Questions from Fall, 2001:

Q1. I'm a little confused on our chapter 17 assignment. Are we supposed to read the whole chapter and focus on the figures you mentioned in the syllabus or are we just supposed to read the sections that are labeled as figures, i.e 17.1; 17.3-4; 17.6; 17.9-10. I don't know if there was a misprint or something.

Answer: There isn't a misprint in the syllabus, but it does take some careful reading. You don't have to read the whole chapter--just figures and section numbers listed in the syllabus. So figure 17.1 means just figure 17.1 (not SECTION 17.1; you don't have to read sections 17.1 or 17.2). The next set of numbers DOES refer to section numbers: 17.3-4; 17.6; 17.9-10 (if I don't write 'figure' in front of the number, I'm talking about a section number); so, do read all the SECTIONS from 17.3 to 17.10 except for 17.5. As for 17.5, you'll see on the handout that I ask you to look at three figures that fall in this section; if these figures don't make sense to you from the figure legend alone, you're advised on the handout to read section 17.5 selectively, otherwise you can skip the text portion of section 17.5.

The handout offers the best roadmap for chapter 17. For instance, I tell you in the handout to use section 17.6 for reference (meaning you don't have to know it in great depth, even though it's part of the assigned reading); and I tell you to read the intro to the chapter (the paragraphs preceding section 17.1).

Q2. The book is really hard for me to understand and I am just curious as to how much of it is on the test that we didnt' cover in class.

Answer: I will not test you on things that are in the assigned readings in the text but that I have not mentioned in class at all.

Q3. So, in class you were talking about how reverse transcriptase was a sloppy DNA polymerase. Do a lot of the viral "progeny" end up being inactivated from their nucleotide sequences getting too muddled up? How can viruses be so flexible with their coding material when it seems like if we screw up just a little bit with our DNA there are big consequences?

Answer: The reason viruses can 'afford' to be sloppy while we cannot is that they produce massive numbers of progeny each time they lyse a host cell. If some of these virus particles have mutations that make them defective, they aren't missed very much; on the other hand, even if one of these particles has a mutation that's advantageous (for example, expands the host range from monkeys to humans, or makes it resistant to an antiviral drug) it's likely to be strongly selected for.

Q4. I have been reading ahead in our biology 319 textbook, and can across a section called regulation by antisense RNA. The book gave an example of transposase gene regulation via antisense RNA. I was wondering if anyone has ever thought of making an antisense strand for a viral protein say HIV, inserting that sequence into a vector say a retrovirus which would insert the sequence into the genome. Expression of antisense RNA would hybridize with viral RNA

blocking expression and shutting down viral replication. I think this might be an effective cure for viral infections.

Answer: Your idea is certainly an interesting one, and it has occurred to other people too. Antisense RNA is widely used in basic and biomedical research, mostly to understand the functional importance of a gene under study (knock out the RNA for that gene using antisense RNA, then look for a phenotype), but its promise as a therapy is being explored as well.

Antisense RNA is being tried against AIDS and a few other life-threatening viral diseases, pretty much as you envisioned it! There are some difficulties, however. For one thing, it wouldn't help a person who has full-blown AIDS; they've already got the virus multiplying and lysing cells. You could theoretically protect a person who is HIV-positive but doesn't have disease symptoms; you could remove their T cells (specifically the CD8+ cytotoxic T lymphocytes or CD 4+ helper T cells), genetically engineer them so they express an antisense RNA that would bind to an important mRNA made by the viral genome (the *gag* gene RNA has been targeted by one company), and return the T cells into their blood.

There are two practical problems with this strategy: 1. Because HIV is genetically diverse, you would have to first determine which strain of the virus the patient has, then make your antisense RNA to match that strain's relevant DNA sequence (and hope that it hasn't, and will not, mutate in your patient). 2. Because T lymphocytes have a limited lifespan, you'd have to do the genetic engineering regularly throughout the person's lifetime to keep AIDS at bay. You could permanently engineer their bone marrow stem cells to express the antisense RNA but there's some risk of insertional mutagenesis (what if the inserted gene plops into an important gene and knocks that out an important gene function? Or, worse, what if it causes activation of an oncogene? In a person who doesn't even have AIDS just yet!). Bone marrow gene therapy remains technically difficult, although it has been tried for thalassemia, a blood disorder; the biggest problem is that bone marrow stem cells are scarce (not a whole lot of tissue to work with) and most of them are inactive and nobody knows how to identify and separate the active and inactive cells.

At present, ribozyme technology appears to hold greater promise in combating HIV. The idea is to synthesize catalytic RNAs (ribozymes) that will destroy certain types of RNA. Many of these 'designer ribozymes' are altered versions of plant ribozymes (remember, plants have no immune system, so they battle viral villains with clever nucleic acid chemistry). Many different types of custom ribozymes directed at the regulatory sites of HIV RNAs (more likely to be conserved, less likely to mutate among strains) have been made, and some have even been tested on patients. Periodic repetitions of the treatment are necessary, because no bone marrow engineering has been tried thus far. In test tubes the ribozymes greatly reduce viral replication, but we don't yet know how the trials in people will turn out.

<u>Questions from Winter, 2001</u>:

Q1. Does polymerase I, II, and III mentioned for *E. coli* apply for eukaryotes? Should we know the basic functions of I, II, and III in DNA replication?

Answer: First, let's be sure we're clear about which polymerases we're talking about.
RNA polymerases: You do need to know the three eukaryotic RNA polymerases, and how they're different from each other.
DNA polymerases: you don't need to know the NAMES of all the different DNA polymerases in prokaryotes (they're named I, II and III) or in eukaryotes (the mammalian ones are named alpha, beta, gamma, delta, epsilon, and so on), but you do need to know the role played by each TYPE of DNA polymerase (I've given them generic names based on function: e.g. replicative or repair DNA polymerases)

Q2. Is it important to know the difference between hnRNP and hnRNA?

Answer: It's useful to get the terminology right. HnRNP means any ribonucleoprotein particle, and it refers to *any* nuclear RNA that is found associated with one or more distinct proteins (hnRNP proteins). Hn stands for heterogenous, and refers to *all* classes of RNA including pre-mRNAs, snRNAs, and mature mRNAs. Thus, all of these are HnRNPs: a mature mRNA complexed with proteins; a pre-mRNA with cleavage factors and poly A polymerase bound to it; snRNAs with snRNA-proteins bound to it (as in U1, U2,...etc).