Glasgow, Feher’s group in San Dieog and Drews’ group in Freiburg) have now obtained crystals of antenna complexes. This holds out the exciting prospect of a high-resolution structure of an antenna complex being determined by X-ray crystallography, and this is eagerly awaited.

In the case of *Rps. capsulata* the structural genes corresponding to the apoproteins of the B875- and B800 850-complexes have been cloned and sequenced (see, e.g. Youvan et al., 1984). The B875-apoproteins are located next to the reaction-centre L- and M-genes on the same operon, whereas the B800 850-apoprotein genes are located elsewhere on the chromosome. In all cases the amino acid sequence deduced from the nucleotide sequence exactly matches that determined by direct amino acid sequencing of the purified apoproteins, i.e. there is no post-translational modification. This type of arrangement of the apoprotein structural genes on the chromosome may well explain how the amount of B875-complex per reaction centre is always constant and how the synthesis of the B800 850-complex can be independently controlled.

Functional assembly of the chloroplast H⁺-ATPase and photosynthetic reaction centres

NATHAN NELSON

Department of Biochemistry,
Rutgers Institute of Molecular Biology,
New Brunswick, N.J. 08903, U.S.A.


transferred to the nucleus. This event probably divided the genes coding for the chloroplast H+-ATPase into two separate loci. In all the organisms tested so far, the gene coding for the β-subunit precedes the one coding for the ε-subunit (Walker et al., 1982; Huttly & Gray, 1984; Walker et al., 1984). The genes coding for the β- and ε-subunits of CF₁ are transcribed on a bicistronic mRNA and those for subunits II, I and α on a polycistronic mRNA (Herrmann et al., 1985). Studies on the organization of the chloroplast genes and sequencing of non-reading frames are likely to yield valuable information in the near future.

So far, studies on the structure, transcription and translation of the chloroplast genes revealed quite a few general properties (see Herrmann et al., 1985). Structural genes are scattered on both strands of the chloroplast DNA. So far, none of the genes coding for proteins are interrupted by non-transcribed regions. The genes coding for chloroplast proteins and located in the nucleus are transcribed as polyadenylated [poly(A)+] mRNA and translated by cytoplasmic ribosomes as larger precursors. All the genes located in the chloroplast DNA are transcribed into poly(A)-mRNA. Most of them translated into their mature size. So far, only cytochrome f has been translated in vitro into a larger precursor (Alt & Herrmann, 1984; Willey et al., 1984). The herbicide-binding protein (33 kDa) is translated as an 'illegal' precursor with an extension in its C-terminal side (Marder et al., 1984). The chloroplast uses a 'universal' code for amino acids and plays no mitochondrial games. In all cases tested so far, ATG seems to serve as the initiation codon. In general the chloroplast genes exhibit prokaryotic features. Even though the chloroplast genes are not organized in operons, some of them are transcribed into polycistronic mRNA. A complex mRNA processing and a biased codon usage may contribute to the control of gene expression during chloroplast biogenesis. There exists a large discrepancy between the amount of a specific mRNA present at a given time and the amount of the corresponding polypeptide in the chloroplast. Therefore the regulation of the chloroplast biogenesis may be governed by post-transcriptional events (Herrmann et al., 1985). A striking example is provided by the genes coding for the 51 kDa polypeptide of Photosystem II, cytochrome b₅, and subunit IV of cytochrome b₅f complex. The former is supposed to be a 'photogenic', because its product is present only after an illumination of etiolated leaves, whereas the expression of the other two genes is not affected by light. Yet it was found that these three genes are transcribed into a single polycistronic mRNA species (Herrmann, 1985; Heinemeyer et al., 1984).

Subunit I of the Photosystem I reaction centre is one of the chloroplast gene products (Nechushtai et al., 1981; Nechushtai & Nelson, 1981). The position of its gene on the chloroplast DNA has been located and sequencing of the region revealed two reading frames with considerable sequence homology (Fish et al., 1985; Alt et al., 1984; Kirsch et al., 1985). The two predicted polypeptides were denoted as subunits Ia and Ib of the reaction centre and their calculated Mᵦ values were 83 000 and 82 000 respectively. So far only the gene coding for subunit Ia has been shown to be translated and assembled into the functional reaction centre. The predicted amino acid sequences of the two polypeptides kindly supplied by Dr. R. G. Herrmann showed that methionine is evenly distributed in polypeptide Ia, and only in the N-terminal side of polypeptide Ib is there an uninterrupted sequence with an Mᵦ of 30 679. The second largest sequence between two methionine residues has an Mᵦ of only about 12 000. Fig. 2 shows that CNBr treatment of isolated Photosystem I reaction centre yielded a polypeptide with an Mᵦ of about 30 000. This experiment suggests that the gene coding for polypeptide Ib is expressed in vivo and that its protein product is assembled into the functional reaction centre.

