Note: This key is written on an earlier draft of the final. The answers are the same as the final version but some of the questions are differently worded.

Biochemistry 465 **Your Name:** _____
Biological Information Processing **Prof. Jason Kahn. Univ. Maryland**
Final Exam (200 points total) **December 19, 2008**

You have 120 minutes for this exam. N=73

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. If you must have more, the last page is blank.

You do not need a calculator for this exam, and no other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. (60 pts) Transcriptional Regulation

(a; 14 pts) Fundamentally there are two different ways that prokaryotic transcriptional activators accelerate transcription initiation (the mechanisms are shared with eukaryotes). Name them and give a brief example for each.

+3 – recruitment or enhanced binding

+2 CAP + RNAP bind promoter cooperatively] or +4 for another correct answer
(+2) (+2)

+3 – catalysis of open complex formation or later steps in transcription

+2 NtrC catalyzes conversion of σ^{54}-RNAP to active form
(+2) (+2)

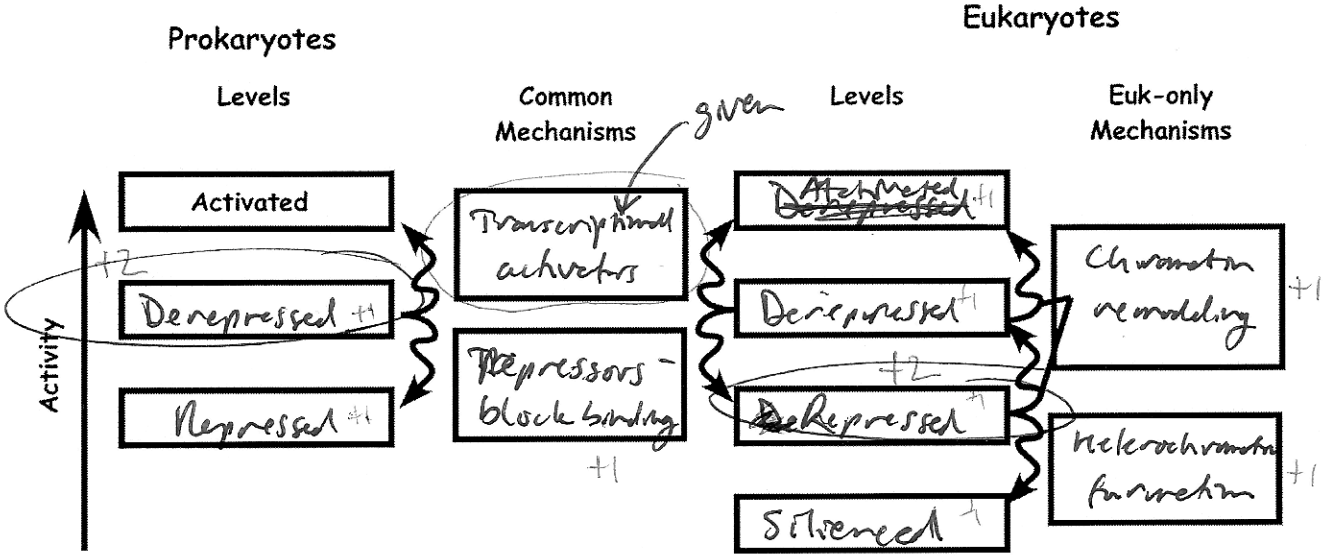
Score for page:_____

(b; 25 pts) We discussed the idea that prokaryotes and eukaryotes are fundamentally different in terms of the levels of regulation of transcription.

+1 each +6 [(*i*) Label the remaining two expression levels for prokaryotes and the four levels for eukaryotes.

+3 [(*ii*) Specify ~~how (in general) transitions~~ are made between levels (fill in the boxes ~~for~~ for mechanisms/factors).

+4 [(*iii*) Indicate which is the baseline state for each kingdom (circle one from column P, one from column E).



6 (*iv*) Explain the heuristics of the baseline states -- why should it be this way?

+3 each [
- Prokaryotes can afford to be and want to be leaky – they never know where they will be in 5 minutes.
- Eukaryotes rigorously repress proteins inappropriate for their cell type

6 (*v*) Explain how the different baseline state is enforced in eukaryotes.

- Chromatin is a repressor – some active process must reveal TF binding sites

Score for page:_____

(c; 11 pts) There are three fundamental mechanisms for chromatin remodeling. One is covalent modification of histones. Give a brief description of the histone code idea. Name one of the other fundamental mechanisms and briefly describe its function.

