Hunt the helix

from E. G. Richards

NO sooner was the first primary structure of transfer RNA worked out, than the clover leaf model for its secondary structure was proposed. This has become an article of faith for molecular biologists and all of the 60 sequences now available conform to it. When the sequence of 5S RNA from *Escherichia coli* was published in 1967, several authors leapt to the challenge and published models for its secondary structure although computer investigations suggested that there were several million possibilities. The favourite tack today is to devise experiments that eliminate at least some of these. Kearns and Wong (*J. molec. Biol.*, **87**, 755-774; 1974) have attempted to go one better and deduce a unique structure from the application of the nuclear magnetic resonance (NMR) method which they and others have so successfully applied to tRNA structure.

Resonances in the range 12-15 p.p.m. arise from hydrogen bonded ring N-H protons from U or G so that the number of resonances gives the number of AU and GC pairs—at least it does if GU pairs are ignored since this pair has two H-bonded ring N-H protons, but this possibility is glossed over. Kearns and Wong find that at 35°C there are 28 ± 3 base pairs on this basis of which but 4 are AU. As the temperature is increased about 10 resonances have melted out at 52°C to leave a 'core' structure which is stable to 62°C. By 73°C a further 9 or so have gone to leave about 9 base pairs. This maximal figure of 28 pairs is at variance with estimations obtained from the analysis of various optical properties which are in the range 38-44 pairs. These optical methods are fraught with assumptions that are difficult to test adequately and it is reasonable to accept Kearns and Wong's result as being the more reliable.

Kearns and Wong have attempted to delineate the precise base pairing scheme involved. To do this they have employed explicit semi-empirical rules for calculating the precise shift expected of an AU or a GC base pair in a variety of different helical environments defined by its nearest neighbours. They thus show that the structure left at 73°C gives a spectrum in close agreement with that expected of the longest and presumably most stable helical region that it is possible to form from the sequence. They have gone on to list all possible helical regions in order of length and GC content and worked out the position of the resonances of each. They thus show that the 'core' present at 52°C could contain the two most stable helices, H₁

and H₂. If these two are present, H₃-H₁₀ are automatically excluded since they each involve residues in common with the first two; moreover, H₆-H₁₀ give resonances that are inconsistent with the 52°C spectrum. The next most stable contender is H₁₁ and the spectrum comprised of the H₁, H₂ and H₁₁ contributions is in nice agreement with the 52°C result and they conclude that the core contains these regions. This core structure is of some interest in that it is essentially three dimensional unlike most proposals for RNA secondary structure which can be neatly drawn on a flat page of a journal without the chain crossing itself—a restriction that would seem to be purely aesthetic in origin.

The helices comprising the core rule out all but 12 of the remaining possible helices and calculation of the spectra of these eliminates all but 6 from further consideration. Not all combinations of these 6 are possible but the NMR data do not allow further progress. Kearns and Wong thus invoke the results of oligonucleotide binding experiments that imply that certain regions of the sequence are non-helical. This serves to eliminate all but H₁₇ and H₇₉. They thus provisionally conclude that the full low temperature structure contains the five helical regions H₁, H₂, H₁₁, H₁₇, and H₇₉ giving a total of 28 base pairs of which 11 are AU. The discrepancy in the number of AU pairs is perhaps not too serious in that the partition of the low temperature spectrum into AU and GC regions is subject to error. The question may be raised as to whether this structure corresponds to the native or denatured forms of the molecule; Kearns and Wong claim that their results are independent of the nature of the solvent and the mode of preparation suggests that at least some of their solutions contained a mixture of both forms. It may be that the two forms are not distinguished by their technique even though it is almost certain that they have different base pairing schemes.

A further question is whether their final model is compatible with other available experimental data which they do not invoke. The results of limited nuclease attack have been interpreted to imply that certain pairs of regions of the structure are not hydrogen bonded to each other but, alas, it can be readily shown that Kearns and Wong's model is incompatible with these interpretations. Maybe these assumptions are at fault or maybe Kearns and Wong were too simplistic in ignoring GU pairs, looped out bases and other possible structural complications. Enthusiasts for these sorts of games await further developments with eager anticipation.

Solid state test tubes

from our Solid State Physics Correspondent

MANY solid state scientists have wondered whether the particular physical system with which they work (possibly a semiconductor surface or a glassy matrix) might be so sensitive to the activity of added chemicals that they could identify the chemical precisely by means of a simple electrical measurement on that solid. The result would be a novel way of following reactions occurring in or on a solid—a sort of solid state test tube.

One example of the fulfilment of such a wish has been developing over the last few years. Giaever discovered in 1960 that the I-V characteristic of metal-insulator-metal (MIM) sandwiches was controlled by tunnelling if the insulator was only 20 Å thick. He realised that the non-ohmic behaviour of the I-V characteristic happened because of the transitions of the tunnelling electron from an energy level in one metal to a different level in the other and that energy-band information—for example the size of a superconducting energy gaps—could be extracted from these I-V characteristics. In fact, the I-V curve not only contained band information but also showed fine structure arising from phonon transitions and other vibrational processes (Jaklevic and Lambe, *Phys. Rev. Lett.* **17**, 1139; 1966). In

Pb-Al₂O₃-Al sandwiches an Al phonon transition can be identified and, if water is present, then the effect of OH bond vibrations are seen. The reason is that the tunnelling electron, driven by the applied field, can either make a loss-free transition to a state of the same energy on the opposite side of the barrier (E_1 in the figure) or it can make a transition to a lower level, E_2 , provided it can lose exactly the right amount of energy ($\hbar\omega_0$ in the figure). This energy can be transmitted to the O-H group by inelastic transfer and the transition provides an increase in the 'inelastic tunnelling current' at a potential corresponding to $\hbar\omega_0$.

It must then have occurred to Lambe