normal and RNase III deficient strains of *E. coli* by gel electrophoresis. They find that the ribonuclease deficient strain the usual pattern of small RNAs is replaced by a large RNA identical with that usually transcribed *in vitro*. Extracts from *E. coli* strains containing ribonuclease III can cleave this large RNA to generate the messengers usually found *in vivo*.

Another maturation event, studied both by Dunn and Studier and also by Nikolaev, Silengo and Schlessinger (*ibid.*, **70**, 3361; 1973), seem to take place in ribosomal RNA synthesis. In the RNase III deficient strain, synthesis of irbosomal RNA is slowed and the usual 16S and 23S sedimenting molecules are replaced by a precursor of 30S or 1.8 – 2.3 × 10^6 daltons (according to the different estimates), large enough to contain both sequences. Hybridisation experiments confirmed that the giant molecule competes with both the mature species. Cleavage *in vitro* with an extract of strains possessing RNase III showed that this activity can generate two molecules of about the size of 16S and 23S rRNAs.

The production of ribosomal RNA in *E. coli* is thus very similar to the pathway previously characterised in eukaryotic cells, although the eukaryotic precursor is some twice the combined size of the mature molecules, so that a great amount of additional material is degraded. It is not clear just how much larger the bacterial precursor is compared with the combined mature molecules. One puzzling feature of these results is that precursors only slightly larger than the 16S and 23S rRNAs have previously been described, a point mentioned in neither of the present articles. One model is to suppose that an initial cleavage generates the precursors found, which are then further degraded and modified to yield mature ribosomal RNAs.

That the cleavage of precursors to yield mature RNAs is found also with a phage messenger is, as well as its importance in excluding other mechanisms previously proposed for phage mRNA synthesis, important for its implication that this might occur with bacterial messengers. Any molecule of RNA possessing the specific sequence recognised by ribonuclease III will presumably be cleaved by the enzyme; future research will no doubt elucidate the recognition sequence and investigate its possible presence in bacterial messengers.

Another striking claim concerning the metabolism of RNA is made by Downey *et al.* (*ibid.*, **70**, 3400; 1973), who report that a cytoplasmic RNA dependent RNA polymerase—in other words an RNA replicases—can be found in lysates of rabbit reticulocytes. The enzyme seems to utilise RNAs as templates *in vitro* to direct incorporation of labelled triphosphates into an acid precipitable product. The enzyme proved inactive when provided with DNA as primer.

The presence of an RNA replicase in the cytoplasm would add a new level of control to expression and could, for example, account for production of haemoglobin after synthesis of mRNA has ceased (instead of postulating that the haemoglobin mRNA is stable). But before this conclusion can be accepted it will be necessary to categorise rigorously the product of the reaction by qualitative means such as hybridisation assays instead of the only preliminary quantitative assay of total nucleotide incorporation. And of course, it is necessary to bear in mind frequent demonstrations of recent years that activities *in vitro* make few implications about enzyme roles *in vivo*.

Function of chloroplast DNA and its replication

from a Correspondent

ALTHOUGH the presence of DNA in chloroplasts has been recognised for more than a decade, surprisingly little is known about its function. Measurements of the molecular weight of the chloroplast genome generally give values of approximately 1–2 × 10^8 daltons. This is sufficient to code for several hundred proteins, yet isolated chloroplasts have only been shown to synthesise the larger subunit of the fraction 1 protein and two membrane-associated proteins (Blair and Ellis, *Biochem. J.*, **127**, 42P; 1972; Ellis and Forrester *ibid.*, **130**, 28P; 1972).

It seems probable that many chloroplast enzymes are coded for by nuclear genes. The genetics of the fraction 1 protein, which catalyses the carboxylation of ribulose diphosphate, are particularly interesting. The gene for the larger subunit of the protein shows cytoplasmic inheritance and is presumably located in the chloroplast whereas the gene controlling the smaller subunit shows Mendelian inheritance and is therefore in the nucleus (Kawashima and Wildman, *Biochim. biophys. Acta*, **262**, 42; 1972; Chan and Wildman, *ibid.*, **277**, 677; 1972). Another part of the chloroplast DNA codes for the 1.1 × 10^6 and 0.5^6 × 10^6 dalton RNA components of the chloreplast ribosomes (Scott and Smillie, *Biochem. biophys. Res. Commun.*, **28**, 598; 1967) and these RNAs have been shown to be synthesised inside chloroplasts (Hartley and Ellis, *Biochem. J.*, **134**, 249; 1973).

On the other hand DNA-RNA hybridisation studies with higher plant systems have previously suggested that a large number of genes for chloroplast ribosomal RNA are also present in the nucleus although there are alternative explanations of these results (Tewari and Wildman, *Proc. natn. Acad. Sci. U.S.A.*, **59**, 569; 1968; Ingle *et al. Symp. Soc. exp. Biol.*, **24**, 303; 1970). Experiments by Scott now suggest that at least in *Euglena* chloroplast ribosomal RNA may be coded for only by chloroplast DNA (*J. molec. Biol.*, **81**, 327; 1973).

Of the total DNA in light-grown *E. gracilis* cells, between 5 and 6% is present in chloroplasts. There are about ten chloroplasts in each cell and each organelle contains 40–80 copies of the chloroplast DNA which has a molecular weight between 1.8 × 10^8 and 0.7 × 10^8 daltons. DNA isolated from the chloroplasts has an average buoyant density in caesium chloride of 1.686 g cm^{-3}, which distinguishes it from the nuclear DNA with an average density of 1.707 g cm^{-3}. When RNA from chloroplast ribosomes is hybridised to denatured chloroplast DNA immobilised on nitrocellulose filters, the saturation values give an estimate of 6% of the DNA coding for chloroplast rRNA. These genes are clustered on the DNA and have a buoyant density of 1.696 g cm^{-3}. There are separate cistrons for the larger and smaller RNAs with between three and six copies of each cistron per chloroplast genome depending on which value for the size of the genome is correct. This works out at 2,400 genes for chloroplast rRNA per cell. By contrast there are 1,000 genes, buoyant density 1.716 g cm^{-3}, coding for the RNA of the cytoplasmic ribosomes. Such a multiplicity of rRNA genes is commonly found in eukaryotes and is consistant with a high metabolic demand for ribosome synthesis.

Euglena cells grown heterotrophically in the dark do not develop chloroplasts. When DNA from dark-grown cells is challenged with chloroplast rRNA, Scott found that although there is some hybridisation this represents a non-specific reaction. Heating the 'hybrids' dissociates the DNA-RNA duplexes and the melting profile thus obtained affords a measure of the degree of base paring involved. The results clearly show that the duplexes have a much lower thermal stability than authentic rRNA–rDNA hybrids.

The conclusion is that DNA from dark-grown cells (largely nuclear DNA) does not contain a large number of genes for chloroplast rRNA. Presumably there must be at least one copy of the chloroplast DNA present in dark-grown cells but even several copies could have remained undetected in these experiments. For this reason the possibility that there are a very small number of chloroplast rRNA genes in the nucleus cannot yet be ruled out.