+4 - Multiple covalent modifications allow a synchronous signalling to other factors, e.g. bromodomains that bind Lys-~~Ac~~Ac

[+3 - ATPase remodeling machines -

Move nucleosomes around to reveal or conceal TF binding sites
+2 +2

[or = Exchange histone proteins to mark special areas like
+3 +2
DSB's or centromeres.
+2

(d; 10 pts) In general, what do AAA+ proteins do? Name one and give its particular function.

+4 | - They use the energy of ATP hydrolysis to cause conformational changes in other proteins

- NtrC burns ATP to alter conformation of σ^{54} and
+3 ~~used~~ catalyze RP_o formation. +3

2. (35 pts) Regulation by RNA

(a; 12 pts) We have described RNAi as likely descended from a sort of primordial immune system that defends against dangerous nucleic acids. What is the chemical nature of the
+2 dangerous nucleic acid ds RNA ? List two sources of this dangerous macromolecule. Describe one main advantage of using siRNA to knock down gene expression relative to a previously available method.

+3 - viruses

+3 - ~~trans~~ retrotransposons = selfish genes

+4 | You can knock down any gene, any time, as opposed to making a transgenic animal that always lacks the gene and is expensive/impossible to make.

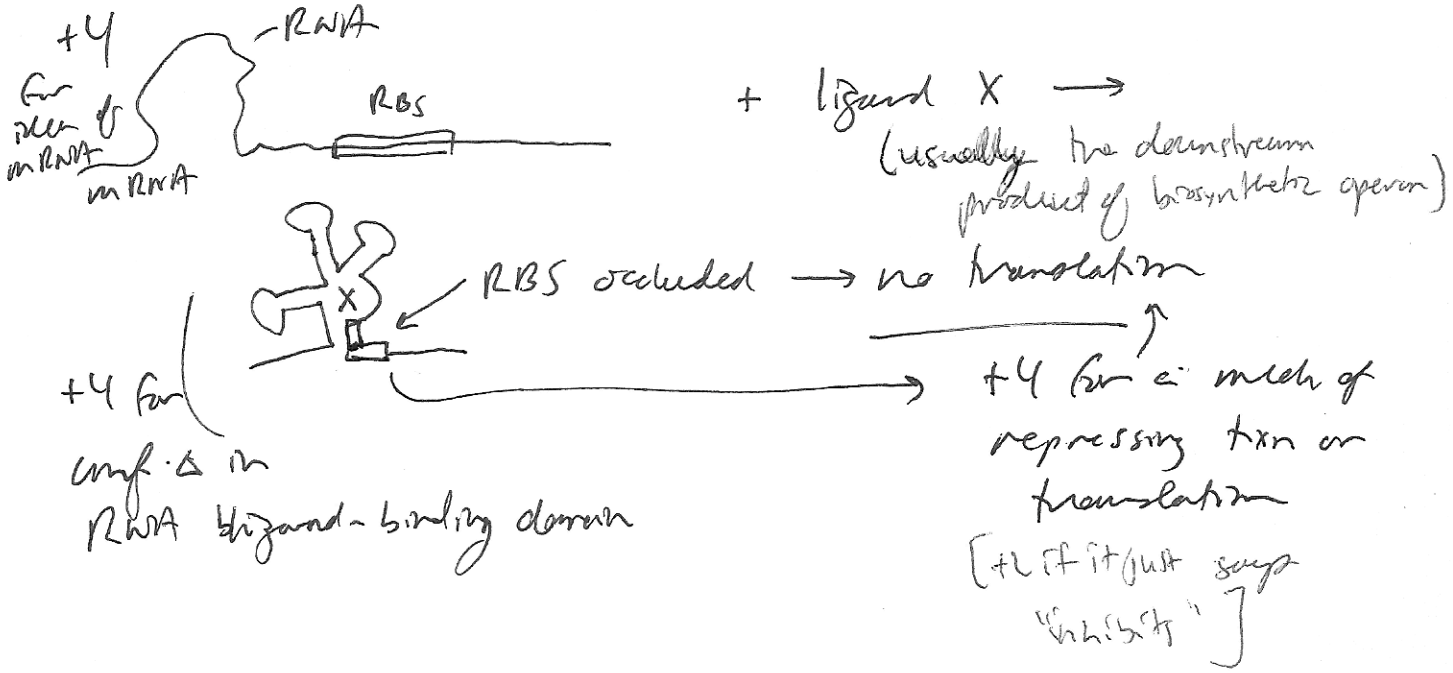
Score for page:_____

(b; 7 pts) Micro RNAs (miRNAs) are processed similarly to siRNA's, although they often cause translation inhibition rather than mRNA cleavage. Give a plausible scenario for the evolution of miRNA regulation from a primordial RNAi immune system, and why it might have prospered.