Regulation of the expression of chloroplast genes by light is not necessarily taking place by 'photogenes' (Rodermel & Bogorad, 1985), since recent evidence has shown that at least part of the regulation is post-transcriptional (see Herrmann et al., 1985). In the last few years we have studied the end results of this kind of
regulation, mainly the amounts of various chloroplast polypeptides during greenning of etiolated seedlings, as well as their amplitudes in response to inhibitors of protein synthesis and photo-inactivation of electron transport (Nechushtai et al., 1981, 1983; Nechushtai & Nelson, 1981, 1985; Ben-David et al., 1983; V. Liveanu, C. F. Yocum & N. Nelson, unpublished work; R. Rott & N. Nelson, unpublished work). Electrotransfer from sodium dodecyl sulphate/polyacrylamide gels into nitrocellulose and immunodetection with polypeptide-specific antibodies were used throughout these studies (Nelson, 1983, 1985).

The conclusions drawn are the following. All of the subunits of cytochrome b_{6}/f complex, the H_{2}O-ATPase complex and ribulose-1,5-bisphosphate carboxylase are present in etiolated leaves in amounts (per wet wt.) approaching that in green leaves. All of the subunits of these complexes are assembled via a concerted mechanism. On the other hand, the Photosystem I reaction centre is assembled step by step (Nechushtai & Nelson, 1985). Subunit I (Iα + Ib) is present in etiolated leaves at about 30% of its amount in green leaves. None of the other six subunits could be detected in etiolated leaves. After about 2 h of illumination, subunit II was revealed, when it assembled into a partial complex. It was suggested that the presence of subunit II in the chloroplast membrane may serve as a signal or template for the other subunits to follow suit and to assemble into the functional complex one after the other (Nechushtai & Nelson, 1981, 1985). This step-by-step mechanism of assembly may be significant for the partial reactions required during biogenesis of the chloroplast membrane.

On the other hand, the Photosystem II reaction centre has no known partial activity having physiological significance. Etiolated leaves contain large amounts of the three subunits of the Photosystem II reaction centre which are soluble in 0.8 M-Tris-HCl, pH 8 (V. Liveanu, C. F. Yocum & N. Nelson, unpublished work; Pyrie et al., 1984). None of the other subunits of the reaction centre are present in etiolated leaves. Only after 6–8 h of illumination can all of the other subunits be detected, and they assemble into the functional complex at nearly the same rate. Fig. 3 depicts the changes in the events during biogenesis of the Photosystem II reaction centre.

Etiolated seedlings are very convenient for studies on chloroplast biogenesis. However, they do not necessarily reflect all of the events which occur during biogenesis of a protein complex in green leaves. Exchange of certain subunits may be a major process during steady-state biogenesis of protein complexes. Recent studies in our laboratory, using inhibitors of protein synthesis and light inactivation, indicated that some of the subunits of the Photosystem II reaction centre are hardly exchangeable, whereas the others are readily exchanged for newly synthesized ones (R. Rott & N. Nelson, unpublished work). Many of these studies were possible because of newly developed immunological techniques, and future development of genuine transformation of plant cells should give fresh insight into mechanisms in biogenesis and assembly of protein complexes in the photosynthetic membrane.

Fig. 3. Light-induced biogenesis and assembly of Photosystem II reaction centre

The symbols for the various subunits correspond to their apparent M_{r} values (e.g. 35 K = M, 35000) on gels, except that 55 S is cytochrome b_{6}/f; LH is the light-harvesting chlorophyll protein, which is composed of at least two different polypeptides; 35 K, 23 K and 18 K are the 'Tris-soluble' subunits; P_{680} is either the 47 K or the 51 K protein and 34 K is the herbicide-binding protein; 33 K is the '34-like protein'.