Biochemistry 465 **Your Name:** _____

Biological Information Processing **Prof. Jason Kahn**

Final Exam (200 points total) **May 17, 2011**

You have 120 minutes for this exam.

Explanations should be concise and clear. I have given you more space than you should need.

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 exam 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. (8 pts) Mechanistically, why is the ground state of transcription in eukaryotes more repressed than it is in prokaryotes, and why does this make sense in terms of the cell biology?

(+4) – Chromatin is a repressor – eukaryotic transcription is much less leaky.

(+4) – To specify 214 cell types and to avoid uncontrolled growth, it is important to minimize inappropriate expression in eukaryotes

2. (4 pts) What does the CBP/p300 protein do?

(+4) Interfaces between transcription factors bound to DNA and the chromatin remodeling machinery. ↳like the enhanceosome and/or the basal transcription machinery

3. (10 pts) List two catalytic activities of TFIIH. Why is TFIIH dispensable for transcription of supercoiled templates?

- (+2) Kinase – phosphorylation (+2) of RNAP CTD
- (+2) Helicase – Hydrolyzes ATP to open up DNA (XPB, XPD)
- On a supercoiled template, relief of writhe provides a driving (+2) force for opening of the transcription bubble.

(+2)

Score for the page ARH

4. (12 pts) Describe the changes in kinetic partitioning between ~~near~~ cognate and ~~non~~ new-cognate tRNAs that describe the function of the EF-Tu GTPase as a molecular clock to increase the fidelity of translation? Interaction between the 30S subunit and EF-Tu•GTP stimulates the GTPase. Suggest a reason for this.

(+1) EF-Tu·GTP·tRNA bound to the ribosome hydrolyzes GTP (OK if implicit)

(+3) EF-Tu·GDP·tRNA is metastable, will dissociate irreversibly at k_{off}

(+4) Cognate: accomodation is faster $k_{acc} > k_{off}$ → most of the tRNA goes on
Near-cognate: " " slower $k_{acc} < k_{off}$ → most of it falls off

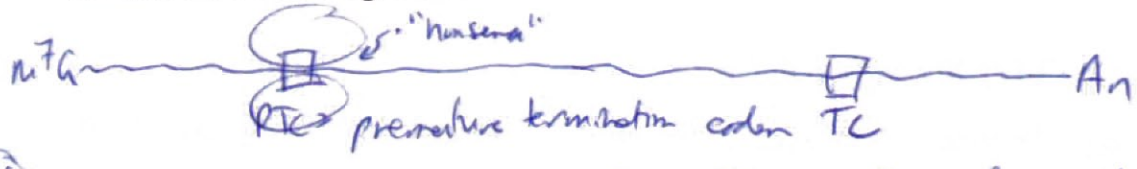
(+4) 30S stimulation ↑ speed of translation and ↓ indirectly ↓ wasted GTP hydrolyzed by free EF-Tu·GTP·tRNA

5. (6 pts) The components of translation include several examples of molecular mimicry. What does this term mean? Give one example.

(+3) – Two macromolecules of different sequences + 2° structure that have similar 3-D structure.

(+3) – EF-Tu·tRNA·GTP ↔ EF·G·GTP ↔ RF1 or 2 ↔ RRF
Any pair

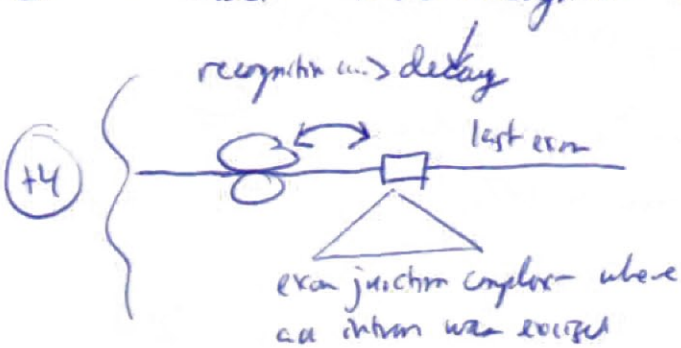
6. (18 pts) Briefly describe the fundamental idea of NMD and why it is a good idea. There are two models for PTC recognition. Name them both and draw a sketch for how one of them might work.

