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THE NATURE OF THE LIGHT-HARVESTING COMPLEX AS DEFINED BY SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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Reelectrophoresis of the oligomer form (CP II*) of the chlorophyll *a/b* light-harvesting complex (LHC) from the green alga *Acetabularia* yields two green bands which run at the position typical of the monomer (CP II). The upper green band (CP II_1) is enriched in the 27 kDa polypeptide of the LHC, while the lower is enriched in the 26 kDa polypeptide. The fact that both bands have both chlorophyll (Chl) *a* and *b*, and in the same ratio, implies that the LHC is made up of two Chl *a/b* proteins. Neither of these bands can be attributed to the Chl *a/b* complex 'CP 29' (Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432). Resolution of CP II_1 and CP II_2 of spinach can be obtained if sucrose gradient fractions of an octylglucoside extract are subjected to SDS-polyacrylamide gel electrophoresis. CP II_1 and CP II_2 are interpreted as being fundamental subunits of the light-harvesting complex as it is defined on SDS-polyacrylamide gels.

Introduction

The light-harvesting Chl *a/b* complex (LHC) accounts for approx. 50% of the chlorophyll in the thylakoid membrane [1] and most, but not all, of the Chl *b* [2–4]. It has no photochemical activity, but is believed to function primarily as an antenna for Photosystem II [5]. It is absent from pea seedlings grown under intermittent light [6,7] and from mutants deficient in Chl *b* synthesis [5], although both of these have high rates of both Photosystem I and Photosystem II activity. It has been identified as the outer shell of 140 Å particles found on the exoplasmic fracture face of thylakoid membranes [8,9]. It is believed to be involved in the regulation of cation-dependent energy distribution between the two photosystems [10,11]. It is also thought to be involved in the formation of grana stacks, but the evidence for that is somewhat equivocal, since mutants of barley and pea which lack Chl *b* still show some grana formation [12].

In spite of all that is known about the light-harvesting complex, there is still no agreement about its subunit composition [13]. Much of the controversy is due to the fact that 'LHC' is an operational definition. The LHC was originally defined as the major chlorophyll-protein complex, usually called 'CP II', separated by SDS-polyacrylamide gel electrophoresis from thylakoids solubilized with SDS or sodium dodecyl benzenesulfonate [1]. Defined in this way, it is reported to have one [14,15], two [2,16,17] or more [18–20] polypeptides. On the other hand, if the LHC is defined as the Chl *a/b* complex purified from Triton extracts by sucrose gradient centrifugation and cation precipitation, it contains two major polypeptides and one minor one [21,22]. If the Triton extract is fractionated on controlled-pore glass, it has one major polypeptide [23]. The com-

Abbreviations: Chl, chlorophyll; LHC, light-harvesting Chl *a/b* complex; CP II*, predominant oligomeric form of LHC on SDS-polyacrylamide gels

plex isolated from digitonin extracts using isoelectric focussing and column chromatography also contains a single polypeptide [24]. Since these methods employ different detergents and different separation techniques, the fragments of membrane they sequester may quite legitimately be somewhat different.

This paper is concerned with the LHC as defined on SDS-polyacrylamide gels under conditions which optimize preservation of chlorophyll-protein complexes from the green alga *Acetabularia* and a number of higher plants [3,25]. In the course of studying the dissociation of the LHC oligomer (CP II*) from *Acetabularia*, we found that two green bands could be resolved at the position of the monomer (CP II). It was therefore possible to ask if one or the other chlorophyll was preferentially associated with one of the green bands, and whether these two chlorophyll-protein complexes plus CP 29, another Chl *a/b* complex [3], would account for all the observed heterogeneity in the CP II band.

Methods

Cultivation of *Acetabularia cliftonii* [26], preparation of washed chloroplast membranes [3,26], octylglucoside solubilization [3,4] and SDS-polyacrylamide gel electrophoresis [3,4,26] were as previously described. Spectra were obtained directly from gel slices containing the relevant complex [4]. For polypeptide analysis, the gel slice was dispersed in a small volume of 2% SDS, 65 mM Tris-HCl (pH 6.8), 10% glycerol, 5 mM dithiothreitol and boiled for 60–90 s.

