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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