Questions Translation

November 2021

1) For each of the following sequences, place a check mark in the appropriate space to indicate the process most immediately affected by deleting the sequence. Choose only one process for each sequence (i.e., one check mark per sequence)

	Process most immediately affected by the deletion			
Sequence deleted	Replication	Transcription	RNA processing	Translation
ori site	✓			
3' splice-site conensus			✓	
sequence for poly(A)tail			✓	
start codon				✓
-10 consensus sequence		✓		
Shine Dalgarno				✓

2) Several experiments were conducted to obtain information about how the eukaryotic ribosome recognizes the AUG start codon. In one experiment, the gene that encodes methionine initiator tRNA ($tRNA_i^{Met}$) was changed. The nucleotides that specify the anticodon on $tRNA_i^{Met}$ were mutated so that the anticodon in the tRNA was 5′-CCA-3′ instead of 5′-CAU-3′. When this mutated gene was placed in a eukaryotic cell, protein synthesis took place, but the proteins produced were abnormal. Some of the proteins produced contained extra amino acids, and others contained fewer amino acids than normal.

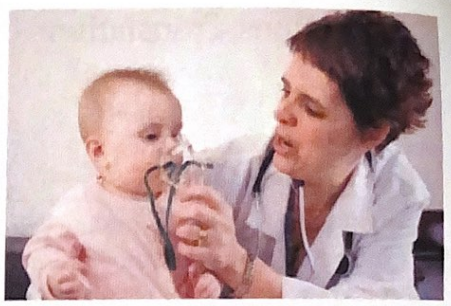
a) What do these results indicate about how the ribosome recognizes the starting point for translation in eukaryotic cells? Explain your reasoning.

Obviously the start site is not clear anymore. The ribosome migrate along the mRNA to find first AUG, but the tRNA can't bind properly. The results suggest that, to initiate translation, the mRNA is scanned to find the appropriate start sequence.

b) If the same experiment had been conducted on bacterial cells, what results would you expect?

The initiation of translation in bacteria requires the 16S RNA of the small ribosomal subunit to interact with the Shine-Dalgarno sequence on the mRNA. This interaction serves to line up the ribosome over the start codon. If the anticodon has been changed such that the start codon cannot be recognized, then protein synthesis is not likely to take place.

3) Mutations that introduce stop codons cause a number of genetic diseases. For example, from 2% to 5% of the people who have cystic fibrosis possess a mutation that causes a premature stop codon in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). This premature stop codon produces a truncated form of CFTR that is nonfunctional and results in the symptoms of cystic fibrosis.



In *Drosophila*, such nonsense mutations can be cured by introducing a suppressor tRNA into the genome of the fly. Using a $tRNA^{Tyr}$ gene, how would you change the anticodon to rescue an amber (UAG) nonsense mutation? *Drosophila* has only one type of $tRNA^{Tyr}$. Tyr = UAU or UAC.

anticoden in $tRNA^{Tyr}$ = NUA where N is the wobbling base that recognizes C or U in the wobbling position.

4) Genetic Code stop codons are UAA. UAG. UGA.

by changing the anticodon in the $tRNA^{Tyr}$ to CUA the stop codon UAG will be read.

Using the one letter code for amino acids, translate the mRNA on the following page into the corresponding protein sequence.

