

Recitation 02 Problems

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WARM UP

(EXAM 1, 1994)

You add poly(ACCA) RNA to a cell-free E. Coli protein synthesis system. Although inefficient, you are able to monitor some protein synthesis. What polypeptide product(s) would you expect to isolate?

QUESTIONS

1. You are given the mRNA sequence which contains 5' ~ ~ C C C A **A** U G U C ~ ~ 3'. How can you prove that the genetic code is non-overlapping by just mutating the bolded nucleotide to a "C"?
2. *(Exam #1 2002, Question #4)*
Imagine yourself a UROP student in a lab wishing to study the E. coli phenylalanyl-tRNA synthetase, the enzyme that uses ATP to specifically attach Phe to the phenylalanine tRNA (tRNAPhe). Your task is to purify this enzyme, and to do this you need an assay.
Propose an assay for determining the amount of Phe tRNA synthetase activity in the fractions coming off your column. A colleague in your lab has a preparation of total E. coli tRNA that you can use for your assay. When describing your assay, mention that the molecules that you will add to the fractions and what procedure you will perform after adding these molecules.
3. *(3/6/96 Exam 1 Question 3)*
tRNA synthetases "charge" tRNA molecules in a two-step reaction. Suppose that you carried out both steps of this reaction in the presence of radioactive ATP (32P incorporated at all phosphorous atoms in ATP). Where would you expect the radioactivity to be located after charging was complete? Explain briefly.
4. How would the tRNA synthetase discriminate between Aspartate and Asparagine? How about between Tyrosine and Tryptophan?

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5. *(Exam #2 2002)*
An mRNA molecule encoding a protein of 100 amino acids is translated in a cell-free preprotein synthesis system in which ATP, GTP, and amino acids are added.
- Suppose that for every protein molecule produced, an average of 272 GTP molecules are hydrolyzed to GDP. Provide a detailed accounting for how this number of GTP molecules are likely to have been consumed (i.e., for each part of the translation process that uses GTP, state the number of GTP molecules used, and provide a rationale for choosing that number).
 - Suppose that for every protein molecule produced, an average of 117 ATP molecules are hydrolyzed to AMP. Account for how this number of ATP molecules would be consumed.
6. Explain how the lack of proofreading in Reverse Transcriptase is responsible for making it such a “deadly and dangerous” enzyme.
7. Reverse transcriptase has ribonuclease activity as well as polymerase activity. What is the role of the ribonuclease activity?
8. *(Gumport, Chapter 28, Problem 5)*
When mammalian genes are cloned, a strategy that is frequently followed involves the isolation of mRNA rather than DNA from a cell and the preparation of a complementary DNA (cDNA) by the enzyme reverse transcriptase (this method is used to generate cDNA libraries). Suppose that mRNA isolated from a cell specialized for the production of protein X is used as a template for the production of cDNA. What major difference or differences would you expect to find between the structure of that cDNA and genomic DNA for protein X?
9. *(PSET 2, 2003)*
The inhibition of telomerases is being explored as a way to stop cancer. After puberty, most of the body does not express telomerases, so targeting them is almost equal to targeting the cancerous cells.
- Explain why telomerases are present in cancerous cells, but not in most other cells in the body. Briefly explain how the telomeres are maintained in such cells.
 - Explain why inhibiting telomerases can possibly stop cancer.
 - Why aren't drugs that inhibit telomerases used to treat bacterial infections?