NUCLEIC ACIDS

Genes Made Manifest

from our Molecular Biology Correspondent

ONE of the prettiest techniques to bubble up from the cauldron of molecular biology in recent years is Inman's denaturation mapping of DNA. The DNA is brought to a precisely defined temperature, or *p*H, at some point within its narrow melting range. Formaldehyde is introduced to prop open the melted parts of the chain, and a population of molecules so treated is examined in the electron microscope. The denatured regions are then indexed along the chain for a sufficient number of molecules on the grid, and correspond to tracts of sequence relatively lower in (G+C) than the rest. An interesting new application for this approach has now been found by Wensink and Brown (*J. Mol. Biol.*, **60**, 235; 1971), namely the identification of a gene, using its characteristic denaturation map as a fingerprint.

It is known that the DNA corresponding in eukaryotic cells to the ribosomal RNA is present in the form of many clustered copies, apparently interspersed by spacer segments, much richer in (G+C). Moreover this ribosomal DNA is separately amplified, and occurs as an extra-chromosomal component, differing apparently only in that none of its cytosine residues is methylated. Such a DNA, when subjected to the denaturation mapping technique, displays in the electron micrographs an immediately apparent periodicity, repeating up to fifteen times in one molecule. To ensure, however, that this effect is no trick of the eye or mind, Wensink and Brown have devised a means of testing for the presence of a periodicity and determining its precision and spacing. The measured distribution is matched against a Fourier function, the wavelength of which is varied until a correlation appears and is maximized. A sharp peak comes up at a periodicity of $5.4 \pm 0.4\mu$, whereas a denaturation map of T2 phage DNA shows only a flat background. From a calibration of the length measurement against T7 DNA, as a molecular weight standard, the repeating unit turns out to correspond to a molecular weight of some 8.7×10^6 which is the same as the molecular weight determined by Dawid and his colleagues of 9×10^6.

The repeating unit consists of a rather readily denaturable region, and another, somewhat shorter and much more resistant to melting, which may therefore be surmised to be richer in (G+C). This is exactly what would be expected from the size and composition assigned to the spacer region between successive copies of the ribosomal RNA gene. A comparison with Inman's results on λ phage DNA, which can be divided into two halves differing by 20 per cent in their (G+C) content, indicates that there is a similar difference between the ribosomal RNA tract and the spacer.

This is a very satisfactory result, but there is still more information in the denaturation maps, for the map of the low-melting segment contains a highly reproducible fine structure, which persists over a wide range of melting. Dividing the repeating unit into 100 elements, a histogram of the fraction of chains in which each element is melted shows a relatively broad region of melting, followed by a trough, corresponding to a much more resistant sequence, then another, narrower peak, followed in turn by a narrow and extremely resistant minimum, and then a mirror image of the first pair of separated peaks. The pattern is thus symmetrical about a short, highly (G+C)-rich segment on which the patterns are most readily indexed. This whole pattern corresponds to the 5×10^6 molecular weight of the 40S ribosomal RNA precursor, which contains the 28S and 18S chains of the large and the small subunit. In conversion of the precursor to these products a small portion of the chain is lost. This portion is believed to be rather rich in (G+C), and may be presumptively identified with one of the two shallower troughs in the denaturation map. This latter must therefore separate the 28S and 18S parts of the chain. The short very stable region can also be accounted for, because partial enzymatic degradation of 28S ribosomal RNA from other animal cells has led to the isolation of a sizable fragment (200,000 molecular weight) containing no less than 80 per cent (G+C). Wensink and Brown have compared finally the denaturation maps of the chromosomal and amplified ribosomal RNA, and found them to be identical.

Lord Kelvin's criterion that only that which can be measured and expressed in figures is science, can also be applied to another interesting essay in electron microscopy. Freifelder (*ibid.*, 401) has given experimental proof to the proposition that intercalative binding in DNA causes a change in the pitch of the double helix with a consequent increase in length. Using monodisperse phage DNA he has measured the length distribution of the molecules as a function of the concentration of added ethidium bromide. The standard deviation of measured lengths in each binding state is only 3 per cent, and the increase in length with ligand concentration reaches a sharp saturation level. At this point the chain has stretched by some 27 per cent. Moreover, the molecules look quite different: instead of a rather crinkly thread, broadly curved filaments are seen. Circular DNA–dye complexes have the appearance of smooth ellipses. It seems then that the hydrodynamic stiffness of the molecule must change on intercalation, so that direct inferences about the length from viscosity or sedimentation measurements are not in fact justified.

Locating a Quasar

OUTSIDE the circle of observational astronomy it sometimes comes as a surprise to learn that the exact positions of celestial objects are not always known, and in particular that there is considerable difficulty in relating positions measured by optical and radio techniques. A little thought, however, makes it seem more remarkable that identifications at optical and radio frequencies can be compared at all in some cases, at least to the accuracy required before theories can be built upon the observations. Even optical positions can only be measured relative to certain reference stars, and at radio frequencies sources can only be pinned down by a technique such as occultation, or, most commonly, by finding an optical counterpart to the source. The embarrassing aspect of the second technique is that it is less than adequate when the object of the exercise is to determine whether an optical source and a radio object are one and the same.

A classic example of the success of the occultation technique was provided by the identification of the source 3C 273, the first known quasar, by timing the eclipse of the radio source by the Moon. But it has proved impossible, until now, to state unequivocally that the small component 3C 273B really is coincident with the associated QSO, because of a difference of 0.7 arcsec between the best optical and radio positions published. Dr C. Hazard and his colleagues at the University of Cambridge and the Royal Greenwich Observatory have rectified this situation, and publish in next week's *Nature Physical Science* positions which confirm the association of the radio and optical objects to within 0.3 arcsec. Unlike the earlier recorded difference (0.7 arcsec) this figure is well within the limits of experimental error. Indeed, the best of the lunar occultation observations gives a radio source position exactly at the mean of the optical positions. Because of the differing reference systems, however, such good agreement is not to be taken at face value. There is no reason to prefer either the radio position or the optical position, so that the best position now available for 3C 273B should be taken as the average of the two positions determined by this work.