Quiz 1 Summary: answers & score distribution

1. Please describe the main features of the DNA structure. (10 points)

Key features:

(1) Double helical structure **(4')**

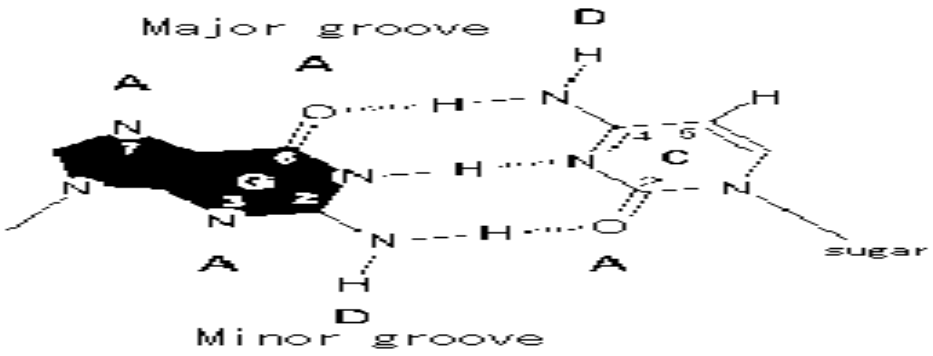
(2) Two strands are held together in an antiparallel orientation **(3')** by base paring **(1')**.

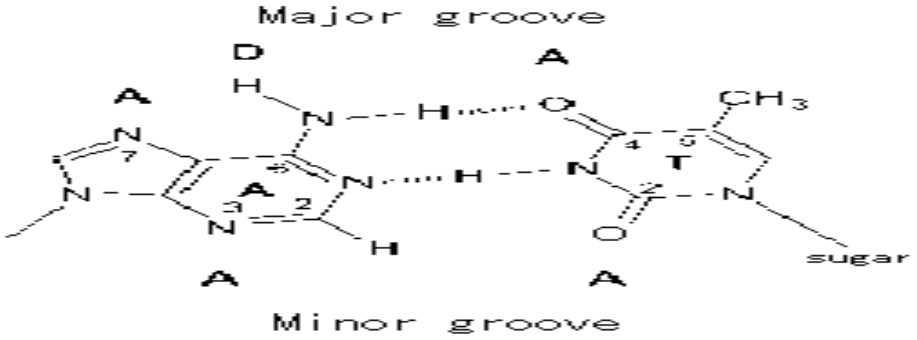
Other features

Composed of deoxyribose, phosphate (backbone), bases (A, T, G, C);
Hydrogen bonds (A=T, G≡C) and base stacking force to keep stability;
Major groove and minor groove;
A, B, Z forms of DNA;
DNA can be melted by heat…

1' each

2. The following is the two Watson-Crick base pairs, please (1) indicate which sides are in the major groove, and which sides are in the minor groove; (2) indicate the hydrogen bond acceptors and donors in each groove. (10 points)





(1) Correctly indicate the major and minor groove of the two base pairs. **(5')**

(2) Correctly indicate the 11 hydrogen bond acceptors and donors in each groove. (0.5 point each, **5'** in all)

3. Describe the structure of the nucleosome? (10 points)

(1) A nucleosome is comprised of a histone core and the core DNA wrapping around it. **(3')**

(2) A histone core is an octamer of histones, consisting of two H2A, H2B dimers and a H3, H4 tetramer. **(4')**

(3) The core DNA wraps around the histone core in 1.65 turns, which is usually 146 bp long. **(3')**

4. Through what mechanisms that the compact chromatin structure is achieved? (10 points)

The chromatin is compacted through three sequential mechanisms.

1) The DNA chain coils around core histone octamers to form nucleosomes. This arrangement allows the chromatin to achieve a six-fold compaction. **(3')**

2) Nucleosomes then form 30-nm fiber with the help of linker histone H1. About 40-fold compaction is further achieved through this organization. **(3')**

3) The chromatin was further compacted by assembling to DNA loop structure. **(3')**

Note: 6-fold and 40-fold share **1'**.

5. How does the replication at the E.coli origin is initiated? How the leading strand and lagging strand in a replication fork are synthesized? How the DNA synthesis is coordinated to ensure that both strands are simultaneously copied toward the moving direction of a replication fork? (30 points)

How does the replication at the E.coli origin is initiated? (10 points)

(1) Recognition and binding of OriC by DnaA-ATP. **(4')**

(2) Helicase (DnaB) loading and DNA unwinding. **(3')**

(3) Primase synthesizes RNA primer, and DNA Polymerase III synthesizes the new DNA strand. **(3')**

How the leading strand and lagging strand in a replication fork are synthesized? (10 points)

Because DNA is only synthesized by elongating the 3'-OH end, only one of the two exposed template strands can be replicated continuously.