For the experiments shown in Figs. 4 and 5, the membranes were suspended in 65 mM Tris-HCl (pH 6.8), 10% glycerol, 5 mM dithiothreitol, SDS added to give an SDS/Chl ratio of 20, and the complexes resolved by electrophoresis in the cold on a 7.5% polyacrylamide gel [26]. The free pigment front was 6.5 cm from the start of the running gel when the run was terminated. For reasons which had nothing to do with the subject of this paper, the membranes of this particular experiment had been washed with 10 mM $Na_4P_2O_7$-HCl (pH 7.6) [27], containing the protease inhibitors *p*-aminobenzamidine (6 mM), phenylmethylsulfonyl fluoride (1 mM) and ϵ-aminocaproic acid (4 mM). Electrophoresis sample buffer contained the same inhibitors. However, we have no evidence that these inhibitors have any effect on chlorophyll-protein complexes, and CP II* resolved into CP II_1 and CP II_2 in experiments where they were not used.

For separation of the two LHC monomer forms, the oligomer band (CP II*) was cut into small pieces and a small amount (about 5 μg Chl) was loaded into a 1.5×10 mm sample slot of a 10% polyacrylamide gel topped by a 20–25 mm 5% stacking gel. Best results were obtained with a low level of current (15 mA per 125×1.5 mm slab). Gels were run for about 6 h at 4°C.

To demonstrate the spinach monomers, an octylglucoside extract [3] was first layered on a 10–40% sucrose gradient containing 2 mM Tris-maleate (pH 7.0) and 30 mM octylglucoside, and centrifuged at $100000 \times g$ for 16 h. Fractions were removed with a pipette and applied directly to a cold SDS-polyacrylamide gel for separation of complexes.

Results and Discussion

The dissociation of the Acetabularia oligomer

The *Acetabularia* LHC oligomer produced by SDS solubilization was purified on preparative gels by Apel [28] and shown to contain two polypeptides of similar amino acid composition and antigenicity, which had similar but not identical peptide maps. His data suggested that only one of them bound chlorophyll. However, when *Acetabularia* CP II* is reelectrophoresed under conditions which minimize loss of chlorophyll (4°C, dark), some of it breaks down to give two green bands at the monomer position, labelled II_1 and II_2 (Fig. 1b). The original oligomer has a characteristic LHC spectrum (Fig. 1a) and the spectrum of the oligomer remaining after the second electrophoresis is similar (Fig. 1c). The spectra of the two monomer bands are identical to each other and appear to have lost some Chl *a*. Similar results were obtained with oligomer from either SDS- or octylglucoside-solubilized membranes, although complexes tended to be more stable and lose less Chl *a* if they were originally solubilized using octylglucoside. Preferential loss of Chl *a* has been reported by others [15], and may suggest that Chl

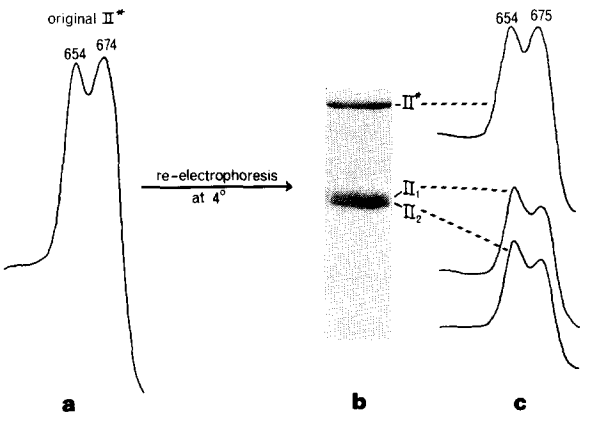

Fig. 1. Reelectrophoresis of LHC oligomer from *Acetabularia cliftonii*. Washed membranes solubilized with SDS and subjected to a first electrophoresis on 7.5% acrylamide/0.1% SDS. (a) Spectrum of 7.5% gel slice containing oligomer (CP II*). (b) Oligomer reelectrophoresed on 10% acrylamide in the cold. Unstained gel. (c) Spectra of gel slices containing the respective green bands from 10% gel.