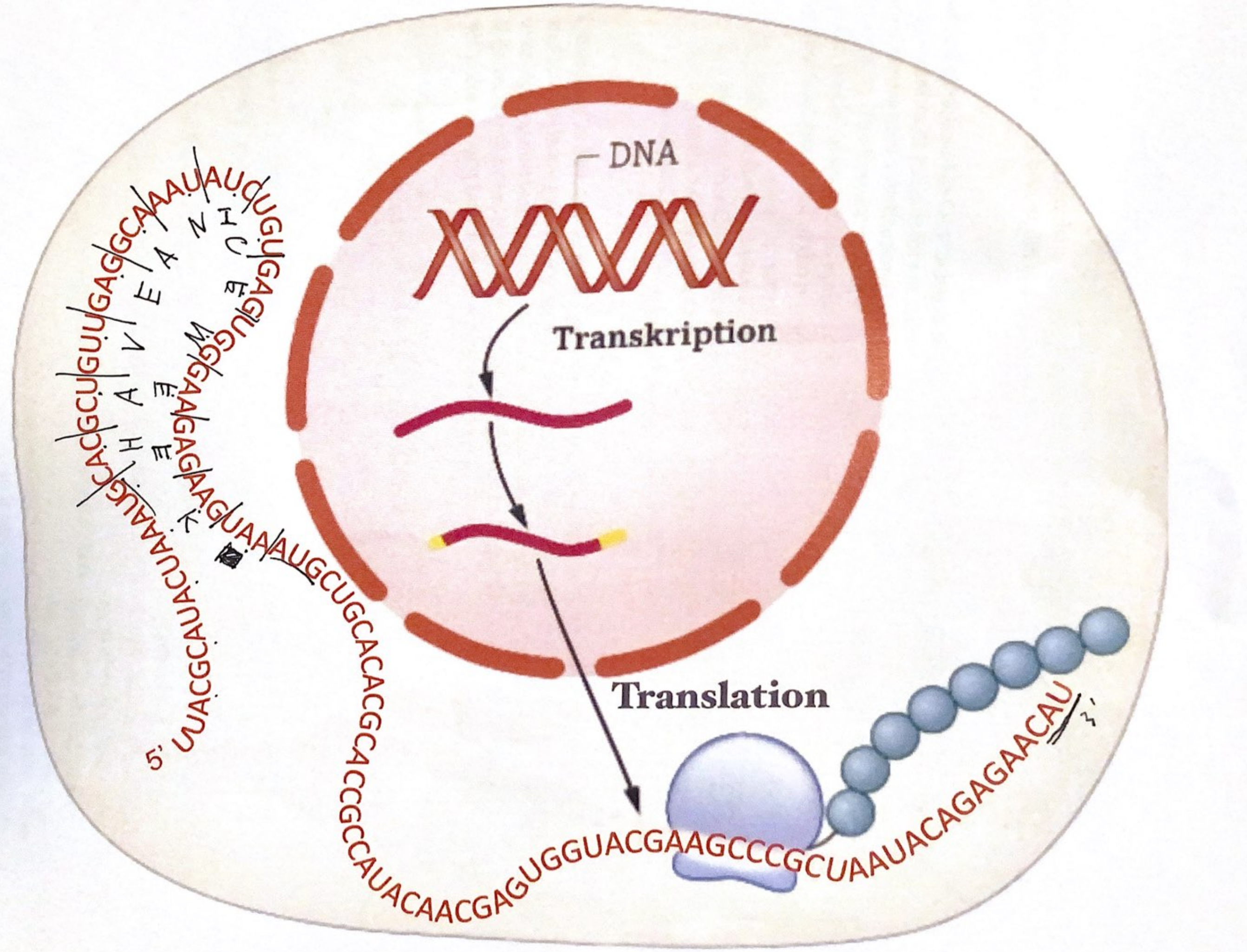
anticodon in $tRNA^{Tyr}$ is (either AUA or) GUA

→ change to CUA

$tRNA^{Tyr}$ ··AUA·· ··AUG·· ··AUC··· 3′

mRNA 5′ ···UAU·· ··UAC·· ··UAG···

Tyr Tyr amber stop


DNA
Transkription
Translation
5' UUACGCAUACUAAAUGCACGCUGUUGAGGCAAAUAUCUGUGAGUGGGAAGAGAAGUAAAUGCUGCACACGCACCGCCAUACAACGAGUGGUACGAAGCCCGCUAAUACAGAGAACAU

Some additional example questions from the book you should be able to answer. You will find the solution at the end of the book.

