

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?

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

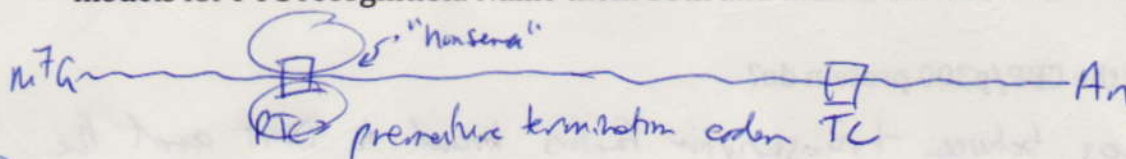
4. (12 pts) Describe the changes in kinetic partitioning between ~~near~~^{cognate} and ~~non~~^{near}-cognate tRNAs that describe the function of the EF-Tu GTPase as a molecular clock to increase the fidelity of translation? Interaction between the 30S subunit and EF-Tu•GTP stimulates the GTPase. Suggest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

- (+1) EF-Tu•GTP•tRNA bound to the ribosome hydrolyzes GTP (OK if mylicit)
- (+3) EF-Tu•GDP•tRNA is metastable, will dissociate irreversibly at k_{off}
- (+4) { Cognate: accommodation is faster $k_{acc} > k_{off} \rightarrow$ most of the tRNA goes on
Near-cognate: " " slower $k_{acc} < k_{off} \rightarrow$ most of it falls off
- (+4) { 30S stimulation \uparrow speed of translation and \uparrow delivery \downarrow wasted GTP hydrolyzed by free EF-Tu•GTP•tRNA

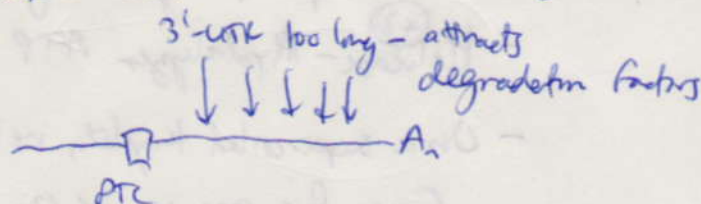
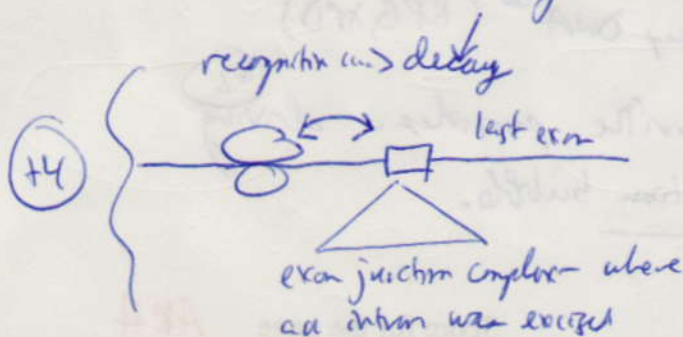
5. (6 pts) The components of translation include several examples of molecular mimicry. What does this term mean? Give one example.

- (+3) - Two macromolecules of different sequences + 2nd structure that have similar 3-D structure.
- (+3) - EF-Tu•tRNA•GTP \leftrightarrow EF•G•GTP \leftrightarrow RF1 or 2 \leftrightarrow RRF
Any pair

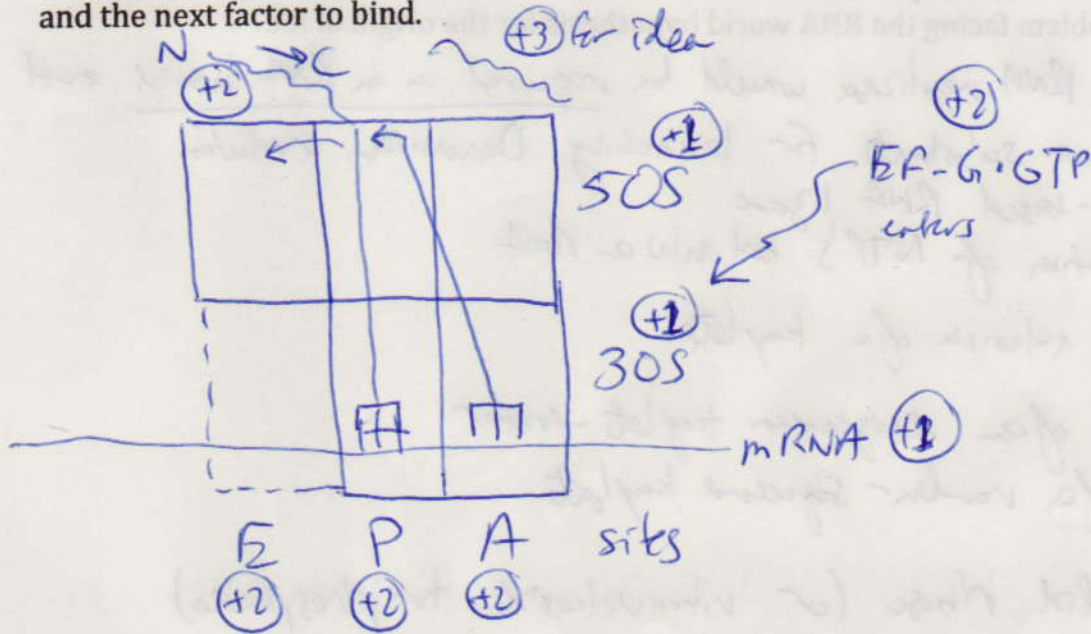
6. (18 pts) Briefly describe the fundamental idea of NMD and why it is a good idea. There are two models for PTC recognition. Name them both and draw a sketch for how one of them might work.



