cell's DNA if that DNA is modified, and what are the consequences of such an attack for DNA replication.

The result of the experiment could scarcely have been more clear cut; the incorporation of HMC into the DNA of the restriction positive cell leads to a 95 per cent or greater inhibition of further DNA replication even when deoxycytidine triphosphate is also present. By contrast, incorporation of HMC by the restriction negative cell has virtually no effect on the rate of continued DNA replication. In short, after incorporation of HMC into DNA further DNA replication in restriction positive cells is irreversibly inhibited even though the cell's DNA is not degraded.

Once HMC residues have been incorporated into the DNA, the cell's restriction enzyme presumably cuts double strand nicks in the replicated daughter strands and this inhibits the further replication of the parental duplex of the replicating chromosome, even though the parental DNA is unmodified. The replication machinery is, it seems, very sensitive to what is going on just behind its back, a conclusion which others have reached from less neat experiments designed to show that the integrity of a chromosome is essential for its replication.

RIBOSOMES

Controlling Synthesis

from a Correspondent

ALTHOUGH the rate of ribosome biogenesis in animal cells is usually linked closely to the cell growth rate, the mechanism of this control and the steps at which it operates have remained obscure. An increase in the growth rate in several systems stimulates both the transcription and the processing of the 45S rRNA precursor in concert, while inhibition of protein synthesis by cycloheximide or by amino-acid starvation reduces both processes.

In a recent article, Pederson and Kumar (*J. Mol. Biol.*, **61**, 655; 1971) report the dissociation of these two effects when they bring about a gradual decline in the rate of protein synthesis in HeLa cells by transferring them to hypertonic culture media. They show that following this transfer the rate of 45S rRNA processing to the 32S intermediate is greatly reduced at a time when its transcription remains unimpaired. Analysis of the nucleolar ribonucleoprotein by isopycnic banding in CsCl gradients shows that a proportion of the particles synthesized in hypertonic media are deficient in protein. As ribosomal precursors synthesized in standard media are processed normally for at least the first few minutes after transfer of cells to hypertonic media, Pederson and Kumar suggest that the rate of 45S rRNA processing is a function of the protein content of the particles rather than the enzymes of the processing system.

Much evidence for the physiological control of ribosome synthesis at the level of processing has come from Cooper's laboratory. The most recent report (Cooper and Gibson, *J. Biol. Chem.*, **246**, 5059; 1971) also demonstrates the dependence of processing on the rate of protein synthesis but implicates rather a different mechanism. Cooper's previous work on the control of rRNA transcription and processing in cultured human lymphocytes has shown that in non-growing lymphocytes less than half the 45S rRNA precursor molecules eventually give rise to mature rRNA. The remainder are degraded without leaving the nucleus, a process which can readily be detected as the 18S rRNA is degraded almost immediately but the 32S precursor remains in the nucleus for some time, distorting the normal 32S+28S:18S rRNA ratio. Activation of the lymphocytes by phytohaemagglutinin not only stimulates the rates of both transcription and processing, but also rapidly reduces the wastage of the 45S precursors. The reduction in wastage does not depend on the increase in transcription, as addition of the stimulant in the presence of actinomycin still reduces the wastage of preformed precursors.

In their latest article, Cooper and Gibson have confirmed the existence of rRNA precursor wastage in lymphocytes by a method independent of the 32S+28S:18S rRNA ratio, and have also shown that the efficiency of processing of 45S rRNA is dependent on the concurrent rate of protein synthesis. Not only is the rate of appearance of mature 18S rRNA virtually abolished when protein synthesis is inhibited by cycloheximide but, more impressively, wastage was much reduced when the rate of protein synthesis was increased by briefly elevating the temperature of incubation to 40° C. Furthermore, wastage was also reduced and the amount of 18S RNA processed remained constant when rRNA synthesis was partially inhibited by very low doses of actinomycin, implying that the wastage in lymphocytes is caused by transcription of more 45S rRNA than the processing system can cope with.

The inescapable conclusion from these two papers is that the rate of biogenesis of ribosomes is potentially and, at least in the case of lymphocytes, physiologically controlled by the rate of processing of 45S rRNA, which is in turn under the control of the rate of synthesis of one or more proteins. What is less clear is the relationship between the steps in ribosome maturation studied by the two groups. The rate of protein synthesis may, of course, affect rRNA processing at more than one point. But, as Pederson and Kumar confined their

POLYOMA VIRUS

New Forms of DNA

from a Correspondent

THE induction of polyoma virus DNA replication in ts-a-3T3 (a mouse cell transformed by a temperature sensitive mutant of polyoma) gives rise to large quantities of oligomeric viral DNA (Cuzin *et al.*, *J. Mol. Biol.*, **47**, 317; 1970). The finding of such unusual forms of DNA seemed to be especially curious because they were thought to be absent in the normal replication of wild-type polyoma. There has been much speculation, therefore, that these unusual forms of DNA may be a clue to the temperature sensitive lesions of ts-a, one of which is the temperature sensitivity of the establishment of transformation. It has been thought, for example, that these multimeric forms of DNA may reflect an aberration of integration of the ts-a genome into that of the 3T3; perhaps that it is integrated in a tandemly replicated form.

These ideas have now been made somewhat less likely by a recent report of Meinke and Goldstein (*J. Mol. Biol.*, **61**, 543; 1971) which shows quite clearly that multimeric forms of polyoma DNA are also produced during the replication of wild-type polyoma. These forms have a sedimentation constant of 25S in neutral CsCl and are mostly catenated dimers. This contrasts markedly with the circular oligomers found by Cuzin *et al.*, though the isolation procedure used by Cuzin *et al.* might well have missed catenated dimers if a large proportion of them had had a nick in one of the monomers. Also in contrast to the ts-a oligomers, some radioactive label can be chased through the catenated dimers suggesting that they may be close to, if not actually on, the pathway of DNA replication.

Thus yet another form of DNA has been added to the plethora of intermediates of polyoma replication. There is still no clear idea of the relationship between them all, but at least this latest discovery makes the oligomeric forms of Cuzin *et al.* seem a little less bizarre, and makes it seem more likely that ts-a has a lesion which affects primarily the normal replication of viral DNA.