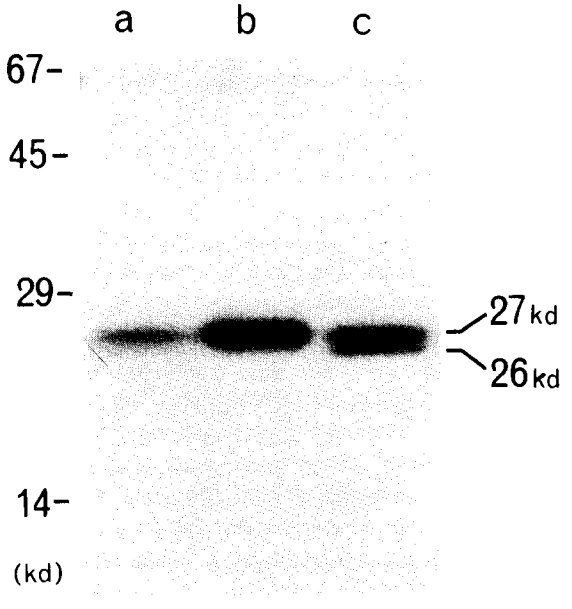

Fig. 2. Polypeptides of *Acetabularia* LHC oligomer and monomer bands. (a) Oligomer (CP II*), (b) CP II_1, (c) CP II_2. Complexes dissociated by heating in 2% SDS and electrophoresed on 10% acrylamide/0.1% SDS. Molecular mass markers: bovine serum albumin, (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), ribonuclease (13.7 kDa). Stained with Coomassie blue. kd, kilodalton.

a is located more to the surface of the complex [29,30] and is thus more susceptible to detergent.

When the bands were cut out of the gel in Fig. 1 and the complexes dissociated by heating in 2% SDS, CP II* was found to contain polypeptides of 26 and 27 kDa (Fig. 2) as previously reported [26,28]. Assuming that both polypetides stain equally well with Coomassie blue, the ratio of 27 to 26 kDa polypeptide ranged from 2:1 to 3:1 in various samples of CP II*. The CP II_1 band was enriched for the 27 kDa polypeptide, while the CP II_2 band contained about equal amounts of the 27 and 26 kDa ones.

The most interesting thing about the two monomer bands is that they have identical Chl *a/b* ratios. Considering the fact that the two monomers were not completely resolved, the enrichment of each green band with one polypeptide strongly suggests that each monomer represents one polypeptide plus its bound chlorophyll. This means that both polypeptides bind both chlorophylls to the same extent, i.e., it is not a case of one binding Chl *a* and the other Chl *b*. CP II* is therefore composed of two Chl *a/b*-protein complexes.

The population of the Acetabularia CP II band

Fig. 3a shows a typical result of an SDS solubilization (SDS/Chl = 20) followed by electrophoresis on a 7.5% polyacrylamide gel containing 0.1% SDS. Note that the CP II band is not homogeneous in density. In order to determine the species present in this region, it was sliced into an upper, a middle and a lower section, labelled fractions 1, 2 and 3, respectively. The spectra are shown in Fig. 3b. Fraction 1 had a Chl *a/b* ratio greater than unity, fractions 2 and 3 had spectra typical of LHC, with lower Chl *a/b* ratios than fraction 1.

When the three fractions were reelectrophoresed on a 10% gel, each was resolved into two green bands (Fig. 4). The upper band from reelectrophoresis of fraction 1 migrated behind the two bands from CP II*. Its spectrum was typical of CP 29 [3,4]. The lower band, however, had a spectrum typical of LHC. The presence of CP 29 in fraction 1 explains the higher Chl *a/b* ratio of the latter. The two green bands from fractions 2 and 3 had identical spectra which were very similar to those of CP II_1 and CP II_2 (Fig. 1).

