Multiple choice (5 points each):

1. Which of the following statements about E. coli RNA polymerase is false?
   A) Core enzyme selectively binds promoter regions, but cannot initiate synthesis without a sigma factor. Book answer!
   B) RNA polymerase holoenzyme has several subunits.
   C) RNA produced by this enzyme will be completely complementary to the DNA template. I accepted this because ‘completely’ is unclear
   D) The enzyme adds nucleotides to the 3’ end of the growing RNA chain.
   E) The enzyme cannot synthesize RNA in the absence of DNA.

2. After binding by E. coli RNA polymerase, the correct order of events for transcription initiation is:
   A) closed complex formation, open complex formation, promoter clearance, start of RNA synthesis.
   B) closed complex formation, open complex formation, start of RNA synthesis, promoter clearance.
   C) open complex formation, closed complex formation, start of RNA synthesis, promoter clearance.
   D) start of RNA synthesis, closed complex formation, open complex formation, promoter clearance.
   E) start of RNA synthesis, open complex formation, closed complex formation, promoter clearance.

3. The 5'-terminal cap structure of eukaryotic mRNAs is a(n):
   A) 7-methylcytosine joined to the mRNA via a 2',3'-cyclic linkage.
   B) 7-methylguanosine joined to the mRNA via a 5'→3' diphosphate linkage.
   C) 7-methylguanosine joined to the mRNA via a 5'→5' triphosphate linkage.
   D) N6-methyladenosine joined to the mRNA via a 5'→5' phosphodiester bond.
   E) O6-methylguanosine joined to the mRNA via a 5'→5' triphosphate linkage.

4. Which one of the following is not true of the mRNA for ovalbumin?
   A) Exons are used for polypeptide synthesis.
   B) Introns are complementary to their adjacent exons and will form hybrids with them.
   C) The mature mRNA is substantially shorter than the corresponding region on the DNA.
   D) The mRNA is originally synthesized in the nucleus, but ends up in the cytoplasm.
   E) The splicing that yields a mature mRNA occurs at very specific sites in the RNA primary transcript.
5. Compared with DNA polymerase, reverse transcriptase:
   A) does not require a primer to initiate synthesis.
   B) introduces no errors into genetic material because it synthesizes RNA, not DNA.
   C) makes fewer errors in synthesizing a complementary polynucleotide.
   D) makes more errors because it lacks the 3’ → 5’ proofreading exonuclease activity.
   E) synthesizes complementary strands in the opposite direction from 3’ → 5’.

Essay questions (15 points each) - Answer any 5.

1. Back in Chapter 24 it was stated that up to 45% of the human genome is made up of transposons. What is a transposon? How are simple and complex transposons different? What is the difference between direct transposition and replicative transposition? And, finally How are Eukaryotic transposons different from prokaryotic ones, and what is the tie between Eukaryotic transposons and RNA?

A transposon is a piece of DNA that can ‘jump’ from one site to another. In a simple transposon the piece of DNA only contains the sequences required for transposition and the genes for the transposase that does the transposition. In a complex transposon some additional gene are carried with the ‘jumping’ element. In a direct transposition the DNA simply moves from one site to another. In a replicative transposition one copy remains at the original site while a second copy of the DNA is integrated into a new site.

In the last part of the question I was fishing for you to say that many (but not all) eukaryotic transposons go through an RNA intermediate, and may have evolved from RNA viruses.

2. Recombination allows a human to make millions of different antibodies even though the human genome only has a TOTAL of about 29,000 gene. Tell me about the structure of the immunoglobulin genes, and how recombination is used to generate millions of different immunoglobulins from these genes.

A diagram like figure 25-46 is a good place to start.
Each immunoglobulin is made from 2 heavy and two light chains. The gene for the light chain is made up of around 300 V segments, 4 J segments and 1 constant segment. As the cell producing a given immunoglobulin differentiates, it uses recombination events to chose a single V and J segment to connect to the constant region for each heavy and light chain. This can give rise to 300x4 or 1,200 possible sequences from a single gene. On top of that, the splicing events and the subsequent splicing of the mRNA is variable so this single gene actually give rise to about 3000 different chains. The gene for the heavy chain is similar in structure and splicing, and gives rise to about 5000 different gene products. Thus 2 genes can express as many as 3000x5000 or 1.5x10^7 different proteins.
3. To make a piece of mRNA, the RNA polymerase need to ‘know’ where to start and stop. Tell me about the signals and proteins involved in both initiating and terminating RNA polymerase activity in E. Coli.

