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The Function of Chloroplast Ribosomes

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Chloroplasts contain prokaryote-like ribosomes that can represent up to 30% of the total leaf ribosomes. Experiments to discover the function of chloroplast ribosomes have involved the treatment of greening cells with 70S ribosomal inhibitors. The results suggest that most of the soluble proteins of the chloroplast are made on cytoplasmic ribosomes and subsequently transferred across the outer membrane of the plastid; chloroplast ribosomes appear to be necessary to synthesize only some chloroplast membrane proteins, some chloroplast ribosomal proteins and Fraction I protein (Boulter *et al.*, 1972). However, conclusive answers can come only from studies of protein synthesis by isolated chloroplasts, since there are difficulties in the interpretation of the inhibitor experiments. Blair & Ellis (1972) have already reported that intact isolated chloroplasts synthesize the large subunit of Fraction I protein. We now report that intact chloroplasts also synthesize membrane-bound protein.

Chloroplasts were isolated by the rapid method of Ramirez *et al.* (1968) from 7–10-day-old pea plants (*Pisum sativum*) and incubated for 40 min at 20°C with either [³⁵S]methionine or [¹⁴C]leucine. Red light was used as energy source in the absence of either added ATP or catalysts of photophosphorylation; this ensures that incorporation of amino acids into protein occurs only in intact chloroplasts. After incubation the chloroplasts were dialysed against hypo-osmotic buffer and treated with 1% sodium dodecyl sulphate. Analysis of the whole incubation mixture by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis in borate buffer, pH 8.3, revealed two major radioactive peaks. Neither peak is found in chloroplasts incubated in the dark or in the presence of chloramphenicol. The peaks do not coincide with the chlorophyll–protein complexes derived from either photosystem I or photosystem II.

When the dialysed extracts are centrifuged at 10000g for 10 min the slower-moving peak remains in the supernatant, where it runs exactly with the large subunit of Fraction I protein. The faster-moving peak occurs solely in the pellet and cannot be removed by washing in hypo-osmotic buffer. This peak is present when the chloroplasts are treated with 10 mM puromycin near the end of the incubation, and when the chloroplasts are boiled in sodium dodecyl sulphate immediately after incubation to inactivate proteases.

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Effects of α -Amanitin on Protein and Nucleic Acid Synthesis in Chick-Embryo Fibroblast Cells

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α -Amanitin, a bicyclic octapeptide from the toadstool *Amanita phalloides* (Fiume & Wieland, 1970), inhibits DNA transcription by binding specifically to RNA polymerase form II of eukaryotic organisms without affecting the activity of RNA polymerase form I (Kedinger *et al.*, 1970). The effects of α -amanitin on RNA polymerase activity *in vitro* have been well studied, and can be used as the basis of an assay to distinguish form I and form II polymerase activities in whole nuclei (Novello & Stirpe, 1970). Little is known about the action of α -amanitin on growing cells, but it has been reported that total cellular RNA synthesis as measured by [³H]uridine incorporation is relatively resistant to the drug (Rott & Scholtissek, 1970).

To obtain more information on the effects of α -amanitin, we first measured the overall rates of synthesis of protein, DNA and RNA in α -amanitin-treated cells. The growth medium of monolayer cultures of chick-embryo fibroblast cells was supplemented with α -amanitin (20 μ g/ml) and at intervals thereafter the cells were pulse-labelled for 15 min with [¹⁴C]-labelled protein hydrolysate, [³H]thymidine or [³H]uridine before determination of acid-insoluble radioactivity as described by Mahy *et al.* (1972). The rate of protein synthesis was not significantly altered during up to 8 h after treatment with α -amanitin. Incorporation of [³H]thymidine into DNA remained unchanged for 2–3 h after α -amanitin treatment, then declined to 50% of control values by 8 h. Similarly, incorporation of [³H]uridine into RNA

declined between 2 and 8 h, but during the first hour after addition of α -amanitin the rate of RNA synthesis was consistently found to increase by up to 20% above control.

In a further series of experiments chick-embryo fibroblast cells were treated with various doses of α -amanitin up to 30 μ g/ml, and after 1 h the cells were harvested and the nuclei were purified and assayed for RNA polymerase form I and form II activities (Novello & Stirpe, 1970). After doses of 5 μ g or more/ml there was an 80% inhibition of RNA polymerase form II activity of isolated nuclei, but RNA polymerase I activity was stimulated by 50%. The latter finding may account for the observed stimulation in [3 H]uridine uptake in the pulse-labelling experiments after treatment with α -amanitin (20 μ g/ml) for 1 h.

It is clearly not possible to determine the effects of α -amanitin on cellular RNA synthesis simply by measuring the net incorporation of [3 H]uridine into acid-insoluble material; therefore we have measured the effects of the drug on the synthesis of various types of cellular RNA, and the results of these experiments will be described.

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Polarity in the *gua* Operon of *Escherichia coli* K12

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In *Escherichia coli* the purine nucleotide IMP is synthesized in ten steps from 5'-phosphoribosyl 1'-pyrophosphate and is at the branch point for the biosynthesis of AMP and GMP. The biosynthesis of GMP from IMP is catalysed by two enzymes, IMP dehydrogenase and XMP aminase. IMP dehydrogenase is subject to feedback inhibition by GMP (Mager & Magasanik, 1960), and both enzymes are repressed by high intracellular concentrations of GMP (Nijkamp & de Haan, 1967). The structural genes for the two enzymes are linked and are coordinately regulated (Nijkamp & de Haan, 1967).

The present communication gives further information on the regulation of these two enzymes.

A number of mutants blocked in GMP formation were isolated from wild-type cells of *E. coli* K12 and are designated *guaA* (XMP-aminase-less) or *guaB* (IMP-dehydrogenase-less). The mutants, which require guanine for growth, were cultured under conditions where the guanine supply was insufficient to support maximal growth of the cells. Under these conditions enzyme synthesis was no longer repressed.

All *guaA* mutants had de-repressed levels (40–45-fold) of IMP dehydrogenase. By contrast, two classes of *guaB* mutant were obtained. The first class had de-repressed levels (15–25-fold) of XMP aminase whereas the second class had low levels of this enzyme. The latter type of *guaB* mutation showed a polarity effect leading to a low-level expression of the adjacent *guaA* gene. These results are contrary to the findings of Nijkamp & Oskamp (1968), who found that in *E. coli* K12 some *guaA* mutations were polar but that none of the *guaB* mutations showed polarity effects.

Each *guaB* mutant was tested for its ability to produce cross-reacting material. The reaction between anti-(IMP dehydrogenase) serum and cross-reacting material was detected by immunodiffusion in gels on microscope slides. Non-polar *guaB* mutants were either positive or negative with respect to cross-reacting material, whereas all polar *guaB* mutants were negative, as expected if they arose by 'nonsense' mutations.

To establish that the polar mutations lie within the *guaB* gene, a genetic map of the IMP dehydrogenase structural gene was constructed by three-factor reciprocal crosses. The polar mutants were found to lie within the *guaB* gene.

These findings indicate that transcription of the two genes of the *gua* operon proceeds from *guaB* to *guaA*.

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