Problem 1. There are many programs and sites available for folding RNA into what the computer believes to be the most stable structure. This is harder than it might seem, perhaps just as hard as ab initio protein folding, because no RNA (not even tRNA!) is just a bunch of perfect stems connected by short single stranded loops.

Get in the mood by (re)watching) the "RNA folding movie.mp4" with the volume all the way up.

Now take the E. coli asparagine tRNA sequence 5’ UCCUCUGUAGUUCAGUGGUAGAAC GGCAGGCAGCAGCGAUAGUUGUCGAGGUGACGAGGAGGAGCCA 3’ and fold it into a secondary structure both manually and in silico. For the manual route, you assume it forms a cloverleaf much like other tRNAs, and try to maximize the number of G:C and A:U base pairs. The manual approach takes almost forever, but it works, and you get a nice textbook secondary structure because you already knew pretty much what tRNA looks like. For the in silico route, you Google for servers and programs, and finally settle on the old but friendly "Mfold" program of Zuker using the default settings:

http://mfold.rit.albany.edu/?q=mfold/RNA-Folding-Form

After thinking for a microsecond or two, the computer declares three structures to be its best guesses. You can download the structures in jpg, png, and pdf formats; one structure is shown below on the left here. The secondary structure of human tRNA valine is shown on the right. What did the computer get right? What did it get wrong? List and explain several reasons why a structure as "simple" as tRNA might be so hard for a computer to predict.
**Problem 2.** Give 3 reasons why the ribosome is a ribozyme. For extra credit, give a 4th reason. If you know 3 or 4 reasons, but still do not like the idea, give a few reasons why you are not a fan or believer. You will not be labeled a heretic, nor will your grade suffer; indeed, your grade may improve if you make a good point. Science thrives on disagreement; without it, our critical faculties would begin to atrophy.

**Problem 3.** The two hepatitis delta virus ribozymes (one on each strand) cleave the RNA chains leaving a 2',3' cyclic phosphate [Ferré-D'Amaré et al. (1998) Crystal structure of a hepatitis delta virus ribozyme. Nature 395, 567-574] just like the tobacco ringspot satellite virus ribozymes [Rupert et al. (2002) Transition state stabilization by a catalytic RNA. Science 298, 1421-1424]. Explain how the reaction mechanism of these ribozymes enables the circular genomes to replicate while making only minimal use of host enzymes, thus increasing the host range of the infectious genomes.

**Justification:** This problem set is not about evolutionary speculation, it is about the value of evolutionary thinking as a useful perspective on the biology, structure, and function of molecules as well as organisms. The evolutionary questions help to frame the facts we know, and you cannot answer these questions unless your grasp and understanding of the facts goes way beyond memorization.