– Given that RNAi existed, transcription of a small RNA from a separate location could have caused translational silencing → this then developed into a regulated way of repressing target genes that did not rely on evolving new proteins. Can repress a variety of targets

+3 evolution
+4 current advantages

(c; 16 pts) Sketch ~~the~~ mechanism of ~~translational~~ repression in response to a small molecule ligand by a riboswitch. This is an example of a (fill in) negative feedback loop. +2
Consider the Trp repressor, which binds the promoter for Trp biosynthetic genes only when tryptophan is bound to the repressor, and the Lac repressor, which is induced by its ligand allolactose. Which one is part of the same kind of feedback loop: Trp (fill in). +2



Frequent full-credit answer:
Inhibition allows storage of mRNA for later rather than requiring re-synthesis of mRNA.

or – miRNA evolved as ~~exist~~ built-in antiviral machinery w/ endogenous transcripts –
(+5) ~~+3~~ ~~+4~~

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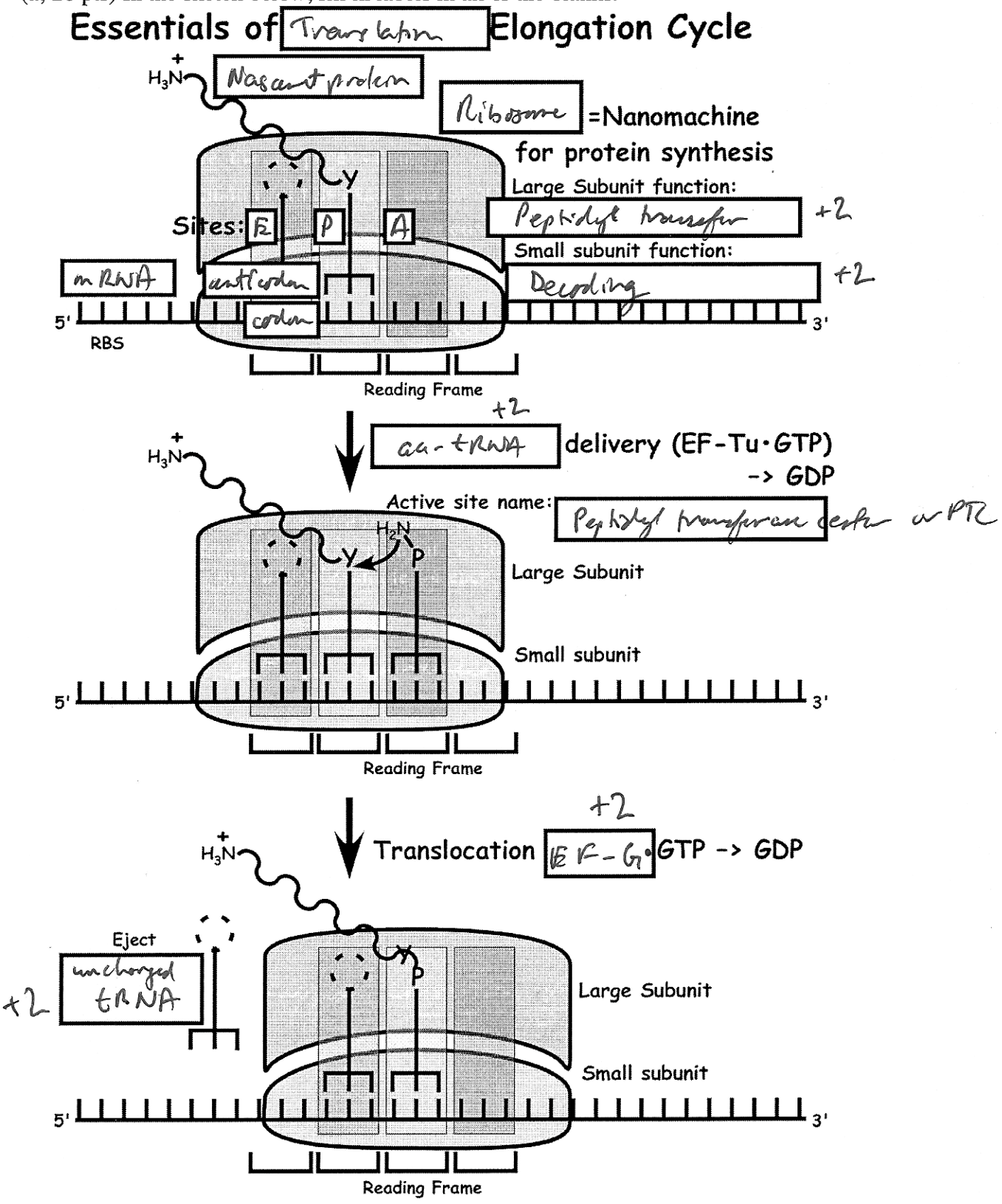
3. (72 pts) A process that is part of the central dogma

(a; 20 pts) In the sketch below, fill in labels in all of the blanks.