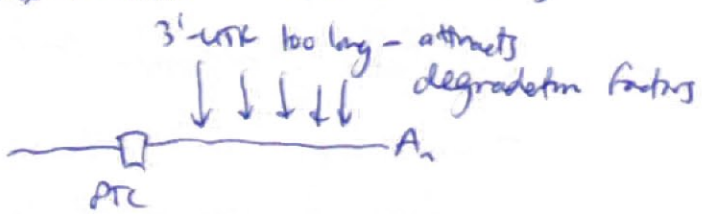


(+4) → If translation stops prematurely the mRNA is degraded – Nonsense-Mediated Decay

(+4) → Avoids production of possibly toxic (dysregulated or dominant ⊖) truncated proteins

(+3) – Model – ETC recognition or "faux 3'-UTR" (+3) (DSE OK too)





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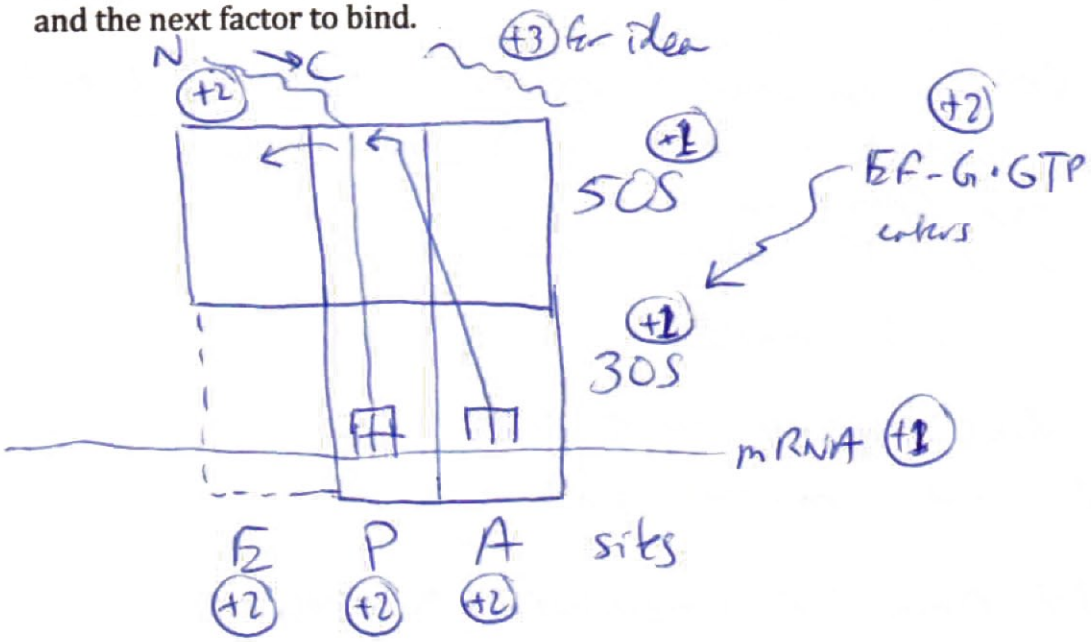
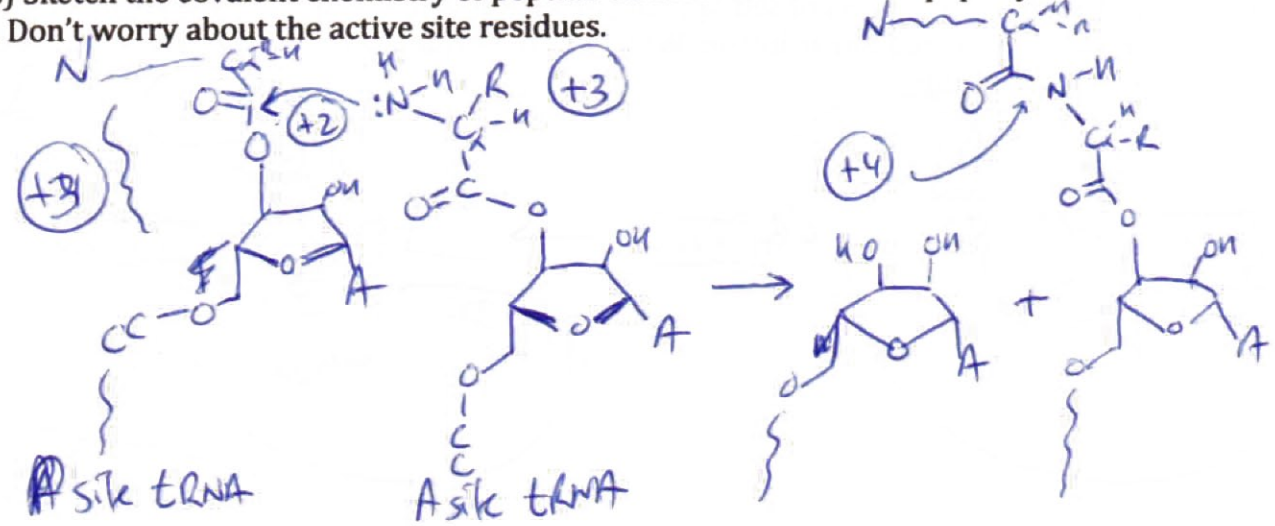
7. (16 pts) Sketch the hybrid states model for translation at the instant that peptidyl transfer occurs. Include the names of the sites, the positions of the two ribosomal subunits, two tRNAs, the mRNA, and the next factor to bind.