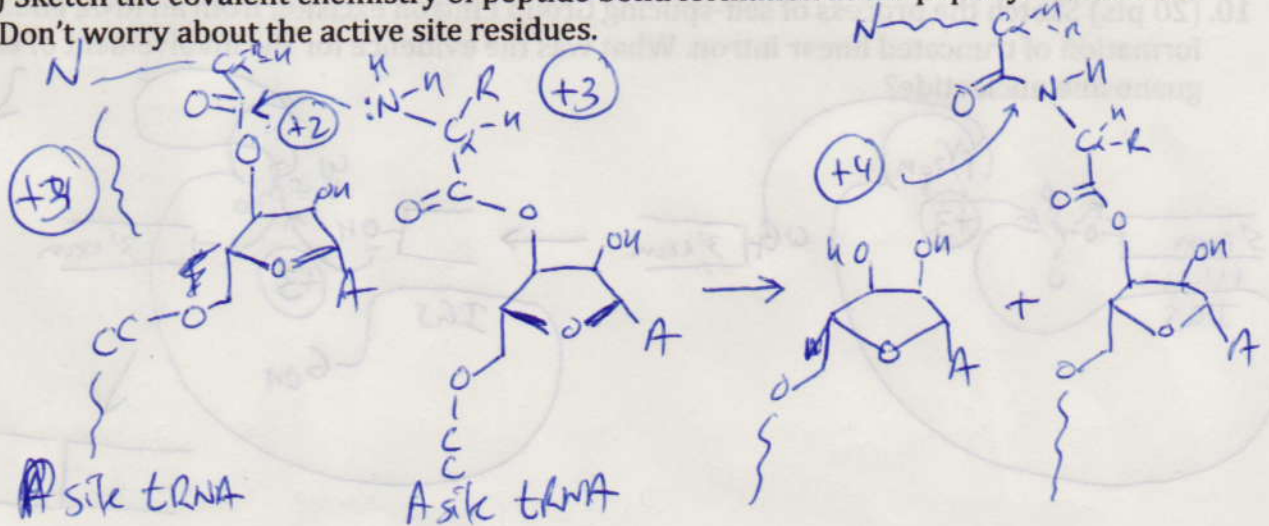
- (+4) \rightarrow If translation stops prematurely the mRNA is degraded - Nonsense-Mediated Decay
- (+4) \rightarrow Avoids production of possibly toxic (dysregulated or dominant \ominus) truncated proteins
- (+3) - Model - EJC recognition or "faux 3'-UTR" (DSB OK too)



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.



