insulin concentration of 12½% (112 to 128 μ units/ml). (b) Thirteen animals, with chambers with islets from New Zealand obese mice, showed increases in body weight, blood glucose concentration and plasma insulin concentration essentially similar to those in group (a). (c) Eleven animals, with chambers with islets from normal albino mice, showed a 19% increase in body weight (27 to 32g), a decrease in blood glucose concentration of 49% (207 to 106mg/100ml) and a decrease in plasma insulin concentration of 65% (152 to 54 μ units/ml). There was no significant difference between groups (a) and (b). Group (c) differed from groups (a) and (b) with a significance of $P < 0.001$ on all three variables monitored.

After 45 days chambers were removed from three animals of group (c) and reimplanted into three other New Zealand obese mice. The body weight, blood glucose concentration and plasma insulin concentration of the former group reverted to values characteristic of New Zealand obese mice. The latter group normalized. It is concluded that the implanting of normal islets into New Zealand obese mice normalizes these animals, suggesting that the basic genetic defect in these obese mice lies in the islets. When normal islets were implanted into *ob/ob* mice, similar results were obtained, confirming the work of Strautz (1970).

NZO mice when given glucose orally respond by producing a typical diabetic tolerance curve. After implantation with normal islets there is an improvement in the diabetic tolerance curve after 1 week. After 3 weeks the glucose tolerance curve is normal and this is maintained for at least 7 weeks.

In an effort to identify a functional difference between the islets of albino and New Zealand obese mice, the activity of adenylate cyclase was examined. No difference was found between the islets of the two groups when they were stimulated with tolbutamide, glucose and glucagon. This suggests that the defect seen by Larkins & Martin (1972), where no response of plasma insulin concentration to glucose, glucagon and tolbutamide *in vivo* was observed, lies beyond the adenylate cyclase system.

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The Synthesis of a Discrete High-Molecular-Weight Ribonucleic Acid Species by Isolated Chloroplasts

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Chloroplasts contain DNA, but so far no specific gene product of chloroplast DNA has been unambiguously identified. Hybridization studies have shown that chloroplast rRNA has complementary sequences in both chloroplast and nuclear DNA (Tewari & Wildman, 1968; Ingle *et al.*, 1970). Although there is indirect evidence that the chloroplast cistrons function in the synthesis of chloroplast rRNA (Surzycki *et al.*, 1970), investigations on RNA synthesis by isolated chloroplasts have failed to demonstrate the synthesis of any discrete species of RNA (Spencer *et al.*, 1971). We now report that intact isolated spinach chloroplasts synthesize a discrete high-molecular-weight RNA species of similar size to rapidly labelled chloroplast RNA synthesized *in vivo*.

Chloroplasts were isolated from the first-formed leaf pair of 14–16-day-old spinach plants (*Spinacea oleracea*) by the method of Ramirez *et al.* (1968). Incorporation of [³H]uridine into RNA showed an absolute requirement for light, and did not occur in lysed chloroplasts. Fractionation of the product synthesized *in vitro* by polyacrylamide-gel electrophoresis revealed a major product of mol.wt. 3.0×10^6 and two minor products of mol.wt. 1.25×10^6 and 0.5×10^6 . Chloroplasts isolated from excised leaves labelled via their petioles with [³²P]P_i for 1 h contained three major radioactive RNA species of mol.wt. 3.0×10^6 , 1.25×10^6 and 0.67×10^6 and a minor component of mol.wt. 0.5×10^6 . With increasing times of labelling, radioactivity appeared in the chloroplast rRNA species (mol.wt. 1.1×10^6 and 0.56×10^6), and during this transition the radioactivity in the rapidly labelled RNA species became a progressively smaller proportion of the total. The RNA species of mol.wt. 3.0×10^6 synthesized *in vivo* and *in vitro* coincided exactly with each other on co-electrophoresis. When actinomycin D was applied after a 1 h pulse-label with [³²P]P_i *in vivo*, further incubation resulted in an increase in the specific radioactivities of the chloroplast rRNA species with a decrease in the radioactivity of the rapidly labelled RNA species. This is consistent with the latter being the precursors of the former. We suggest that the rapidly labelled chloroplast RNA of mol.wt. 3.0×10^6 is a likely candidate for a large precursor to chloroplast rRNA, and is transcribed from chloroplast DNA.

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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