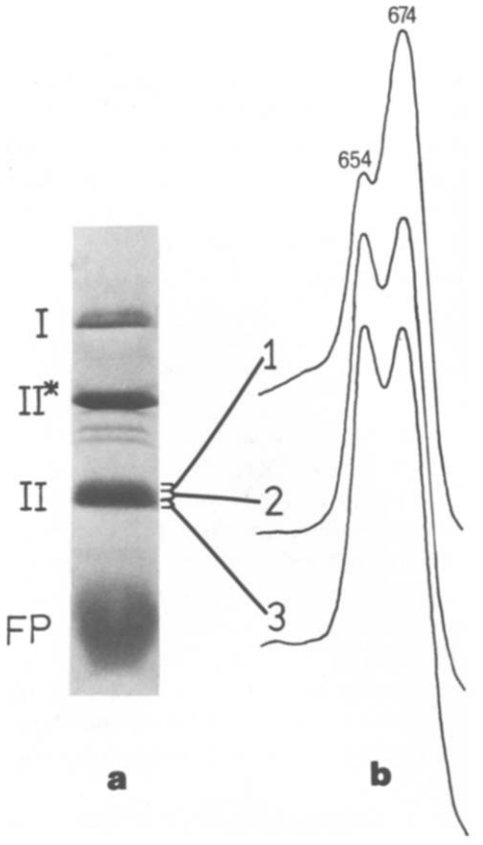

Fig. 3. CP II region: First electrophoresis. (a) First electrophoresis of SDS-solubilized washed membranes on 7.5% acrylamide. Unstained gel. The CP II region was sliced into three fractions as marked. FP, free pigment. The three minor bands between II* and II are the dimer of CP 29 (upper band) [25], and the two minor Chl *a* complexes (lower bands). (b) Spectra of the three fractions.

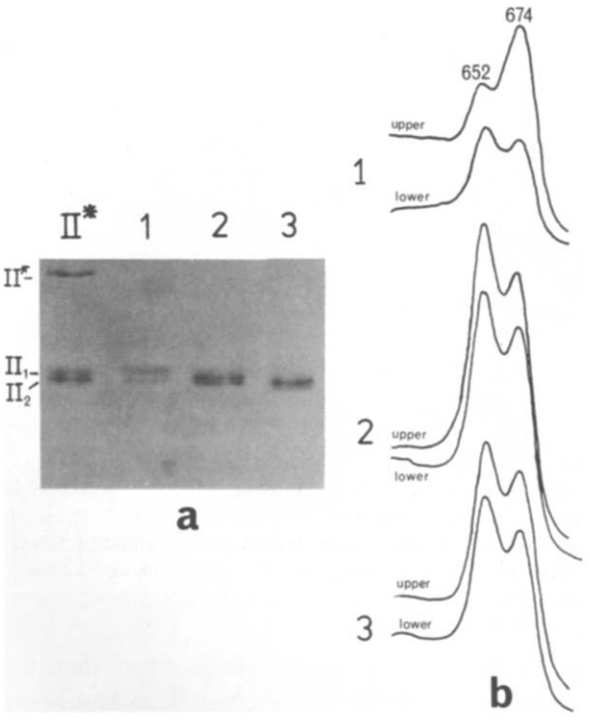

Fig. 4. CP II region: Second electrophoresis. (a) Reelectrophoresis (on a 10% gel) of fractions 1, 2 and 3 from 7.5% gel of Fig. 3. Unstained. (b) Spectra of upper and lower bands resulting from reelectrophoresis of the three fractions.

It should be noted that to get a visible separation of green bands in the CP II region, it is essential to apply a rather small amount of chlorophyll to each gel slot. Any more than 3–5 μg Chl per 1.5 × 10 mm slot leads to single broad, blurred bands having a very heavy leading edge at the same position, even the one from reelectrophoresis of fraction 1. In initial experiments, this gave the impression that parts of the original CP II band were reversing their positions.

The polypeptides of each subfraction were examined (Fig. 5). The upper band from reelectrophoresed Fraction 1 contained the 29 kDa polypeptide of CP 29 as well as two other polypeptides. The lower band from fraction 1 contained the 27 kDa polypeptide of the LHC as expected, as well as a 25 kDa polypeptide which was the major polypeptide in the section of gel between the two green bands marked '–' in figure). It is not surprising to find a lower molecular mass polypeptide in this fraction, because polypeptides with chlorophyll attached migrate faster than their apoproteins on 7.5 or 10% gels [26], and in the green gel, CP II_1 would have been comigrating with the 25 kDa polypeptide.