(1) Leading strand refers to the newly synthesized DNA strand that is continuously copied from the template strand by a DNA polymerase after the first RNA primer was made by a primase. A sliding clamp is usually loaded to the DNA polymerase to increase the polymerase processivity. The 5'→3' direction of the leading strand is the same as the moving direction of the replication fork. **(5')**

(2) Lagging strand refers to the newly synthesized strand that is discontinuously copied from the template strand. The 5'→3' direction of the lagging strand is opposite to the moving direction of the replication fork. Primase makes RNA primers periodically after the template strand is unwound and becomes single-stranded. DNA polymerase extends each primer to synthesis short DNA

fragments, called Okazaki fragments. The polymerase dissociates from the template strand when it meets the previous Okazaki fragment. RNA primers are digested by an RNase H activity, and the gaps are filled by DNA polymerase. At last, the adjacent Okazaki fragments are covalently joined together by a DNA ligase to generate a continuous, intact strand of new DNA. **(5')**

How the DNA synthesis is coordinated to ensure that both strands are simultaneously copied toward the moving direction of a replication fork? (10 points) [Figure 8-21]

(1) HOLOENZYME: In order to copy the two antiparallel parental DNA strands simultaneously, *E. coli* uses a holoenzyme complex containing two complete copies of DNA Pol III, with one DNA Pol III being engaged in synthesizing the leading strand, and one for the lagging strand. Because these two copies of DNA Pol III are physically linked together, synthesis of the leading and lagging strands is therefore physically coupled. **(5')**

(2) MECHANISM: The "trombone" model was developed to explain how synthesis of the Okazaki fragments of the lagging strand matches the continuous synthesis of the leading strand at the *E. coli* replication fork. (i) One DNA Pol III in the holoenzyme efficiently extends the leading strand immediately after the template strand is released from the replication fork by the DNA helicase. (ii) However, the template strand for the lagging strand synthesis is coated by the single-stranded DNA binding proteins (SSB). (iii) Then the primase periodically associates with the DNA helicase at the replication fork to synthesize a new RNA primer on this lagging strand template. (iv) When the lagging strand DNA polymerase completes the previous Okazaki fragment, it is released from the sliding clamp and the DNA.(3 points). Then, a clamp loader resided in the holoenzyme uploads the freshly established primer:template junction and sliding clap onto the empty DNA Pol III to synthesize the next Okazaki fragment. In this way, synthesis of the lagging strand can match the continuous polymerization of the leading strand toward the moving direction of the replication fork. **(5')**

6. What is the mechanism that ensures the eukaryotic chromosomes to be replicated exactly once per cell cycle? (10 points) [Figure 8-32, page 224-228.]

Pre-replicative complex (pre-RC) formation directs the initiation of replication in eukaryotes. There is only one opportunity for pre-RCs to form, and only one opportunity for pre-RC to be activated during each cell cycle, and Pre-RC formation and activation occur at separated phases of a cell cycle. **(4')**

(1) Pre-RC is assembled during G1 phase. The first step in the pre-RC formation is the recognition of the replicator by the eukaryotic initiator ORC. Once ORC is bound, it recruits two helicase loading proteins Cdc6 and Cdt1. Then the eukaryotic replication fork helicase Mcm2-7 complex is recruited to complete the pre-PC formation. However, the pre-RC is not active in G1 phase. **(3')**

(2) Pre-RC is activated during S phase. Activation of pre-RC requires two protein kinases Cdk (cyclin-dependent kinase) and Ddk. These two kinases

are inactive in G1 phase and become active once the cell enters S phase, and remain active until enter the next G1 phase. Once Cdk and Ddk are activated, they target the pre-RC and other replication proteins to activate the initiation of replication. Meanwhile, the high level of Cdks in S, G2, and M phase inhibits the further formation of Pre-RC during all these phases. Therefore, formation of the new pre-RC has to wait until the next G1 phase. **(3')**

7. What is the eukaryotic chromosome end replication problem? How the problem was naturally resolved? (10 points)

(1) The lagging strands of the eukaryotic cells are unable to copy the extreme ends of their linear chromosomes, because the last RNA primer of the lagging strand is removed or there is insufficient space to synthesize a new primer. **(2')** Therefore, after each chromosomal replication cycle, the 5' end is shorter than the 3' end, and genes at the end of the chromosomes would be lost eventually if no further mechanism to replication the chromosomal end. **(2')**

(2) Fortunately, telomerase is present in eukaryotic cells to replicate the chromosomal ends. Telomerase is a novel DNA polymerase that includes both protein and RNA. **(2')** Telomerase extends the 3' end (leading strand or the longer strand) of the repetitive chromosomal end, called telomere, using its RNA component that base pairs with the telomere sequence as a template to repeatedly synthesize telomere sequences. **(2')** As a result, the 3' chromosomal end becomes long enough to act as the template to continue the synthesis of the 5' end (lagging strand or the shorter strand) by the regular DNA synthesis complex (including DNA polymerase, RNase H, 5'-3'exonuclease and ligase), and thus to prevent the chromosomal end from shortening. **(2')**

(It is better, however not required, to mention the notion that telomerase only exists in germ cells or cancer cells)

8. Can DNA polymerase proofread? If you answer yes, please explain how? (10 points)

(1) Yes. **(4')**

Note: *If you answer "it depends", you can also get 4' if you could cite any example that substantiates your viewpoint.*

(2) The palm domain of the DNA polymerase contains two catalytic sites: one is DNA replication site and the other is the 3'-5'proofreading exonuclease site. **(2')** When a mistake occurs, the rate of DNA synthesis is reduced due to the incorrect positioning. However, the exonuclease active site has a higher affinity for the mispaired last base pairs (or you can answer that they have more time docking at the exonuclease site). Once bound at this active site, the mismatched nucleotide is removed in a 3'-5' and non-specific way. After that, a correct primer:template junction is reformed and polymerization resumes. **(4')**

Note: *Proofreading refers to any mechanism for **correcting errors** in protein or nucleic acid synthesis that involves scrutiny of individual units **after** they have been added to the chain.*

Some students answered that the ability of DNA Pol to distinguish between ribo- and deoxyribonuleoside triphosphates and the kinetic selectivity of correct base pairing at the DNA replication site are examples of proofreading functions. Unluckily, they are not proofreading.

Score Distribution Chart