MITOCHONDRIA

Where the Genes Reside

from our Cell Biology Correspondent

THE notion that mitochondria are the evolutionary descendants of some form of bacterium, or bacterial like organism, is nowadays widely accepted. The evidence for this suggestion, of course, comes from comparative biochemistry. Mitochondria have their own protein synthesizing machinery which has many of the characteristics of that of bacteria and can readily be distinguished from that of the eukaryotic cell cytoplasm in which the mitochondria reside. Furthermore, mitochondria have their own genomes or, to be more precise, circular DNA molecules. But the amount of DNA in a mitochondrion is not sufficient to specify all the proteins and RNAs involved in mitochondrial protein synthesis let alone all the enzymes and structural proteins of these organelles. Quite clearly, if mitochondria have evolved from symbiotic microorganisms, during that evolution many of the genes essential for the survival of mitochondria have come to reside in the host cell chromosomes, and the evolutionary advantage to mitochondria of an autonomous protein synthesizing apparatus becomes far from obvious.

The specific chain elongation factors required for the synthesis of proteins on the "70S" mitochondrial ribosomes are a case in point. According to Parisi and Cella (*FEBS Lett.*, **14**, 209 ; 1971), in the yeast *Saccharomyces cerevisiae* these enzymes, although required specifically for mitochondrial protein synthesis, are coded by nuclear DNA. For Parisi and Cella have simply shown that two strains of "petite" yeast contain elongation factors capable of catalysing protein synthesis by bacterial ribosomes even though the mitochondria of these strains are known not to support protein synthesis and their DNA is believed to be unable to specify active proteins. Moreover, they have also shown that chloramphenicol, which inhibits bacterial and mitochondrial protein synthesis, does not block the synthesis of these elongation factors on 70S ribosomes in either wild type or "petite" yeast. It seems therefore that these elongation factors, like the structural proteins of *Neurospora* mitochondrial ribosomes, are specified by nuclear genes and are also synthesized on cytoplasmic ribosomes.

By contrast, Rabbitts and Work (*ibid.*, 214) report experiments which indicate that the 18S and 12S RNA moieties of the mitochondrial ribosomes of chick liver cells are specified by mitochondrial DNA. After labelling the chick cells with ^{3}H-uridine, they isolated the RNA associated with mitochondrial fractions and resolved it into three components—28S, 18S and 12S—by gel electrophoresis. Only two of these types, the 18S and 12S RNAs, can be isolated from a preparation of mitochondrial ribosomes, which, in the conditions Rabbitts and Work used, sediment at 55S.

To decide whether the 18S and 12S RNA are specified by the mitochondrial DNA rather than by nuclear DNA, they exposed cells simultaneously to ^{3}H-uridine and ethidium bromide, a drug which allegedly selectively inhibits the transcription of RNA from the circular mitochondrial DNA. After such treatment, the amount of ^{3}H-uridine label in the mitochondrial RNAs was markedly reduced, but ethidium bromide had no effect on the incorporation of this precursor into the 28S and 18S RNAs of cytoplasmic ribosomes. Rabbitts and Work conclude therefore that the RNAs of mitochondrial ribosomes are specified by the mitochondrial DNA ; it would, of course, be interesting to see whether hybridization tests bear out this prediction.

And what is the site of synthesis of the outer and inner membranes of mitochondria? According to Neupert and Ludwig (*Europ. J. Biochem.*, **19**, 523 ; 1971), who have separated these two membranes of *Neurospora* mitochondria and characterized each by electron microscopy, associated enzyme activities and associated pigment, the single chief protein component of the outer membrane is made on cytoplasmic ribosomes. Its synthesis *in vivo* is susceptible to inhibition by cycloheximide. By contrast, at least some of the twenty different proteins found in the inner membrane seem to be synthesized within the mitochondrion, for their synthesis *in vivo* and *in vitro* is insensitive to this drug. But whether the genes for these proteins made on mitochondrial ribosomes reside in the mitochondrial rather than in the nuclear DNA is another and unresolved question.

Calling the False Reverse Transcriptases

SINCE the discovery of reverse transcriptase (the enzyme in tumour viruses which is capable of using the single stranded viral genome as a template for the synthesis of a double stranded DNA) was announced by Temin and Mizutani and Baltimore just a year ago, much confusion has arisen about the distribution of this enzyme. For as soon as Spiegelman reported that synthetic RNA/DNA hybrids are very efficient templates for these viral enzymes, numerous groups began to use these synthetic nucleic acids to search for reverse transcriptase activity in extracts of cancer cells and normal cells as well as tumour viruses. Polymerase activities capable of using these hybrids as templates for DNA synthesis were duly found in all these sources, but, as Todaro and his colleagues have now proved, such findings do not necessarily mean that cancer cells and normal cells contain the same enzyme as tumour viruses. Their report published in *Nature New Biology* next week is a timely antidote to rampaging oversimplification.

Their experiments are in essence simple enough. They have partially purified and compared the DNA polymerases in mouse leukaemia virus particles, untransformed mouse cells and mouse cells transformed by mouse sarcoma virus, using calf thymus DNA and synthetic DNA/RNA hybrids, poly rA.dT of different sizes, as templates. Furthermore, they have prepared antisera against the reverse transcriptase in mouse leukaemia virus particles and tested its inhibitory effects on the DNA polymerizing enzymes purified from transformed and untransformed cells.

Mouse leukaemia virus particles have a single enzyme capable of using both these templates for DNA synthesis ; this enzyme is reverse transcriptase as defined by Temin and Baltimore and it can also use the viral genome as a template. From extracts of untransformed mouse cells they purified a DNA polymerase which can use calf thymus DNA but not the synthetic hybrid as a template, and a second enzyme which can use both templates. This enzyme, however, behaves very differently from virus reverse transcriptase on chromatography. Finally, in mouse cells transformed by mouse sarcoma virus they find three DNA polymerases, the two that are in untransformed cells and the reverse transcriptase that is in murine sarcoma virus particles. Only this latter activity in the transformed cells is inhibited by antisera against the reverse transcriptase of mouse leukaemia virus.

The moral of this story is plain enough. The DNA polymerase activities detected in normal, transformed and cancer cells with synthetic DNA/RNA hybrid templates are normal cellular enzymes unrelated to the reverse transcriptases of the animal RNA tumour viruses. Furthermore, synthetic nucleic acid templates, useful though they are for characterizing reverse transcriptase once its existence has been proved, are no substitute for the natural, single stranded RNA templates when it comes to searching for the enzyme in new situations.