The 25 kDa contaminant was also found in the upper band from reelectrophoresed fraction 2. Both bands showed the polypeptide profiles expected if fraction 2 is mainly CP II_1, i.e., the upper band almost exclusively 27 kDa and the lower mainly 27 kDa. A sample of fraction 3 which was not reelectrophoresed (second lane from right) had the profile expected if it was enriched in CP II_2 but still contained CP II_1, i.e., it contained about equal

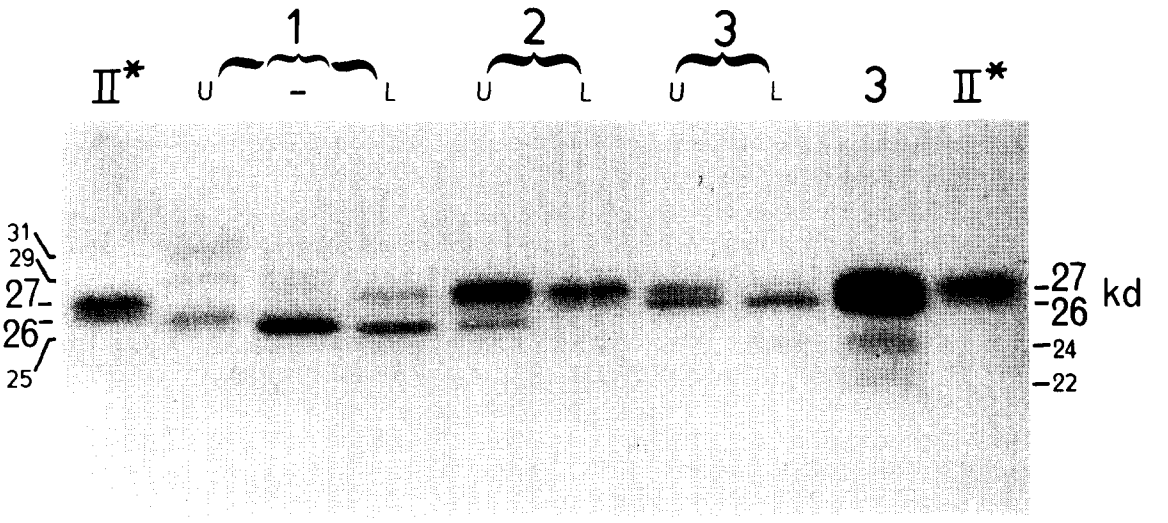

Fig. 5. Polypeptides of fractions from fig. 4. U, upper; L, lower. –, region of gel between upper and lower bands of fraction 1 in Fig. 5. '3' without subscript was part of the original fraction 3 sample (Fig. 3) which was not subjected to reelectrophoresis. Conditions as in Fig. 2.

amounts of the two polypeptides rather than the 2:1 or 3:1 ratio of CP II*, as well as two lower molecular mass contaminants. After reelectrophoresis of fraction 3, the upper band subfraction contained both polypeptides and the lower one was highly enriched in the 26 kDa polypeptide.

This supports the idea that CP II_1 is the 27 kDa polypeptide plus its chlorophyll, which migrated originally at an apparent molecular mass of 25 kDa, and CP II_2 is the 26 kDa polypeptide plus chlorophyll, with an apparent molecular mass of 23–24 kDa [26]. These two complexes and CP 29, as well as various colorless polypeptides, are al present in the so-called CP II region, and vary in concentration across it. The upper part of CP II (fraction 1, Fig. 3) contains CP 29, CP II_1 and colorless polypeptides. The middle part of the CP II region (fraction 2) contains CP II_1, some CP II_2 and another polypeptide. The lower part (fraction 3) contains some CP II_1, most of the CP II_2, and some lower molecular mass polypeptides.

In Fig. 4, the upper and lower green bands from fraction 3 seem to have electrophoresed ahead of CP II_1 and CP II_2 from CP II*, even though they are similar to the latter complexes in polypeptide composition and in Chl *a/b* ratio. This was not observed in other experiments and is probably due to an irregularity in the sample slot in this particular gel.

Resolution of spinach CP II_1 and CP II_2

It has not been possible to demonstrate CP II_1 and CP II_2 by electrophoretic dissocation of CP II* from any higher plant we have examined.

