Polyadenylated mRNA for the Light-harvesting Chlorophyll a/b Protein
Its Presence in Green and Absence in Chloroplast-Free Plant Cells

Michael Müller, Maija Viro, Christiane Balke, and Klaus Kloppstech*
Institut für Botanik, Universität Hannover, Herrenhäuser Straße 2, D-3000 Hannover 21, Federal Republic of Germany

Abstract. In thylakoid membranes isolated from green plants of parsley, pea, and barley, the light-harvesting chlorophyll a/b protein complex (LHCP, mol. weight: 25,000), is a major constituent. Poly(A)RNA isolated from these species was translated in a wheat germ, cell-free system. The in vitro translation products were treated with antibodies raised against the LHCP. This treatment resulted in the precipitation of a precursor protein (mol. weight: 29,000). Poly(A)RNA was also prepared from a cell culture of Petroselinum that does not develop chloroplasts upon illumination. This poly(A)RNA is capable of stimulating amino acid incorporation in the in vitro translation system, however, it does not direct the synthesis of LHCP.

Key words: Cell cultures – Chlorophyll protein – Photosynthesis (light harvesting) – Poly(A)RNA – Thylakoid membranes.

Introduction

Light induces prominent changes in the structure and function of chloroplasts during the transition of etioplasts to chloroplasts (Armond et al. 1977). Among these changes is the insertion of LHCP into the thylakoid membrane. The appearance of the functional LHCP complex is controlled by light at least at two levels, namely the light-dependent synthesis of the chlorophylls (Bogorad 1967) and the light-induced transcription or activation of mRNA for the light-harvesting chlorophyll a/b protein (Apel and Kloppstech 1978).

Cell cultures of parsley – originally isolated from parenchymatic root tissue (Seitz and Richter 1970; Spieß and Seitz 1975) – contain leucoplasts but do not turn green after illumination (Spieß and Seitz 1975). In these cultures, light induces the appearance of the phenylalanine ammonia-lyase (PAL) (Schröder et al. 1979), which poses an interesting question as to whether, under similar conditions, the mRNA for LHCP is also induced. The answer to this question might contribute to the problem of the activity of nuclear genes in cells which are deficient in both a transcribed chloroplast genome and the synthesis of chloroplast pigments.

The experiments presented here show that the nuclear genes which code for chloroplast proteins are not expressed under conditions of reduced chloroplast gene expression. Parts of the results have been published in preliminary form (Müller et al. 1979).

Materials and Methods

Growth Conditions

Barley plants (Hordeum vulgare, cv. Aramir) and parsley cell cultures (Petroselinum crispum Mill.) were grown in the dark for 6 d at 23°C and illuminated for 12 h (2,000 lx) before harvesting. The cultivation of parsley callus cultures has been described in detail elsewhere (Seitz and Richter 1970). Green plants of parsley were grown in a greenhouse and freshly harvested. Pea (Pisum sativum L. cv. Alaska 7) plants were cultivated on vermiculite for 7 d at 23°C under a 12:12 h dark-light regime.

Extraction of RNA and Purification of Poly(A)RNA

The plant material was frozen in liquid nitrogen and ground to a fine powder. One gram of powder was suspended in 10 ml of extraction buffer, and poly(A)RNA was extracted by the phenol method (Kloppstech and Schweiger 1976). The poly(A)RNA was purified on oligo(dT)-cellulose (1 g cellulose/1.5 g leaf tissue) and stored under liquid nitrogen (Apel and Kloppstech 1978; Kloppstech and Schweiger 1976).
In Vitro Protein Synthesis and Immunoprecipitation of Translation Products

Saturating amounts of poly(A)RNA (30 µg/ml assay) were translated in a wheat germ, cell-free system for 90 min at 25°C (Roberts and Paterson 1970). The translation in a commercial reticulocyte system was carried out following the guidelines of the manufacturer. 80 µg of poly(A)RNA/ml assay mixture were used. The samples were analyzed with 7.5-15% gradient PAGE (Neville 1971). The gels were stained with Coomassie blue (Meyer and Lamberts 1965), destained, and treated for fluorography as described elsewhere (Bonner and Laskey 1974).

Preparation of Thylakoid Membranes

The thylakoid membranes were prepared according to Neville’s method (1971). A chloroform-methanol (C:M) extraction of the membranes was accomplished according to Chua et al. (1975). The samples were analyzed as described above, but they were not prepared for fluorography.

Source of Materials

[^5S]Methionine (22 TBq mmol⁻¹) was obtained from Amersham Buchler, Braunschweig. The reticulocyte lysate came from New England Nuclear, Boston.

Results and Discussion

SDS gel electrophoresis of thylakoid proteins from barley, pea, and parsley chloroplasts shows a prominent protein band with an apparent molecular weight of 25,000, which presumably represents LHCP. This finding corresponds well with the data described for barley (Apel and Kloppstech 1978; Armond et al. 1977) and spinach (Chua and Blomberg 1979).

Neither the positions of the protein bands to each other nor the apparent molecular weight of the LHCP of these three species show any significant differences. The small amounts of protein isolated from the membranes of parsley, relative to the initial fresh weight of the material, are due to the presence of gum resin that complicated the isolation of the chloroplasts.

LHCP and a few other membrane proteins were extracted from thylakoid membranes by treatment with chloroform-methanol (C:M, 2:1, v/v), (Fig. 1). Apparently, the majority of the membrane proteins are not soluble in C:M. A quantitative extraction of the LHCP is not accomplished under these conditions. However, taking advantage of its solubility and the capability to bind chlorophyll, it is possible to identify the 25,000 molecular weight band as LHCP. The results are in agreement with those of Chua et al. (1975).

In order to compare the precursor proteins of the LHCP (preLHCP), poly(A)RNA fractions of the three plant species were translated in wheat germ and reticulocyte cell-free systems. Those translation products of the poly(A)RNA, which presumably represent preLHCP, were precipitated by antibodies against the LHCP. The presumptive preLHCPs of the three species under investigation apparently have the same molecular weight. This finding compares well with the similarity in molecular weight of the mature LHCP of these species. As has recently been shown for the preLHCP of barley (Apel and Kloppstech 1978), the precursor proteins of pea and parsley also possess an additional peptide chain with a molecular weight of about 4,000. No significant difference was detected when the RNA was translated in the wheat germ or reticulocyte system (Fig. 2, tracks 2 and 3). The concurrence between the results obtained...