MCDB 3500
Exam #4
Fall, 2004

75 minutes, closed everything. Please ask if any question is not clear.

Name Key

ID

Q 1 (20 points)

Q 2 (20)

Q 3 (20)

Q 4 (20)

Q 5 (20)

Total (100)

Grade

Second position

First position (5'-end)	U	C	A	G	Third position (3'-end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

1. Imagine that you are Dr. Jesse Castenada, a molecular surgeon of the 21st century. A patient comes to you with Norrie Disease (ND), an X-linked defect limited to eye development, that nevertheless leads to complete blindness due to malformation of the retina. You sequence ND mRNA from affected and unaffected sons from the same family. Here are message sequences covering crucial regions within the ND gene:

Blind: …`CCU`AAG`AAG`UAA`UAC`GGG`GUC`…

Normal: …`CCU`AAG`AGU`AAU`ACA`GGG`GUC`…

Ticks indicate the reading frame.

Starting with RNA from both individuals, how did you get the sequences?

SEQUENCE THE 5' END OF THE ND mRNA WITH ddNTP & REVERSE TRANSCRIPTASE, USING A SPECIFIC PRIMER.

How did you recognize the crucial region?

IT HAS A SEQUENCE CHANGE THAT EXPLAINS THE DISEASE.

How did you guess the reading frame from mRNA sequences alone?

(POSSIBILITIES) a. I FOUND AN ORF.
b. I CHOSE THE PHASE USING THE 1ST AUG/KOZAK CONSENSUS.
c. I STARTED IN PHASE TO THE UAA/UAG/UGA TERMINATOR
d. I LOOKED UP SOME AMINO ACID SEQUENCE
e. OTHERS

What is, most likely, the cause of this genetic disease?

AN INSERTION & DELETION
PUT A UAA STOP IN PHASE.

Given that the "delivery problem" (that of introducing arbitrary new sequences into the many nuclei of a multicellular organism) has now been solved, what is the simplest way (requiring the smallest genetic change) to correct this defect using gene therapy?

(POSSIBILITIES) a. INSERT A tRNA THAT SUPPRESSES THE UAA STOP.
b. INSERT A NEW ND GENE; SINCE X-LINKED GENES ARE HAPLOID ANYWAY, THIS HAS AN INCREASED CHANCE TO WORK

2. What follows are 5 flagrant, despicable lies about the molecular biology of translation. Your answer should be two or three sentences (no more) that:

Say exactly what the lying assertion is

Supply a revised, corrected statement about the topic.

a. Translation of bacterial mRNAs is said to be "functionally circular" because the same ribosomes translate the message over and over.

FUNCTIONAL CIRCULARITY = INTERACTION of 5' & 3' END

Translation is said to be "fc" because a message works best when poly A · poly A binding protein interacts with initiation factor (eIF4G), in array

b. The double sieve model of aminoacyl-tRNA synthesis explains how an aaRS can distinguish amino acids and tRNAs at the same time.

not (aa + tRNA), but amino acids alone

The double sieve is activation (sieve #1 = lets small aa thru) plus editing (sieve #2), which destroys aa that are too small.

c. The synergistic effects of capping and poly A formation on translation can be understood in terms of an effect on message half-life.

The joint effect of capping & polyA is on initiation of translation.

d. The peptidyl transferase can be shown to be a normal enzyme by using an antibiotic (pactinomycin) that binds stably to the active site.

not "normal", it's a ribozyme & not pactinomycin, but puromycin.

The peptidyl transferase can be shown to be a ribozyme by using CCdA, Puro, which binds stably to the active site

or "puromycin"

e. Some mammalian viruses inactivate human cellular translation by modifying release factors so they work only for viral messages.

initiation factors (not eRF's) are modified

Mammalian viruses inactivate initiation factors so they work only for uncapped viral messages.

3. Define

molecular mimicry

Translational factors look like EF-Tu • tRNA sometimes.

ribosome recycling factor, RRF

The protein that disrupts the ribosome • mRNA • tRNA complex after termination.

Shine-Dalgarno

In bacteria, the homology to the 3' end of the 16S rRNA which occurs just upstream of the initiation codon.

Y, Wyosine

Modified nucleotide that occurs just 3' of the anticodon in tRNA.

Formyl-methionine

The bacterial initiator amino acid.

assembly map

The description of the order of assembly of proteins onto rRNA to make the ribosome

exon junction complex

Proteins that mark exon-exon boundaries after splicing, deposited by the spliceosome.

polysome

A mRNA plus multiple ribosomes making protein.

