a nucleosome, so I will give some latitude here.

A nucleosome is a protein DNA complex consisting of roughly 200 bp of DNA and 9 histone proteins. The core of the nucleosome consists of copies each of histones H2A, H2B, H3 and H4. Each histone, in turn is a small protein of about 100-200 amino acids that has a large preponderance of Lys and Arg, giving thei histoe a large positive charge a neutral pH.

About 150 bp of DNA wraps around the outside of this protein core in a left-handed superhelical coil. The remaining 50 bp of DNA serves as a linker between nucleosomes, and usually bind histone H1. although the amount of H1 binding varies dramatically, depending on the ranscriptional activity of the DNA. IF the DNA is being actively transcribed very little H1 is bound, if DNA is not begin transcribed, then about 1 H1 bind for every nucleosome.

4. (10 points) Describe what is currently known about the structure of eukaryotic chromatin.

DNA bound to nucleosomes are folded together to make a 30 nm (thick) fiber.

These fibers are then looped around the nuclear scaffolding proteins to make structures called rosettes. The rosettes are coiled on top of each other to form the cromatids, or the visible arms of the condensed chromosomes.

5. (10 points) Define he following terms:

Primase A protein that synthesizes the RNA primer necessary to start the replicaiton of DNA

Ligase A protein that uses ATP to seal nicks in a DNA sugar-phosphate backbone

Helicase A protein that uses ATP energy to open up a loop in a DNA double helix

Replicase (or replisome) A large complex of several proteins that is used to replicate DNA in a cell. Enzyemes activities found in this complex incluse primases, helicases, and DNA polymerases

processivity A measure of hoe efficiently a polymerase operates, that is how often it dissociates form a template nucleic acid in te course of a polymerase reaction. An eneyme with high processivity come off the template very infrequently, while an enzyme with low processivity falls of the template strand often.

exonuclease An enzyme that cleaves nucleotides off of DNA (or RNA) starting at the ends

endonuclease An enzymes that cleaves DNA (or RNA) in the interior of the strand

lagging strand - At a replication fork one strand of DNA is being synthesized in a 3'-5' continuous fashion, while the other strand must be synthesized a discontinuous manner using pieces of 3'-5' DNA that are then linked together by a ligase go give synthesis on this second strand. This strand, where the discontinuous replication of small fragments is done is called the lagging strand. .

replication fork The point at which double stranded DNA has been opened up to yield 2 single strand of DNA for replication.

catenane 2 circular pieces of DNA that are intertwined with each other and cannot be separated because their covalent backbones are twisted around each other.

6. (15 points) Describe the elongation step of DNA replication in *e coli*.

DNA is unwound by a helicase to expose two single strands

The two single strand are protected and prevented from re-annealing by the binding of single strand binding protein (SSB)

The DNA is then replicated on the leading and lagging strand by a large protein complex on the leading strand the primase binds once to synthesize about 10-60 nucleotides of RNA before the DNA polymerase III starts adding DNA to this primer.

The lagging strand is synthesized in a similar manner, but because this it the 5' strand, and DNA can only be make in a 5' to 3' direction, only a short pieces of DNA, called Okazaki fragments, are made to fill in this strand in a discontinous manner.

The replication of both strands is coordinated by having DNA replicated on a DNA polymerase III dimer, with the lagging strand of DNA being looped around so both polymerases make DNA in the same direction. This looping of the lagging strand is further coordinated in a large enzyme complex that includes the τ protein scaffold, the DnaB helicase, a clamp loading assembly that brings the primase in when needed on the lagging strand and cycles the β clamping subunint on and off the lagging strand as needed.

A figure like 25-14 would help this explanation

The lagging strand must be further processed. The RNA primer is removed and replaced with DNA using DNA polymerase I, and the nicks between the Okazaki fragments must be filled using a DNA ligase.

7. (15 points) What are the different DNA repair systems found in most cells?

Mismatch repair system - recognize incorrectly paired bases in newly synthesized DNA
locate the mismatched base on the <u>new</u> strand of DNA, remove a portion of the DNA around that incorrect base, then replace the missing DNA

Base-excision repair - used to repair certain lesions of DNA bases - particularly C and A deaminations - repairs the base by excising the base for the sugar phosphate backbone, then other enzymes cur the DNA at the AP site and around the bad base site

Nucleotide excision repair - used to repair other lesions of DNA bases - usually photo-induced dimers. Endonucleases cut the bad DNA base + several flanking bases and the DNA is then replaced

Direct Repair - Enzyme that reverse the cyclobutane pyrimidine dimers by re-modifying the DNA bases back to their original form.

Error Prone Repair system - a last ditch SOS repair system to replace damaged DNA, even if the repairs are not exactly correct

Recombination Repair system - use genetic information for homolgous genes to repair DNA at single or double strand breaks

8. (10 points) How does the formation of Holliday intermediates in homologous genetic recombination differ from their formation in site-specific recombination?

Problem 13, chapter 25. During homologous genetic recombination , a Holliday structure may be formed almost anywhere within the two paired, homologous chromosomes. Once formed the branch of the intermediate may move extensively by branch migration. In site-specific recombination the Holliday intermediate is formed between two specific sites, and branch migration is generally restricted by heterologous sequences on either of the recombination sites.

9. (10 points) Describe the chromosomal structure of the human IgG kappa light chain protein. How does this structure account for the highly variable structure of this protein.

The chromosomal structure of the human IgG gene consists of 1 copy of a constant (C) region, 4 different copies of a joining (J) region of about 12 residues, and about 300 different copies of a 95 residue variable (V) region. The cell uses recombination events to link a single copy each of C, J, and V regions together to make a single protein and that single protein is the only human IgG protein expressed in that cell. This combination of 1 X 4 X 300 allows cell to express at least 1200 different proteins from the same piece of DNA. The recombinatnion event used to join these regions together is somewhat sloppy, so in reality, close to 3000 different genes can be produced from this piece of DNA.