NUCLEIC ACIDS

Finer Structure

from our Molecular Biology Correspondent

THERE is evidently no end to the strange structural details in DNA that increasing technical ingenuity is capable of bringing to light. The most diverting example in some while is a closed circular DNA, which contains a ribonucleotide in one strand so that treatment with ribonuclease causes the supercoiled structure to untwist. The discovery has been made by Blair *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 2518 : 1972) in a colicinogenic factor, that is to say an extra chromosomal DNA, or plasmid, which occurs in *Escherichia coli*.

Previous work had shown that treatment with chloramphenicol leads to proliferation of the colicinogenic DNA, *col* E_1, whereas chromosomal DNA synthesis ceases after a short period, the number of molecules of *col* E_1 continues to grow, and ultimately increases a hundredfold. The population generated in this way differs in a curious manner from normal *col* E_1. When examined by equilibrium density gradient sedimentation in alkaline calcium chloride, it bands predominantly at the buoyant density associated with linear strands. The scission of cyclized strands in alkaline solution was demonstrated by velocity sedimentation after incubation for various times in alkali. As lability to alkali is a characteristic of ribonucleotides as opposed to deoxyribonucleotides, the next step was to see whether ribonucleases had the same effect, and so indeed it proved. Pancreatic ribonuclease opened *col* E_1 chains only from cells treated with chloramphenicol. Ribonuclease T_1, which is specific for guanine residues, did not work, but ribonuclease H did. This latter is a highly discriminating enzyme, the activity of which is confined to double stranded DNA-RNA hybrids. It will not operate on native or denatured DNA, or on single or double stranded RNA. Furthermore, when the open circular DNA, after treatment with pancreatic ribonuclease, was isolated and the strands separated in alkali, a one-to-one correspondence of circular and linear single strands was observed.

The inference is inescapable: after treatment with chloramphenicol the cells manufacture a supercoiled circular DNA, in which one strand contains one or a short run of ribonucleotides, paired with the deoxynucleotides in the complementary strand. Blair *et al.* do not neglect to leave the open-mouthed reader with a plausible explanation. They find that rifampicin, which is a specific inhibitor of RNA synthesis, stops the synthesis of normal *col* E_1, and also of the abnormal form after treatment with chloramphenicol. Moreover in some other systems it has been reported that DNA replication is contingent on RNA synthesis. Perhaps therefore in these situations the DNA polymerase system will not function except in the presence of an RNA primer, which must be slipped into the existing DNA duplex and remains covalently integrated until the strand is complete, when it is finally eliminated by a specific strand scission and repair process. It can then be postulated that enzymes implicated in these final steps are absent in the cells treated with chloramphenicol.

A more familiar feature that has long been recognized in the linear DNA of bacteriophages is the repetition of terminal sequences. This takes the form of the presence of identical sequences, reading in the same direction, at the 5′ and 3′ ends of the same strand. The other strand of the duplex therefore likewise contains identical sequences at its two ends. In order to reveal the existence of this type of repetition it is only necessary to expose the DNA to an exonuclease specific for either the 5′ or the 3′ end. Working inwards on the two strands from opposite ends of the duplex it will leave single-strand tails, complementary to each other. These cohesive ends will base-pair under annealing conditions and cause cyclization. The latest demonstration of such a situation comes from Rhoades and Rhoades (*J. Mol. Biol.*, **69**, 187; 1972) and relates to T5 phage DNA. This differs from other T phages in containing a few natural single-strand breaks, one of which it now seems is in one of the terminal repeated segments, for if all breaks are first repaired with polynucleotide ligase, the degree of exonuclease digestion required to cause cyclization is substantially reduced. When the breaks are present, the exonuclease will cause liberation of what would be the cohesive end by eating away its complementary strand. More digestion will therefore be required to produce a new cohesive sequence. The DNA repaired with ligase, examined in the electron microscope after exhaustive exonuclease treatment and annealing, shows the internal double helices corresponding to the complementary tracts of the single strands so produced. These have a narrow length distribution, with 10,000 or so base pairs, or 9 per cent of the genome. If the DNA is not first repaired, the base paired sections are shorter, and variable in length. It seems likely that the natural break is not confined to a single location within the repeated sequence.

A new variation on terminal sequences has been unearthed in human adenovirus DNA by Garon, Berry and Rose (*Proc. US Nat. Acad. Sci.*, **69**, 2391 ; 1972). In this case, cyclization does not follow exonuclease treatment. Instead denaturation and annealing, especially at low DNA concentration, lead to the formation of single-stranded circles. If the ends of the native molecule are eroded with a 3′ exonuclease to the extent of 2 per cent or more, this transformation no longer occurs. This has been observed for all of a number of serotypes. It seems clear therefore that in this DNA the repetitive sequence occurs at opposite ends of complementary strands, so that there is self-complementarity within each. It remains to enquire whether the repeated sequences are read in the same or in opposite directions. In the one case annealing at the ends would produce a circle with a double stranded stalk, in the other a double helical segment as part of the circle. No structures with stalks are seen in the electron microscope. Denaturation on the other hand does expose a small loop of two separated strands in the circles, suggesting that the complementary sequences may be read inwards towards each other from the ends. Garon *et al.* report that the lengths of the repeated sequences—measured by the extent of exonuclease digestion that inhibits cyclization—correlate with the oncogenic activity of the serotype. They suggest that cyclization in the cell may be a step in the incorporation of the DNA into the host genome.

TECTONICS

Magmatic Differences

from our Geomagnetism Correspondent

As long ago as 1926, Benson (*Mem. 1*, **19** ; National Academy of Sciences, 1926) found it convenient to distinguish between two broadly different types of igneous rock suite. These were the cordilleran, which "always formed during a period of great lateral pressure", and the laccomorphic, which was "not accompanied by marked lateral pressure". Benson made no attempt to relate these categories to the petrology of the rocks concerned, but nevertheless recognized that the two different tectonic settings might well result in different processes of magmatic differentiation. Until very recently, however, Benson's distinction between tectonic settings tended to receive less attention than the superficially more obvious contrast between the Atlantic and Pacific provinces. In other words, most attempts at relating petrology to tectonics have concentrated on the supposed differences between the chief oceans rather than on the possible differences between tectonic features within the oceans—or, to put it another