Problem Set: DNA/RNA

Some exam-like questions....

1. Rich remarks that tRNA denatures upon heating, but “snaps back” into the native conformation upon cooling.

(a) Propose a method for demonstrating the denaturation of a tRNA molecule.

The simplest method is to measure the UV absorption as a function of temperature. As in the case of double stranded DNA, denaturation is revealed as an increase in absorbance at 260 nm.

(b) Using the method described in (a), describe the results you would expect if the “snap back” renaturation that Rich mentions is true.

As the tRNA sample is cooled, its absorbance should fall, and, in particular, should more or less retrace the path it took when the sample was heated.

(c) Predict the effect of increasing the salt concentration in the buffer on the denaturation observed as in (a).

Increasing concentrations of ions should result in increased interaction of cations with the phosphate backbones of the double helical regions, decreasing the repulsion forces and increasing the temperature required to get denaturation.

(d) Nowadays, we can isolate the gene for a particular tRNA, and compare the denaturation and renaturation of the tRNA with the denaturation and renaturation of a double-stranded DNA form of the tRNA gene. When this is done, the two are found to be different in small, but reproducible ways. Describe three molecular differences between the two that could give rise to these differences.

(1) The tRNA will have some bases which are not stacked because they are saturated or in loops, and these will result in a quantitative difference in the absorbance change. (2) Some bases in the tRNA are modified (non-canonical) bases which could affect both the temperature of denaturation, and the absorbance change that results. (3) tRNA complementary sequences are tethered together, so that they will renature faster than the corresponding DNA sequences, which must find each other in a second order reaction. (4) tRNA refolds as mostly A form helices, while the DNA will renature as a B form helix. (5) Some bases in the tRNA form non-Watson/Crick base pairs, which could affect the temperature of denaturation.

2. Consult the paper by Ban et al on the structure of the large ribosomal subunit to answer the following questions.
(a) In addition to the primary large rRNA molecule, the large subunit contains a smaller RNA molecule, called 5S RNA. The secondary structure map of this RNA is given in Figure 4D, and the 3D structure is shown in cartoon form in Figure 4L at the bottom of p. 913. Remarkably, this molecule, although it is found in large ribosomal subunits from all organisms examined, is not highly conserved in sequence. Explain why not, and predict where you would expect to find conserved residues, if any are present at all.

The sequence is not highly conserved because most of the RNA is in the form of double-stranded stems, whose sequence is not very important so long as the bases in the sequence are complementary. Most of the conserved residues are likely to be present in the loops.

(b) Is it reasonable to describe the 5S RNA as a domain? Why or why not?

Yes. The loop at the end of the stem made from the 5' and 3' ends is complex - there are stems and loops which originate in that loop.

(c) Speaking of being conserved, it was remarked in lecture that the amino acids of the tail portions of ribosomal proteins are often more conserved than the amino acids in the globular portion of the molecules. Why is this fact surprising, and what does it say about the function of ribosomal proteins.

The fact is surprising because conserved amino acids are usually important because they are necessary either for their contribution to a catalytic function or, in proteins that are not enzymes, for their contribution to stabilizing a protein fold. The latter function is only possible in the globular portion of the ribosomal proteins. This fact says that the structure of the globular portions of the ribosomal proteins are not very important, and that the interactions of the “structureless” tails with RNA is the most important function of the proteins.

(d) In an accompanying paper, the Yale group remarks that the exit tunnel for the growing polypeptide chain is largely hydrophilic and water filled. Could an RNA-based structure have had any other character? Explain.

While the tunnel must be filled with water if it’s not filled with protein, the lining of the tunnel need not be hydrophilic. RNA has both hydrophilic (sugar -OH and other H-bonding substituents) and hydrophobic (nucleotide base surfaces) characters. If the tunnel had been lined with the latter, it would have been reasonably hydrophobic.