8. (12 pts) Sketch the covalent chemistry of peptide bond formation at the peptidyl transferase center. Don't worry about the active site residues.



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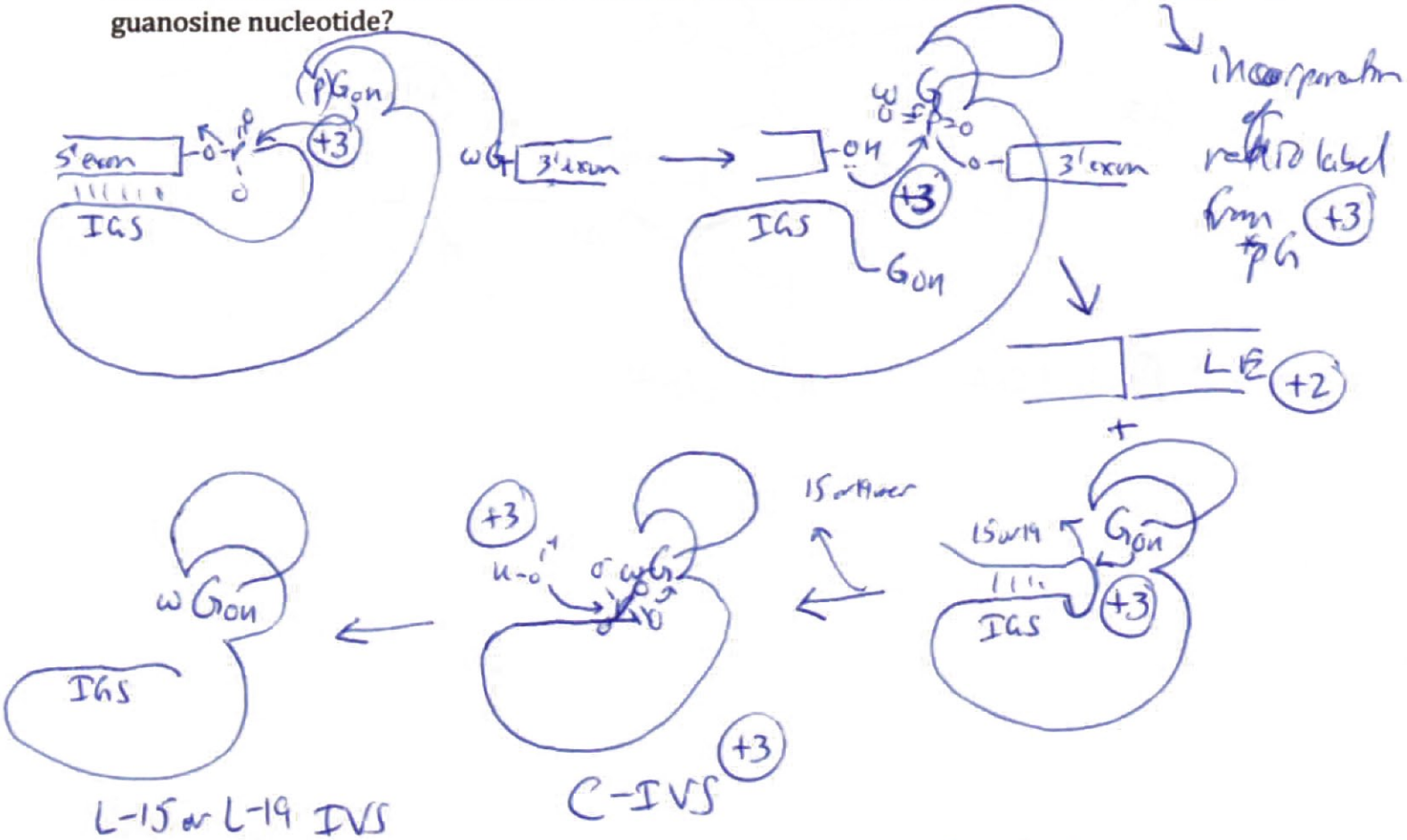
9. (16 pts) Why is RNA-catalyzed replication of an exogenous RNA template a Holy Grail for origin of life research? List two RNA enzymatic activities developed in the lab on the way to this goal. What is the thorniest problem facing the RNA world hypothesis for the origin of life?