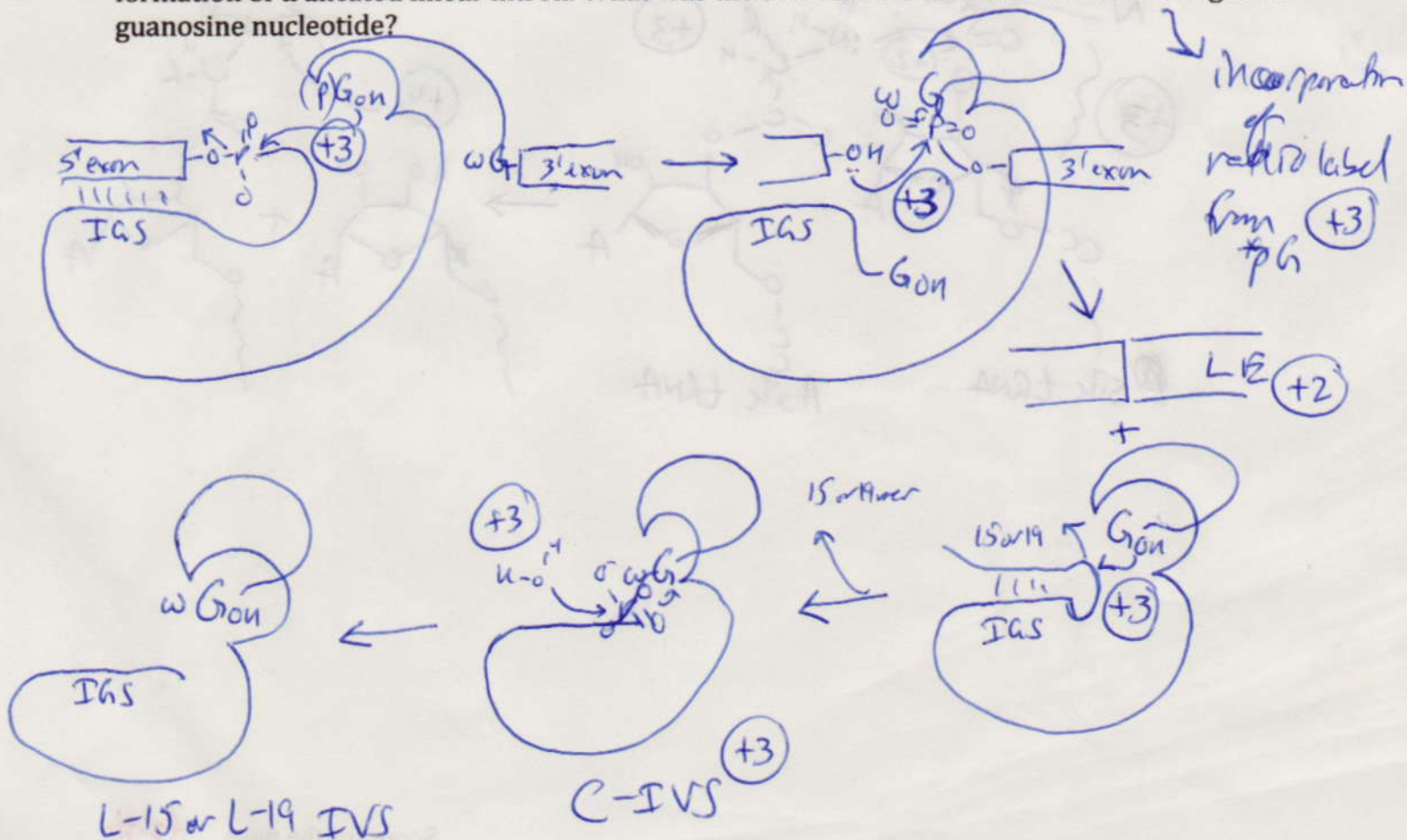
8. (12 pts) Sketch the covalent chemistry of peptide bond formation at the peptidyl transferase center. Don't worry about the active site residues.



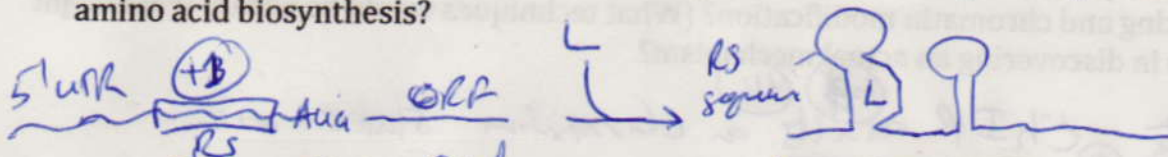
9. (16 pts) Why is RNA-catalyzed replication of an exogenous RNA template a Holy Grail for origin of life research? List two RNA enzymatic activities developed in the lab on the way to this goal. What is the thorniest problem facing the RNA world hypothesis for the origin of life?

- (+4) - An RNA RNA replase 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?



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



- riboswitch folds upon binding ligand and alters RNA 2°
- This can cause transcription termination by introducing a stem-loop then wouldn't else work from
- Or it can stop translation eg. by blocking Shine-Dalgarno sequence
- 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.

- Defence mechanism to destroy dsRNA viruses
- Dicer - processes pre-miRNA to mature miRNA - 21nt duplex
- RISC - uses one strand to inhibit translation / destroy message

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?

- 2 metal ion mechanism
- It requires only exchange of a carboxylate (D or E) for a phosphate chelating active-site metals

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 ⁽⁺⁴⁾ ~~ChIP~~ ⁽⁺⁴⁾ results on chromatin states with ⁽⁺⁴⁾ ~~RNA-seq~~ ⁽⁺³⁾ results on alternative splicing and look for patterns that are consistent in multiple stages / cell types.
- Then go in and try to find the ⁽⁺³⁾ bridging protein (on RNA) factors using X-linking, ~~for~~ co-IP, re-chip, siRNA knockdowns, genetics ⁽⁺²⁾ [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^o of group II self-splicers
 - The U4 is ejected to make a metastable state, so
- ⁽⁺³⁾ the RNA nucleus is only assembled at a bona fide splicing site
- ⁽⁺³⁾ - Analogous to NER w/ UVR A delivering Uvr B

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 reverse needed in MMR
- (+2) c) Between transcription and translation - Polarity of upstream non-coding regions, attenuation at Trp operon
- (+2) d) Between transcription and DNA packaging - RNAi → heterochromatin or heterochromatin → transcription (po P/EV2 position effect variegation)
- (+2) e) Between replication and repair - always need DNAP for BER, NER, MMR, etc. ↳ oops, repair of collapsed forks, p53 → halt 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, 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.

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