The Start
In E coli the place where the RNA polymerase will bind is called the promoter sequence. Each promoter has its own unique consensus sequence at -10 and -35 bases from the actual place where the RNA polymerase begins its work. The polymerase ‘chooses’ which promoter site it will bind to based on which σ factor has been incorporated into the polymerase complex. There are at least 7 different σ factors and each recognizes a different promoter sequence.

The End
We studied to different mechanisms to stop the RNA polymerase, one that uses the ρ factor and one that doesn’t.

In the ρ-independent termination there is a sequence with 3 A’s in the template strand as well as a built in hairpin structure 15-20 residues before the end of the message. When the polymerase hits the 3 A’s in a row it slows down. As it slows down, the hairpin built into the RNA message has time to form, and once it forms, it forces the polymerase to terminate transcription and terminate the message.

ρ-dependent termination uses a ρ-protein that associates with the RNA message as it is transcribed. It probably moves along just behind the polymerase, but is not quite fast enough to catch it until the polymerase hits a ‘rut’ (Rho utilization) sequence that is a CA-rich region. When the polymerase hits this sequence it slows down, and then the ρ-factor catches it. Once it catches it, it forces the polymerase to stop transcription and terminate the message.

4. How are RNA polymerases in Eukaryotes both similar to and different from the RNA polymerase from E. Coli.

E coli RNA polymerase
5 subunits α₂ββ’ωσ total mass ~ 390000

Eukaryotes
3 polymerase
pol I for r-RNA
pol II for m-RNA
pol III for t-RNA

The text did not go into the structure of pol I and pol III, but it did talk about how pol II compares to the e coli polymerase:

12 subunits
RBP1 similar to β’
RBP2 similar to β
RBP 3 & RBP 11 look like σ₂
Has a large CTD (carboxyterminal domain) that has several specialized functions not seen in Ecoli like sites to bind capping and splicing complexes. Will also have lots of interactions with other proteins as part of the more complex control mechanisms seen in eukaryotes.
5. The Eukaryotic rRNA undergoes extensive processing before it takes it mature form in the ribosome. Describe all the process it undergoes. Where in the cell does this processing take place?

A single pre-rRNA is first transcribed using RNA polymerase I. It is then processed in the nucleous to form the 18S, 28S, and 5.8S rRNA’s. This processing includes cleavage reactions by various endo- and exo-ribonucleases and nucleoside modifications, as well as some splicing. It involves upward of 170 non-ribosomal proteins, 70 snoRNA.s as well as the ribosomal proteins that are assembled around the rRNA as it is processed. A 5S piece of pre-rRNA is also transcribed using RNA polymerase III and it is also transported to the nucleous where it is combined the rRNA’s to make the completed ribosome. The ribosome is then exported out of the nucleus into the cytosol.

6. Describe the structure of a typical retrovirus gene and the typical life cycle of a retrovirus.

A retrovirus is a virus that has its genetic information encoded on a single strand of RNA. The simplest virus typically has long LTR’s (long terminal repeats) at each end of the gene. The gene itself can be as simple as a ψ, gag, pol, and env genes, which are usually transcribed and translated as a single large polypeptide which is then cleaved down into virus structural proteins, integrase, protease, reverse transcriptase and virus envelope coat proteins by the protease.

The typical life cycle starts with the virus RNA being injected into the host cell. The RNA is then reverse-transcribed into a strand of DNA using the reverse transcriptase and a t-RNA primer borrowed from the host cell. The reverse-transcriptase then destroys the RNA and replaces it with DNA. The integrase then integrates this double stranded DNA molecule into the host cell genome, where it usually can lay dormant for long periods of time until some signal make the virus start making new virus particles to begin infecting other cells.

7. Up to now, you have probably only heard about t, r, and mRNA. This chapter exposed you to RNA being used in several different ways. Name and describe all the RNA’s other than t, r, and mRNA.

snoRNA - small nucleolar RNA’s used in processing rRNA and building ribosomes in the nucleous

snRNA’s - small nuclear RNA’s used to make snurps that are used to make the spliceosomes that remove the bulk of the eukaryotic introns.

miRNA’s - microRNA’s - noncoding RNA’s involved in gene regulation

Self splicing introns

Rnase P - a RNA cleaving ribozyme
1. A
2. B
3. C
4. B
5. D