Solved problem III: Geneticists interested in human hemoglobins have found a very large number of mutant forms. Some of these mutant proteins are of normal size (but have amino acid substitutions) while others are short, due to deletions or nonsense mutations. The first extra long example was named Hb Constant Spring, in which the β-globin has all of its normal amino acids plus several extra amino acids attached after the normal Cterminal end of the protein.

1. What is the most plausible explanation for its origin? stop is overread/ deletion of a few nucleotides or point mutations
2. Is it possible that Hb Constant Spring arose from failure to splice out an intron? most unlikely
3. Estimate how many extra amino acids might be added to the C terminal end of the mutant protein. Statistically there is one stop codon per 21 codons

Question 7: The following diagram describes the mRNA sequence of part of the *A* gene and the beginning of the *B* gene of phage φX174. In this phage, some genes are read in overlapping reading frames. For example, the code for the *A* gene is used for part of the *B* gene, but the reading frame is displaced by one base. Shown here is the single mRNA with the codons for proteins A and B indicated.

```
aa# 5   6   7   8   9   10 11 12 13 14 15 16
A    AlaLysGluTrpAsnAsnSerLeuLysThrLysLeu
     GCUAAAGAAUGGAACAACUCACUAAAAACCAAGCUG mRNA
B             MetGluGlnLeuThrLysAsnGlnAla
aa#           1   2   3   4   5   6   7   8   9
```

Given the following amino acid (aa) changes, indicate the base change that occurred in the mRNA and the consequences for the other protein sequence.

a. Asn at position 10 in protein A is changed to Tyr. 5'AAC(Asn)→5'UAC(Tyr) In protein B CAA(Gln) to Leu(CUA)

b. Leu at position 12 in protein A is changed to Pro. 5'CUA(Leu)→5'CCA(Pro) In protein B ACU(Thr) to ACC(Thr)

c. Gln at position 8 in protein B is changed to Leu. CAA(Gln) → CUA(Leu) In protein A AAG(Lys) to stop(AUG)

d. The occurrence of overlapping reading frames is very rare in nature. When it does occur, the extent of the overlap is not very long. Why do you think this is the case? It's difficult for ORFs to evolve under constraint to encode functional proteins in two reading frames (Statistically every 21aa there is a stop codon

Question 31: The human genome contains about 500 genes for tRNAs.

a. Do you think that each one of these tRNA genes has a different function?

b. Can you explain why the human genome might have evolved so as to house so many tRNA genes? No, there are only 61 codons to be read and that's it. and some of the tRNAs recognize more than one codon.

redundancy is here to have enough tRNAs ready for efficient translation

Question 43:

43. The following is a list of mutations that have been discovered in a gene that has more than 60 exons and encodes a very large protein of 2532 amino acids. Indicate whether or not each mutation could cause a detectable change in the size or the amount of mRNA and/or a detectable change in the size or the amount of the protein product. (Detectable changes in size or amount must be greater than 1% of normal values.) What kind of change would you predict?

a. Lys576Val (changes amino acid 576 from lysine into valine)
b. Lys576Arg
c. AAG576AAA (changes codon 576 from AAG to AAA)
d. AAG576UAG
e. Met1Arg (at least two possible scenarios exist for this mutation)
f. promoter mutation
g. one base pair insertion into codon 1841
h. deletion of codon 779
i. IVS18DS, G–A, + 1 (this mutation changes the first nucleotide in the eighteenth intron of the gene, causing exon 18 to be spliced to exon 20, thus skipping exon 19)
j. deletion of the polyA addition site
k. GtoA substitution in the 5′ UTR
l. insertion of 1000 base pairs into the sixth intron (this particular insertion does not alter splicing)

Mutations possibly causing a detectable change in protein

size: d, e, g, i

In protein amount (assumes all mutant proteins are equally stable): e, f, j, k

In mRNA size: i, j

In mRNA amount (assumes all mutant mRNAs with poly-A tails are equally stable):

f, j