(+4) – An RNA RNA replicase would be required in an RNA world and would be a substrate for launching Darwinian evolution.

(+4 each)
- An RNA-based RNA ligase
- Incorporation of NTP's into an RNA
- Processive extension of a template
- Extension of an exogenous template-primer
- Extension of a random sequence template

(+4) – Where did ribose (or ribonucleoside triphosphate) come from in the absence of metabolism?

10. (20 pts) Sketch the process of self-splicing Group I intron excision from an RNA, ending with the formation of truncated linear intron. What was the evidence for the involvement of exogenous guanosine nucleotide?



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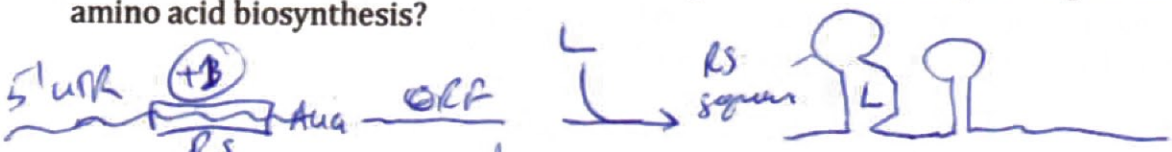
11. (12 pts) How can a riboswitch work as a negative feedback loop for regulation of pathways like amino acid biosynthesis?



- ribos with folds upon binding ligand and alters RNA 2° (+2) (+3)
- This can cause transcription termination by introducing a stem-loop that wouldn't otherwise form (+3)
- or it can stop translation eg. by blocking Shine-Delgarno sequence (+3)
- So the presence of Ligand shuts down machinery needed to make more

12. (15 pts) What is thought to be the evolutionary origin of RNA interference? List the two major complexes needed along the pathway of translation inhibition by a micro RNA and their functions.

- Defense mechanism (+2) to destroy ds RNA viruses (+3)
- Dicer (+3) - processes (+2) pre-miRNA to mature miRNA - 21nt duplex
- RISC - uses one strand to inhibit translation (+2) / destroy message (+3)

13. (6 pts) What chemical mechanism is common to ribozymes and DNA polymerases? Why does it make sense that a primordial RNA active site could have been readily replaced by a protein active site?

