news and views

Chloroplast protein synthesis

from Harry Smith

READERS of the more distinguished biochemistry and molecular biology journals could be forgiven for concluding that plants do not have genes, nucleic acids, proteins, enzymes and intriguing regulatory processes such as are possessed by respectable organisms like *E. coli* and HeLa. The omissions are doubtless due to the small amount of work in progress in these areas of plant science, which is, in turn, largely caused by lack of interest in, and knowledge of, plants on the part of traditionally trained biochemists. It is, therefore, refreshing to see a group tackling one of the central problems of plant molecular biology and in the process producing a concept of interorganelle regulation which may have important implications for other organisms.

R. J. Ellis and his colleagues at the University of Warwick have for several years now been studying the complex problem of the synthesis of Fraction 1 protein in leaves. This protein, which may represent up to 50% of the total soluble leaf protein, is only found in its intact state within the chloroplasts, where it carries the catalytic function of ribulose-1,5-diphosphate carboxylase, the enzyme responsible for photosynthetic carbon fixation. The molecule has a molecular weight of about 525,000 and probably consists of eight identical large subunits (molecular weight about 55,000) and eight to ten identical small subunits (molecular weight about 15,000). Genetic experiments have shown that the functional gene for the large subunit is present in the chloroplast genome, whereas that for the small subunit is in the nuclear genome. Moreover, earlier inhibitor experiments indicated that the large subunit is synthesised only on chloroplast ribosomes *in vivo*, and the small subunit on cytoplasmic ribosomes. There is thus a fascinating problem of interorganelle communication and integration.

In 1973 Blair and Ellis (*Biochim. Biophys. Acta*, **319**, 223–234) showed that intact, isolated pea chloroplasts when driven by light energy, incorporated labelled amino acids into the large subunit of Fraction 1, the only soluble polypeptide to be synthesised. Its identity was established by comparing a tryptic map of the labelled peptides with that from authentic Fraction 1 large subunit. Subsequently, Eaglesham and Ellis (*Biochim. Biophys. Acta*, **335**, 396–407; 1974) showed that isolated chloroplasts in the light incorporate label into six separate membrane-bound products which were considered to be membrane proteins. Other workers have obtained similar results using spinach chloroplasts, but in this case upwards of four soluble proteins, and nine membrane proteins, became labelled (Bottomley, Spencer and Whitfield, *Archs Biochem. Biophys.*, **164**, 106–117; 1974). The concept has thus developed that at least Fraction 1 protein large subunit and a number of chloroplast membrane proteins are synthesised on chloroplast ribosomes. The location of the genes for the membrane proteins is not yet known.

Hartley, Wheeler and Ellis have now demonstrated (*J. molec. Biol.*, **91**, 67–77; 1975) the existence of the messenger RNA for the large subunit by the only real criterion—its *in vitro* translation into a recognisable polypeptide. Using a cell-free *E. coli* preparation as a heterologous protein-synthesising system, they showed that the Fraction 1 large subunit is synthesised when total spinach chloroplast RNA is presented as the message. Two major labelled products were found, both in the cell-free heterologous system, and as a result of protein synthesis by intact light-driven chloroplasts. One of these, with a molecular weight on SDS gels of 52,000, had a closely similar chymotryptic map to that of authentic Fraction 1 large subunit. The evidence for the identity of the labelled product with Fraction 1 large subunit seems most convincing.

Since the large subunit is coded for in the chloroplast genome, its messenger RNA is present in chloroplast RNA, and it is synthesised on chloroplast ribosomes, some control of synthesis must be exerted such that the relative proportions of large and small subunits are coordinated. In the January issue of *Phytochemistry* (**14**, 89–93; 1975) Ellis puts forward a very intriguing hypothesis for the mechanism of this integration. He shows that MDMP (2(-4-methyl-2,6-dinitroanilino)-N-methylpropionamide), a highly specific inhibitor of initiation on 80S ribosomes, inhibits the synthesis of both the large and the small subunit when applied to intact pea leaves. MDMP does not inhibit protein synthesis in chloroplasts. On the basis of this evidence, a model is proposed in which the small subunit is thought of as a positive factor required for the initiation of either the transcription of the messenger RNA for the large subunit, or for its translocation (see figure). On this model, the synthesis of the large subunit by isolated chloroplasts represents run-off of preformed polyribosomes, which accounts for the observed rapid falling off in the rate of protein synthesis. This elegant and painstaking work convincingly demonstrates that plant molecular biology is not dead, but is merely awaiting the attention of competent and dedicated biochemists.