X107A, Fall 2000. Final exam answer key.

Answer key (brief) …

1. The Gln codons are CA(A/G), written 5′→3′. The complement would appear to be GU(U/C). But we want one tRNA to read both codons, and the wobble rules allow us to use U to read both the A/G. So we have GUU. But this is the complement, so is in the opposite direction, 3′→5′. That is, the best way to write the answer is either 3′-GUU-5′ or 5′-UUG-3′. (Writing GUU alone implies 5′→3′, and is therefore wrong.)

2. Slowing down a reaction allows time for an alternative reaction to proceed. Why is this useful/interesting? Slowing down a "forward" reaction allows time for proofreading of an earlier step, thus increasing the accuracy of a process. The specific example that we discussed the most is the role of EF-Tu in determining the timing of peptide bond formation, and hence the accuracy of tRNA choice (codon-anticodon pairing). Hydrolysis of GTP by EF-Tu triggers peptide bond formation. Looking at it from the other side, peptide bond formation is delayed until EF-Tu leaves (which requires GTP hydrolysis, which itself may be stimulated by proper codon-anticodon pairing). The longer it takes for this to happen, the more time there is for the aminoacyl-tRNA (being delivered by EF-Tu) to diffuse away. The odds that any tRNA that remains is correct increases as time goes on, so the longer it takes EF-Tu to leave, the more accurate protein synthesis is.

> Examples can also be found in DNA replication, where elongation and proofreading compete. One student noted how slow methylation of the nascent strand aids with mismatch repair. Interesting point!
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> The broader issue here is kinetic competition between two processes. One can bias the competition in a particular direction by speeding up one side or slowing down the other. Kinetic competitions of one kind or another are common.
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> I noted in the question that the purpose here is not to raise semantic issues. One can argue that the enzymatic activity of EF-Tu is to hydrolyze GTP, a reaction which EF-Tu does indeed speed up. Our goal is to understand Nature, and we need to resist being trapped in our own definitions. (This may be analogous to the Ch 20 hw question #7, in which we looked at a case where an enzyme breaks hydrogen bonds -- contrary to the common statement that enzymes do not break such "weak" bonds.)

3. a. 9. Each of the 3 positions in the codon can change to 3 other bases. Thus there are 9 possible single base changes. The simple prediction, without looking at the specifics of the code, is that 9 possible single codon changes implies as many as 9 possible amino acid changes.

b. In every case, at least 2 of the resulting 9 codons code for the same amino acid. As one specific example, start with the codon UUU = Phe. There are 9 possible codons it could mutate to, but two of them, UU(A/G) both code for Leu. Also, UUC codes for Phe, just like the original codon, so in this case there is no new amino acid at all.

It is important to emphasize that there are 9 single base change mutations possible. However, some may code for the same amino acid. Once again, we have a critical distinction between genotype and phenotype.

There is an interesting practical perspective here. It used to be a common approach to do random mutagenesis to try to explore the effect of amino acid changes on a protein. The naive assumption was that all possible variations would be uncovered. But as the calculation in part a makes clear, even before adding the practical constraint of part b, only a subset of possible amino acid changes can be uncovered by simple mutational events.

4. a. The mutation must have created a UAG (amber) codon. You know that because the mutation is suppressed by an amber suppressing tRNA.

b. Since one amber suppressor works and another does not, the most likely explanation is that serine is not an acceptable amino acid at the mutated codon position.

Weaver p 591. Ch 18 hw #11.

You do not know that Gln is required (or that it is the original amino acid). You only know that it is acceptable at this position, and Ser is not.

5. b. & c. Should look something like the right hand side of Fig 20.6b. Key features: a fork, strands are anti-parallel, lagging strand is discontinuous.

d. Just "ahead" of Pol, where it promotes unwinding of the DNA to prepare it for replication. (The replication helicase is actually on the lagging strand, but I didn't hold you to that for grading.)

e. One with each polymerization "head" (i.e., one sliding clamp on each strand), to hold the Pol onto the DNA. Technical term: The sliding clamp increases processivity.

f. Between two Okazaki fragments on lagging strand, to join them. Note that ligase per se does not fill in the gap, but only seals a nick. (Fig 20.27 cd; look at "nick".)

6. AUG. Protein synthesis always starts with methionine; the observed serine at the N-terminus is due to modification of the protein after synthesis. The major start codon is AUG. (p 536)

7. a. Lower left end. (The 3′ end that appears "indented", so that a template is available.)

b. Upper right end. (The 3′ end that is overhanging.) (Fig 21.35c.)

Many answers to a and b were unclear, because you did not follow the instructions for how to mark answers. I tried to make sense of what you wrote, but didn't feel a great obligation to give you benefit of doubt when you don't follow instructions!

c. It carries its own template (in the form of an RNA). All DNA Pol need a 3′ end to grow onto and a template, to know what to add.

8. tRNA. (And no, the anticodon is not in Dade-Broward-Palm Beach counties. It is over on the other side, facing the Gulf of Mexico. Facing Texas. Fig 19.35a -- and your favorite atlas.)

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