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(b; 10 pts) What are the functions of burning each of the two GTP's in the process above? How was GTP hydrolysis represented in the movie shown in class?

EF-Tu : increasing fidelity because EF-Tu•GDP acts as a kinetic proofreading mechanism
+4

EF-G : mechanical motion – translocation
+4

A guy in a red suit with a puff of smoke.
+2

(c; 12) What is the evidence that the ribosome is a ribozyme? Why has RNA catalysis by the ribosome been preserved when so many other presumed primordial catalysts have been replaced by protein? Why has RNA catalysis by the spliceosome been preserved? (Different answers)

+4 – The PTC is all RNA – no proteins are near active site [or – Noller deproteinization expt]

+4 – Replacing the ribosome is like changing your ~~car~~ distributor cap while driving – tends to be fatal – frozen accident

+4 – RNA is good at processes that require base pairing and large conformational changes.

Score for page:_____

(c; 15 pts) Amino-acyl tRNA synthetases are the guardians of the genetic code. They catalyze two sequential reactions. Write them out (names, not structures) and give the overall reaction. Fundamentally, why must they use ATP hydrolysis (why must the chemical step of protein synthesis be coupled to an exergonic reaction)? What sort of enzyme catalyzes the ~~reverse reaction?~~ of protein reaction that reverses protein synthesis?

+3 Amino acid + ATP → aa-AMP + PP_i

+3 tRNA + aa-AMP → aa-tRNA + AMP

+3 aa + tRNA + ATP → aa-tRNA + AMP + PP_i

+3 – peptide bond formation is thermodynamically unfavorable [+1 for "charged tRNA"]

+3 – proofreading

(d; 15 pts) What difficult and interesting problem do Amino-acyl tRNA synthetases face in maintaining the genetic code? List ~~four~~ ways in which the instructions in DNA could give rise to a protein with an incorrect ~~amino acid.~~ sequence.

should have emphasized correct DNA

+6 – The aaRS's must reject all other aa's and all other tRNA's. (+3 for reject; +3 for all other aa's and all other tRNA's)

+3 – Mistake in transcription

~~+3 – Mistake in RNA splicing~~ OK

+3 – Incorrect tRNA charging (+4/16 for two ways to do this)

+3 – Incorrect tRNA inserted by the ribosome.

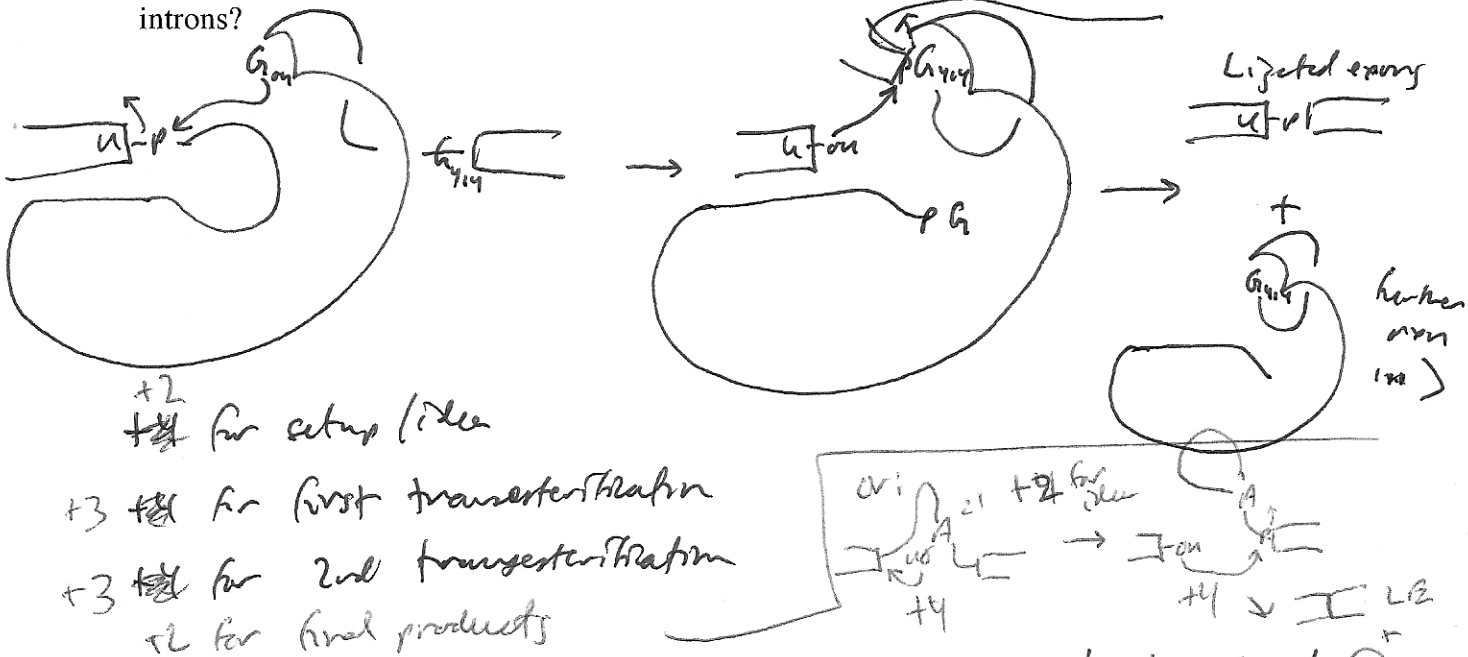
+3 – ribosomal frameshift +3 C→U after transcription

