MITOCHONDRIAL DNA

Models of Replication

from our Cell Biology Correspondent

JUST as seeing a favourite dish in an otherwise chips-with-everything menu whets the appetite and sharpens expectancy, so seeing in the contents list of a journal a report from Vinograd's laboratory makes one tolerant of any amount of mediocrity that might surround it. For Vinograd is nothing if not a professional who always executes his experiments and extracts every ounce of information from their results in an exemplary fashion. Anybody who reads the latest report by Robberson, Kasamatsu and Vinograd in the current *Proceedings of the US National Academy of Sciences* (**69**, 737; 1972) will not be disappointed.

Last autumn this same group reported (*ibid.*, **68**, 2252; 1971) the presence of so-called displacement loop molecules in a population of DNA molecules isolated from the mitochondria of exponentially dividing mouse L cells. Under the electron microscope these molecules can be seen to consist of a short DNA chain hydrogen bonded to the light parental chain of the circular mitochondrial DNA with the corresponding region of the heavy parental chain displaced. Vinograd and his colleagues interpreted these displacement loop molecules as being at the early stages of replication. They have now examined the various molecules that can be found after mitochondrial DNA from exponentially dividing cells has been extracted and fractionated on ethidium bromide—caesium chloride buoyant density gradients, and they have arranged these molecules in a sequence which leads to the formulation of a displacement loop model of mitochondrial DNA replication.

As Robberson *et al.* report, molecules in which the displaced loop of parental heavy strand ranges in size from 10 per cent to greater than 90 per cent of the length of the genome can be seen. Moreover when the displaced, parental, heavy strand exceeds 60 per cent of the genome length it is found to be partially double stranded. In other words, once 60 per cent of the heavy strand has been displaced it seems to act as template for the synthesis of a progeny light strand. This means that the synthesis of the two strands of mitochondrial DNA is highly asynchronous, and not until some 60 per cent of a new progeny heavy strand has been made is the synthesis of the progeny light strand initiated. It is also clear that there is more than one initiation site for synthesis of progeny light strands, but none of these sites becomes available until 60 per cent of the parental heavy strand is displaced

If the two strands are made at roughly the same rate the progeny heavy strand will, of course, be completed long before the progeny light strand, and the liberation of one completed daughter molecule should be accompanied by the liberation of a still replicating molecule, the displaced parental heavy strand and the still replicating progeny light strand. Under the electron microscope such molecules would appear as partially double stranded circles with a single strand region of varying length, and Robberson and his colleagues have observed just such molecules which they have called gapped circular molecules. By hybridization they have proved that in displaced loop molecules it is the parental heavy strand that is displaced and in gapped circular molecules it is again the parental heavy strand which occupies the single strand region, and is therefore acting as template for synthesis of a progeny light strand.

In short, Vinograd's group envisage that the replication of mitochondrial DNA begins at a fixed origin with a single replication fork moving round the parental light strand, making a progeny heavy strand and displacing the parental heavy strand. Not until 60 per cent of the latter strand has been displaced is synthesis of a progeny light strand initiated and completion of the progeny light strand occurs after the two parental chains have separated. Whether other circular genomes which replicate in eukaryotic cells — for example, polyoma virus of SV40 genomes—behave in this way remains to be seen.

ANIMAL BEHAVIOUR

Mimetic Fishes

from our Marine Vertebrate Correspondent

REPORTS of mimicry among fishes are uncommon. This is perhaps more a result of the difficulty of observation underwater than on account of a scarcity of mimetic behaviour among fishes, for since underwater swimming apparatus has been widely available to biologists the number of reports has significantly increased, as is now shown by two recent articles, both the result of underwater observations.

Young specimens of the tropical Indo-Pacific genus *Platax* (batfishes) are known to mimic in form and swimming habit objects of little interest to predators. Juvenile *Platax orbicularis*, for example, have been observed off the Society Islands drifting on their sides among floating yellowish leaves of *Hibiscus tileaceous* which they resemble most closely. John E. Randall and Alan

Origin of SV40 DNA Replication

IN 1961 when the direction of growth of polypeptide chains during protein synthesis was a matter of dispute, Dintzis performed a classic and ingenious experiment which proved that the synthesis of globin chains is initiated at the amino terminal end and completed at the carboxy terminus. His experiment involved pulse labelling nascent and newly initiated globin molecules, and then analysing the pattern of labelled peptides obtained after digesting the variously labelled protein molecules. From the gradient of labelling of the peptides Dintzis established that the C terminal region of the globin molecule is synthesized after the N terminal region.

Nathans and Danna, as they report in next Wednesday's *Nature New Biology* (April 19), have now adapted this experimental rationale to the analysis of the replication of the double stranded, covalently circular DNA genome of SV40 virus in BSC-1 cells, and have shown that the replication of this DNA is initiated at a specific origin rather than at random anywhere in the molecule.

The secret of Nathans and Danna's success lies in their exploitation of a bacterial restriction endonuclease obtained from *Haemophilus influenzae* which, as they have previously shown, digests SV40 DNA to yield eleven fragments, at least eight of which appear in amounts equimolar to the initial amount of intact SV40 DNA. They argued that if the replication of SV40 DNA is initiated at a unique or preferred site by varying the length of pulses of ^{3}H-thymidine, it should be possible to label preferentially either the first part of the molecule to be replicated or the last part to be replicated. In short, just as Dintzis was able to show a gradient of labelling of globin molecules, the synthesis of which is initiated at a unique point, so they should be able to predict a gradient of labelling of the fragments of newly replicated SV40 DNA that are liberated by the *H. influenzae* endonuclease.

When Nathans and Danna digested variously labelled SV40 DNA molecules, sure enough they found a gradient of labelling as predicted. This finding confirms the assumption that the replication of SV40 DNA molecules begins at least at a preferred if not a unique site. They also estimate that under their conditions the whole molecule is replicated in about 5 minutes, and they point out that by refining their technique it may be possible to decide whether or not the replication proceeds in both or only one direction about the circular molecule.