MITOCHONDRIAL DNA

Arrested Replication?

from our Cell Biology Correspondent

IF the exquisite experiments—for once that is no exaggeration—reported by Kasamatsu, Robberson and Vinograd in the current issue of the *Proceedings of the US National Academy of Sciences* (**68**, 2252 ; 1971) are anything to go by, it seems likely that the enigma of the mechanism of DNA replication will be solved by studies of mitochondrial DNA. And on reflexion that claim is not perhaps as surprising as it may seem at first sight, for the mitochondrial chromosome is after all probably nothing more than a heavily pruned bacterial chromosome.

Vinograd's group, in their peerless manner, have found that in two strains of mouse L cells growing exponentially about half the mitochondrial DNA molecules (closed circular DNA duplexes with a molecular weight of about 10×10^6) contain a short segment of triple stranded DNA called a displacement or D loop. This curious structure, revealed in their electron micrographs with a clarity that denies dispute, consists of a short chain of DNA which is apparently base paired to one of the closed circular DNA strands such that a corresponding segment of the complementary closed circular DNA strand is displaced and loops out from the rest of the molecule. By heating mitochondrial chromosomes with these D loops to 90 C in 0.03 M NaCl, the short chain of DNA is released—it sediments at 7S in sucrose gradients—and concomitantly the two closed circular strands snap back together and the D loop is lost.

As Kasamatsu and her colleagues comment, the sedimentation properties of mitochondrial DNA with D loops suggest that the short displacing strand is about 3 per cent of the total length of the mitochondrial DNA molecule. On electron micrographs it measures 3.2 to 3.5 per cent the length of the complete molecule and assuming that the lower estimates of its length are the most accurate the displacing chain must be about 450 ± 80 nucleotides long. This chain hybridizes with the light but not the heavy strand of L cell mitochondrial DNA and it is therefore probably a short segment of the heavy strand. Furthermore, the position of the D loop, and therefore the sequence of the 450 base segment of the heavy strand, seems to be unique.

Vinograd and his colleagues reach this latter conclusion from ingenious argument and experiment. As Clayton, Davis and Vinograd have shown, circular dimeric mitochondrial DNA molecules in human cells consist of two monomeric molecules joined head to tail. If this is also true of dimeric mouse L cell mitochondrial DNAs there should be two D loops per dimer and they should be diametrically opposed and separated by one genome length. Measurements of the position of D loops in electron micrographs of such dimeric molecules fulfil these expectations.

In short the properties of D loops eliminate the idea that they are intermediates in some DNA repair mechanisms, for repair would surely not be restricted to a unique site in the genome. But because they are present in up to 50 per cent of the mitochondrial DNA molecules isolated from exponentially growing cells, D loops may be an early stage in the synthesis of progeny mitochondrial heavy strands during a replication process initiated at a unique origin. Vinograd and his colleagues suggest that the initiation and synthesis of the progeny heavy strand can occur without a nick being introduced into either of the closed circular parental strands but growth of this chain is arrested when it is about 450 ± 80 bases long. Further extension is then dependent upon nicks being introduced into the parental strands to allow their free rotation and unwinding. The occurrence of molecules which have nicked parental strands and displacement loops which range in size up to that of the complete genome supports this interpretation. In brief, closed circular molecules with D loops may well be mitochondrial genomes which have begun their replication but are hung up awaiting the attention of a nuclease without which they cannot continue. No doubt we shall not have long to wait before Vinograd's group can tell us more about this process and also about the synthesis of progeny light mitochondrial DNA strands complementary to the new heavy displacing strand.

Geometric Refinement of Crystal Structures

ALTHOUGH known interatomic distances are often used to find a chemically acceptable crystal structure concordant with an observed diffraction pattern, they are hardly ever used in the subsequent refinement stages of the analysis. In next Monday's *Nature Physical Science* W. H. Baur shows how, in some contexts, they can be incorporated most profitably and even used to overcome shortcomings in the observed crystallographic data.

Least-squares refinement, the most widely used refinement procedure at present, attempts by adjustments of the atomic coordinates and thermal parameters to improve the agreement between the observed and calculated structure amplitudes. It relies on the fact that the number of observed structure amplitudes greatly exceeds the number of adjustable atom parameters, but the ultimate accuracy achieved for any structure depends on the accuracy and extent of the intensity data. In the past, geometrical constraints have only been introduced into the process to treat certain atoms as a rigid group.

Inorganic crystallographers, especially those concerned with silicates, enjoy a situation not shared by many of their colleagues, and this has been exploited by Baur. Inorganic crystals are based on dense packing of anions interspersed by smaller cations and, although the cation angles may be distorted, the wealth of interatomic data now available (much of it derived from accurate single-crystal studies) has shown that contact distances are remarkably uniform. In 1969 Meier and Villiger (*Z. Krist.*, **129**, 411 ; 1969) realized that the number of interatomic distances in these structures exceeds the number of adjustable atom coordinates, described a least-squares procedure for adjusting the coordinates to minimize the spread of distances of a given type and demonstrated the usefulness and scope of the procedure. Baur, who has elsewhere been able to show that changes of interatomic distance are linearly related to changes of bond strength (using an extended electrostatic valence theory) and to derive empirical constants for a number of atom pairs, has now combined these linear relations with Meier and Villiger's least-squares refinement of distances. In particular he has shown how a rather poorly defined structure for the medium-pressure β-polymorph of $MgSiO_4$, originally deduced from powder data, could be refined to produce a far more homogeneous set of distances corresponding closely ($<\Delta d>=0.027$ Å) with those recently derived from a careful single-crystal study of the isomorphous β-$CoSiO_4$. Experience gained with earlier trials had shown that even more preferential weight should be given to the cation-oxygen distances than had been given by Meier and Villiger, and that this weighting is critical. When applied to accurately known structures the approach has proved capable of predicting Si–O distances to 0.01 Å or less.

It is a pity that the method is not more widely applicable: those people who study molecular crystals will surely be forgiven if they seem a little envious of a technique that allows their colleagues to transcend the limitations of their data.