→ +4 total for 3 DNA changes

Score for page:_____

4. (33 pts) RNA Splicing and Processing

(a; 16 pts) Sketch the chemical steps for either Group I intron self-splicing or pre-mRNA splicing. Why do we believe that RNA splicing is descended from self-splicing group II introns?



6 The 2° secondary structure of the U snRNP's bound at splice junctions resembles the 2°S of self-splicing Group II introns that do the same chemistry. +3 +3

(b; 5 pts) We have described the evidence for a primordial RNA world. What complicated enzyme was needed for the transition to a DNA world? Why is DNA preferable as the genomic nucleic acid?

next page ↓

+3 – Ribonucleotide reductase rNDP → dNDP (+2 for reverse transcriptase, not the intended answer)

+2 – DNA is a lot more stable

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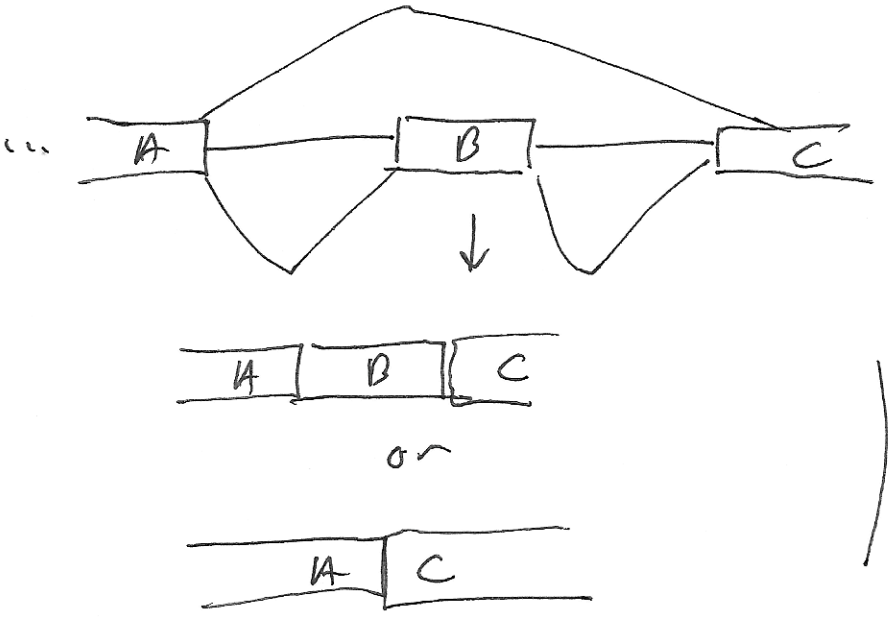
(c; 12 pts) Sketch a generic example of alternative RNA splicing ~~and~~ explain why alternative splicing is much more common in humans than in lower eukaryotes.

It is a relict to the human ego that



+4 for idea

+4 for products

– Alternative splicing allows a small number of genes to make a very large number of proteins. We have the same number of genes but a lot more proteins than lower eukes – we really are more complicated. Besides,

+2 they can't inflate their own self-importance.

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