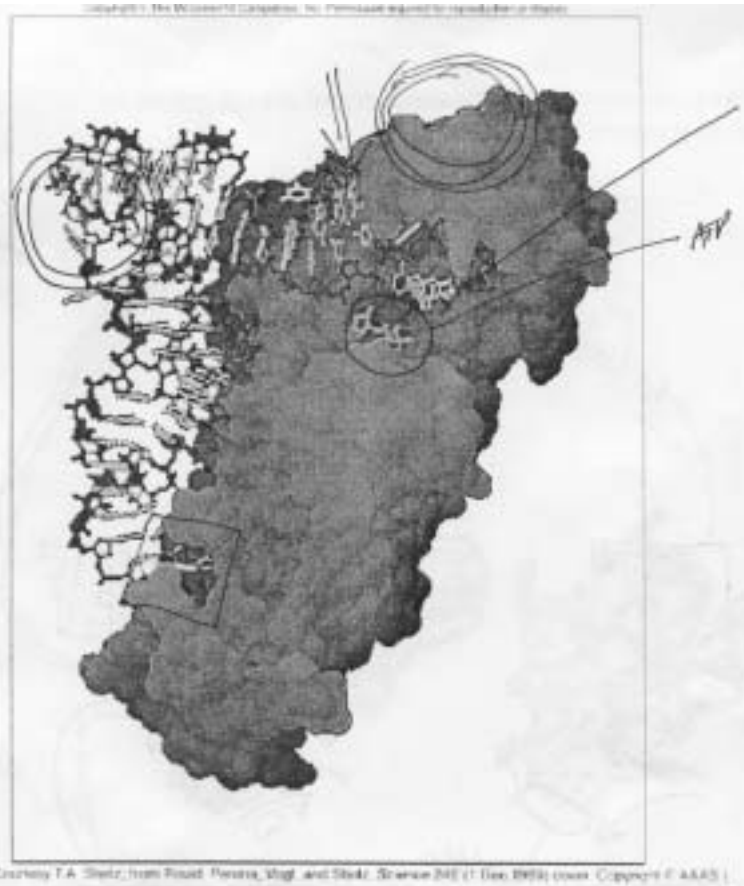
tmRNA

tmRNA ≡ tRNA mRNA; the RNA that captures & tags the protein partially completed on broken bacterial messages.

Kozak consensus

The sequence that must surround active eukaryotic initiators, notably

RCC$\boxed{\text{AUG}}$G.



(Source: Courtesy T.A. Steitz, from Rould, Perona, Söll, and Steitz, Science 246 (1 Dec 1989) cover. Copyright © AAAS.)

4. Circle the site where ATP binds.

Put a square around the anticodon.

Draw a double line around the D loop.

Draw a triple line around the region where you might expect to find an editing site.

Draw an arrow pointing to the 3′ end.

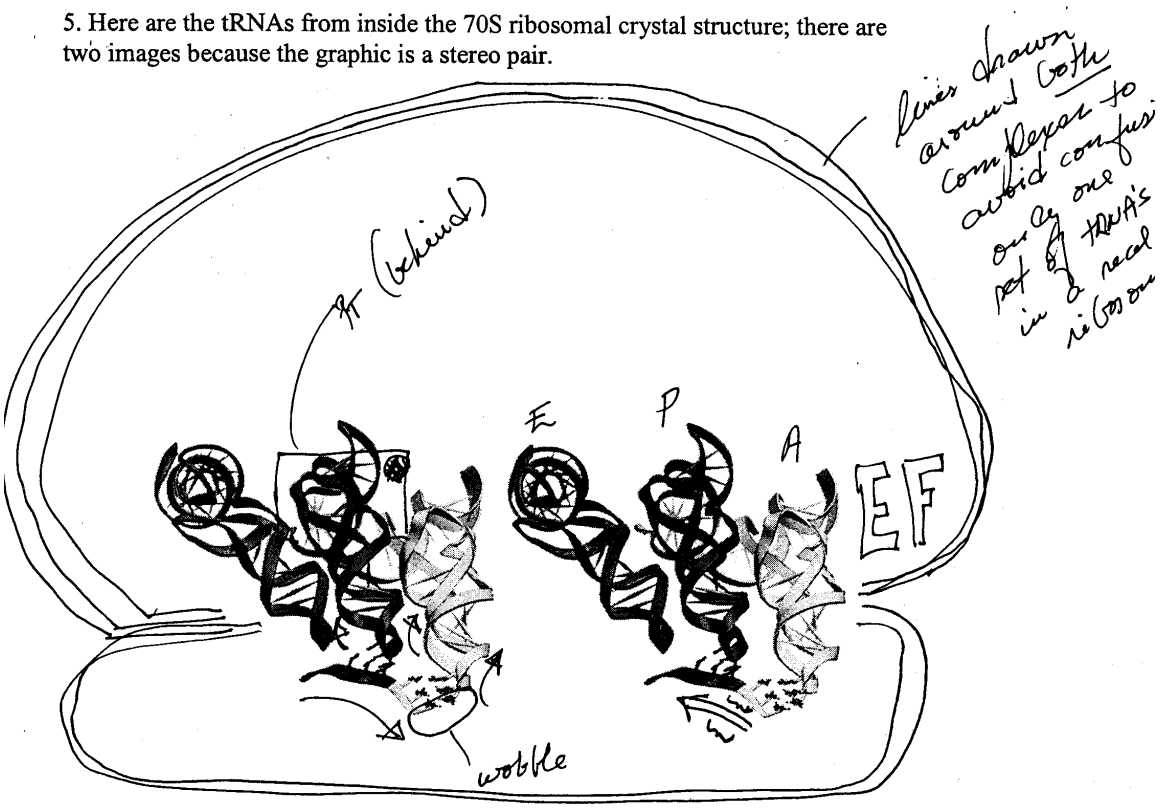
Draw a double arrow pointing to the 5′ end.

This is a type I; what would a type II look like? flip the tRNA over & let protein approach from the other side.

Judging from this structure, what is one purpose of the conserved CCA sequence?

It is single-stranded & can flip between activation & editing sites.

5. Here are the tRNAs from inside the 70S ribosomal crystal structure; there are two images because the graphic is a stereo pair.



Pick the right or left image as a focus, and using conventional arrows, indicate the polarity of all chains.

Circle (or ovoid) the wobble position.

Put a square (or a rectangle) around the position of the peptidyl transferase.

Mark the A-, P- and E- site tRNAs.

Put an approximate double line around the small ribosomal subunit.

Put an approximate triple line around the large ribosomal subunit.

Put a small filled circle (•) at the entrance to the exit tunnel.

Use a thick arrow (⇑) beside the message to indicate the direction EF-G would push during translocation.

Mark with a bracket ({) the area of approach of A_{1492} and A_{1493} of the 16S RNA.

Put  in the region where you expect the EF-T and EF-G binding site.