**Biochemistry 674, Final Exam** **Your Name:**_____

**December 15, 2001** **Prof. Jason D. Kahn**

You have 120 minutes for this exam, which is worth 150 points. Thus you get about the same "points per minute" as for the 80 minute exams.

Explanations should be concise.

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

There will be no formal viewing, but you are welcome to come by after grades have been submitted to see the exam.

Final grades will be available only through MARS. I will send an email to the reflector the moment the grades are submitted, hopefully on Monday.

1. (20 pts) Briefly describe two examples we discussed of proofreading in biological information processing. For one case, specify how energy is dissipated in the process. Why is this necessary? In other words, why does improved fidelity require either that a checking reaction per se or else the step between two checking steps be irreversible?

2. (15 pts) Briefly describe the histone code hypothesis. Histone hyperacetylation has been suggested to alter DNA topology but not the local structure of the nucleosome. How can this be rationalized?

3. (20 pts) RNA can be engineered to do many wonderful things. For example, the system below has been engineered to provide RNA molecules sensitive to allosteric activation by small molecules. The top molecule's left half binds FMN, the right half is a self-cleaving hammerhead ribozyme, whose activity (cleavage at the indicated site) depends on the integrity of all three stems. It cleaves only in the presence of FMN. Note: you do not need to know anything about RNA catalysis for this problem except for what I have just told you.

(a; 5 pts) What might be the origin of FMN-activated self-cleavage of the ribozyme? In other words, how might FMN binding turn on activity?

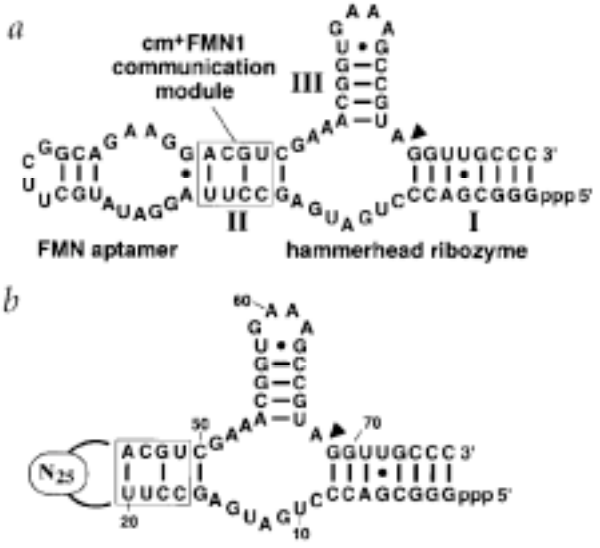


**Fig. 1** The tripartite design for allosteric ribozyme construction. ***a***, Sequence and secondary structure for an FMN-sensitive allosteric ribozyme[17]. In this construct, the cm⁺FMN1 communication module (boxed) separates the ribozyme and aptamer domains. This communication module (cm) is the first sequence class (1) that was previously identified to undergo allosteric activation (•) in the presence of flavin mononucleotide (FMN). Base-paired elements that are required for hammerhead ribozyme activity (I, II and III) are labeled according to Hertel and coworkers[30]. An arrowhead identifies the site of hammerhead-mediated cleavage. ***b***, A tripartite construct carrying a randomized aptamer domain used as the pool to initiate *in vitro* selection. $N_{25}$ represents 25 nucleotides with random base identity.

(b; 15 pts) The system can be repurposed as a general sensor using the pool on the bottom of the figure on the previous page. Sketch a selection protocol which would enable the isolation of RNAs whose self-cleavage is dependent on the binding of cAMP.

4. (10 pts) What is promoter escape in prokaryotic transcription? Why is it hard to leave the promoter? How does promoter escape occur in eukaryotes?

5. (15 pts) Diagram the eukaryotic cell cycle. Briefly describe, in general terms, how the unidirectionality and the continued progression of the cell cycle are assured, and give one specific example.

6. (15 pts) Sketch the nucleosome, indicating the locations of the $(H3/H4)_2$ tetramer and the two (H2A/H2B) dimers. Explain why a rotationally positioned nucleosome also tends to be a stable nucleosome, relative to a nucleosome formed on a non-positioned DNA.

7. (15 pts) Draw a G-C base pair and label the H-bonding specificity determinants in the major and minor grooves. Why do sequence-specific DNA binding proteins tend to bind in the major groove? TBP is a marked exception. What is the basis of TBP's ability to recognize TATA box sequences (one word would do)?

8. (20 pts) Sketch the Pol III replication fork at the end of the synthesis of an Okazaki fragment. Include RNA, DNA, Pol III core, SSB, primase, helicase, tau complex, clamp loader, and sliding clamps. What was the rationale offered for the existence of dimeric Pol III?

9. (20 pts) How does *E. coli* delay the progression of its cell cycle so that initiation occurs once and only once per cell division? Hemimethylation is a part of this process – where else have we seen a role for this? Speculate on likely phenotypes for *E. coli* lacking the dam methylase responsible for GATC methylation.

Do Not Write Below This Line

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|---|---|---|---|
| Score: | 1: | _____ | out of 20: Replication/Repair |
| | 2: | _____ | out of 15: Histone code |
| | 3: | _____ | out of 20: RNA/selex |
| | 4: | _____ | out of 10: Promoter escape |
| | 5: | _____ | out of 15: Cell cyle |
| | 6: | _____ | out of 15: Nucleosome structure |
| | 7: | _____ | out of 15: Protein-DNA recognition |
| | 8: | _____ | out of 20: Replication fork |
| | 9: | _____ | out of 20: DnaA, sequestration |
| | **Total:** | _____ | **out of 150** |