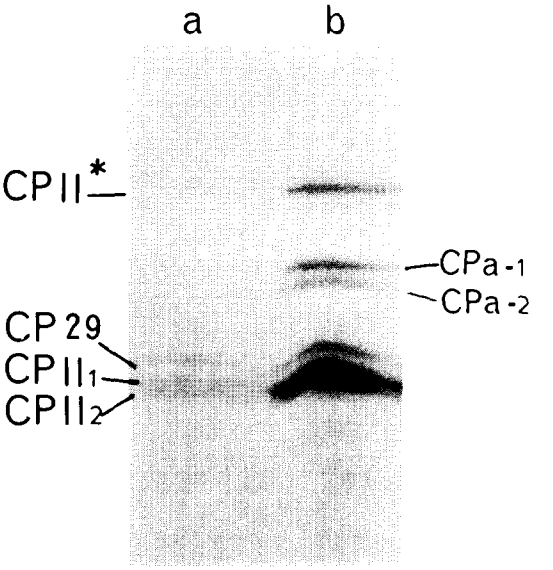

Fig. 6. Electrophoresis of sucrose gradient fractions from spinach. An octylglucoside extract was layered on a 10–40% sucrose gradient containing 30 mM octylglucoside and centrifuged at 100000 × *g* for 16 h. Fractions from: (a) top of gradient, (b) midgradient. CPa-1 and CPa-2 are the two Chl *a* complexes thought to be part of the Photosystem II core [3,22].

However, if an octylglucoside extract of spinach [3] is first fractionated on a sucrose gradient, then the complexes separated by electrophoresis, two bands can be seen at the CP II position (Fig. 6). Lane a is a fraction containing only LHC monomer and CP 29:CP29, CP II_1, and CP II_2 are all clearly separated. Lane b is a fraction comparable in composition to the original octylglucoside extract. It contains CP II* as well as the two minor Chl *a* complexes [3,22], and a partial separation of CP II_1 and CP II_2 can be seen. This separation can only be seen if the gels are lightly loaded. It is not seen if the gradient fractions have been frozen or stored at 4°C.

The resolution of CP II_1 and CP II_2 probably depends on a fortuitous combination of molecular properties, concentration and gel characteristics. It is not due to proteolysis, since the *Acetabularia* complexes were isolated in the presence of protease inhibitors, and both polypeptides were present in the original CP II* sample.

Conclusions

The light-harvesting complex in vivo is thought to consist of a number of chlorophyll-protein subunits clustered around a Photosystem II core [31]. The experiments reported here show that the fundamental subunits of the *Acetabularia* LHC are two related polypeptides of slightly different molecular mass, both of which bind both Chl *a* and Chl *b*. Although we were unable to accumulate enough spinach CP II_1 and CP II_2 to do a polypeptide analysis, we have already shown [3] that both CP II* and the unresolved CP II band contain two polypeptides. This suggests that spinach LHC may also be composed of two Chl *a*/*b*-protein complexes.

As noted in the Introduction, there is still considerable debate over the polypeptide composition of the LHC. The best resolutions published to date [2,3,16,22,26] favor the interpretation that the light-harvesting complex as defined on gels contains two polypeptides, but the possibility of additional polypeptides which could be resolved by other gel systems cannot be ruled out. It has recently been reported [32] that *Chlamydomonas* LHC consists of five or six different polypeptides. However, this was based on the use of the CP II region of a gel, and the region was not fractionated before reelectrophoresis. As our Fig. 5 shows, reelectrophoresis on a different concentration of acrylamide is not sufficient to remove comigrating nongreen polypeptides. In our experience, it is important to start with the oligomer form of LHC to avoid extraneous polypeptides, and to be very careful not to overload the gel in the CP II region because of the effects of localized heating on the rate of migration of chlorophyll-protein complexes. It would be most interesting to reexamine the *Chlamydomonas* LHC using our methods, especially since CP 29 has not been reported in that organism.

The complex CP 29 is not part of the LHC as defined on SDS gels. However, this does not mean that it is not part of the LHC as defined in some other operational mode. Physicochemical evidence indicates that all the Chl *b* in the plant plays a light-harvesting role [1], and we have hypothesized elsewhere that CP 29 is an internal antenna in Photosystem II [33]. CP 29 appears to be coextracted with the LHC and to sediment with units of the LHC in a sucrose gradient (Fig. 6). In the absence of a functional assay for light-harvesting activity, the LHC isolated by other techniques may well include CP 29 or other polypeptides.

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