(+3) - 2 metals in mechanism

(+4) - It requires only exchange of a carboxylate (D or E) for a phosphate chelating active-site metals

Score for the page ARH

14. (16 pts) How could you use modENCODE data sets to look for a connection between regulated alternative splicing and chromatin modification? (What techniques would be used?) What might be the next step in discovering an actual mechanism?

- correlate ChIP results (+4) on chromatin states with RNA-Seq (+4) results on alternative splicing and look for patterns that are consistent in multiple (+3) stages / cell types.
- Then go in and try to find the (+3) bridging protein (on RNA) factors using X-linking, ~~co~~ co-IP, re-chip, siRNA knockdowns, genetics [(+1) for any one technique]

15. (12 pts) Give two pieces of evidence that pre-mRNA splicing evolved from Group II self-splicing RNA. Why is the U4 snRNP ejected before chemistry is done during the assembly and activity of the spliceosome? What DNA repair system is analogous?

(+3) - The chemistry is the same, based on the lariat linked at the 2'5'-A at the branch

(+3) - The secondary structure of the U snRNP's can be mapped onto the 2° of group II self-splicers

- The U4 is ejected to make a metastable state, so
(+3) the RNA nucleases only assembled at a bona fide splicing site

(+3) - Analogous to NER of UvrA delivering UvrB

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16. (10 pts) List connections (one each):

(+2) a) Between repair and recombination – repair of collapsed replication forks requires recombination – and DSB's

(+2) b) Between replication and recombination – replication needed to complete integration

↳ repair of collapsed replication forks, pol III replicase needed in MMR

(+2) c) Between transcription and translation → Not just the central dogma!

– Polarity of upstream nonsense codons, attenuation at Trp operon

(+2) d) Between transcription and DNA packaging

– RNAi → heterochromatin or heterochromatin ⊣ transcription (pos PEV = position effect variegation)

(+2) e) Between replication and repair

– always need DNAP for BER, NER, MER, etc. oops, repair of collapsed forks, P53 → halts replication and ↑ repair

17. (3 pts) What is the evidence from genomics that there is at least as much important non-coding sequence as there is coding sequence in the human genome?

(+3) – The non-coding DNA is just as conserved between organisms as the coding DNA. Could be nc RNA, or other RNA genes, or control sequences.

+2 for just saying what it is

18. (4 pts) List one topic that we covered in class that you found uninspiring, and one of which you would like to hear more.

(+2) each for any answers

Bad (Less or better): DNA topology ||||||||| ; persistence length || ; Molecular recombination techniques ; modENCODE ||||||||| ; RNA splicing || ; nucleosome structure ; Euk. txn regulation ; DNA/RNA structure ||| ; next gen sequencing ||| ; RNA processing ; Bioinformatics || ; Jmol ; molecular mimicry ; History of Nobel prizes || ; DNA replication ; RNA world || ; histone code ; NMD ; transcription + repair ; nearest neighbor rules ; sequencing ; DNA repair "assembly" ; cell cycle ||| ; intron mechanisms ; Z-DNA

Good (More): repair mechanisms ; protein-DNA recognition ; splicing ; RNA drugs ; translation ; transcription initiation mech + regul. ; bioinformatics ; thermodynamics of C.D. ; chromatin modification ; Reg of gene expression ; Future of biochemistry (current research) ; Transposons ; topology ; λ recombination ; Discovery timeline ; Next gen techniques ; NMD ; Regulation + ncRNA || ; Epigenetics |||| ; cell differentiation ; Kinetics + enzyme energetics ; SDM ; RNAi ||||||||| ; DNA repair ; cell cycle ||| ; modENCODE ||| ; next gen sequencing || ; Translore ; Topology ; narrow achieves ; complicated figures ; RNA world |||||| ; 2 metal ion mech ; gene regulation

Page	Score
1	/22
2	/36
3	/28
4	/36
5	/33
6	/28
7	/17
8	—
Total	/200

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