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# THE RELATIONSHIP BETWEEN CHLOROPHYLL–PROTEIN COMPLEXES AND CHLOROPLAST MEMBRANE POLYPEPTIDES

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## SUMMARY

The profile of polypeptides obtained by sodium dodecylsulfate–polyacrylamide gel electrophoresis of green, lipid-containing chloroplast membrane preparations has been compared with that obtained following the electrophoresis of lipid-extracted membrane preparations. We show that two of the Group II polypeptides whose molecular weight is in the range of 30 000 are part of the light-harvesting chlorophyll–protein complex, and that they are seen upon the electrophoresis of both types of preparations. From the study of mutant strains of *Chlamydomonas*, *Hordeum* and *Pisum* in which there are little or no grana formation we propose that the Group II polypeptides are associated with the capacity of chloroplast membranes to stack and that these polypeptides are active at the chloroplast membrane surface both in establishing the position of the light-harvesting chlorophyll and the contacts between membranes. We have also studied the polypeptides that are associated with the chlorophyll–protein complex of Photosystem I. The 110 000-dalton polypeptide seen upon the electrophoresis of lipid-containing preparations is not detected in the electrophoresis of lipid-free preparations; two Group I polypeptides of molecular weight around 60 000 appear instead. We show here that these polypeptides are derived from the 110 000-dalton polypeptide of the Photosystem I chlorophyll–protein complex.

Studies of wild-type and mutant strains presented here and elsewhere show that the two polypeptides are not involved in membrane stacking. They may, however, play a role in establishing the site of the chlorophyll *a* of Photosystem I.

## INTRODUCTION

The electrophoresis of chloroplast membranes on sodium dodecylsulphate–polyacrylamide gels results in the separation of at least 16 polypeptides [1–7]. With lipid-extracted membranes, it has been shown that one group of the polypeptides (Group I) is associated with a membrane fraction enriched for Photosystem I activity,

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whereas another group of polypeptides (Group II) is found in a membrane fraction enriched for Photosystem II activity [4, 7]. For the most part the function of these polypeptides is unknown, though it appears that some of those of Group II are required for stacking of the chloroplast membranes into grana [6, 7].

In this paper we have compared the polypeptide profiles obtained on electrophoresis of green, lipid-containing chloroplast-membrane preparations with those of lipid-extracted membrane preparations. We show that two of the polypeptides of Group II are part of the chlorophyll–protein complex of Photosystem II [8–10], which has been renamed the light-harvesting chlorophyll–protein complex by Thornber and Highkin [11]. We also suggest that the two main polypeptides of the Group I region seen on electrophoresis of polypeptides from obtained lipid-free membranes are derived from the chlorophyll–protein complex of Photosystem I [8, 9, 12].

## METHODS

Wild-type and mutant strains of barley (*Hordeum vulgare* L.) [13, 14] and pea (*Pisum sativum* L., var. Greenfeast) [15] were grown in vermiculite as described previously [7]; spinach was obtained from the local market. Chloroplasts were isolated and either lysed in distilled water and washed in buffer and 1 mM EDTA as previously described [7] to give EDTA-washed chloroplast membrane fragments, or washed three times by suspension in 50 mM Tris buffer, pH 8.0, containing 2 mM $MgCl_2$ and centrifugation at $10\,000 \times g$ for 15 min.

*Chlamydomonas reinhardi* wild-type cells (strain 137C) were cultured in 250 ml of Tris–acetate–phosphate medium under standard conditions [16] with sodium [$^{14}$C]acetate (0.3 mCi) for 48 h. The cells were harvested in the logarithmic phase of growth and chloroplast-membrane fragments were prepared by the procedure of Hoober [1].

The washed chloroplast membranes from these various sources were homogenized in 50 mM Tris buffer, pH 8.0, containing 2 mM $MgCl_2$ and 0.5 % sodium dodecylsulphate using a Potter–Elvehjem homogenizer; the final ratio of sodium dodecylsulphate/chlorophyll was 10/1 (w/w) and the extract was kept at 4 °C. Any insoluble material was removed by centrifugation at $15\,000 \times g$ for 15 min.

One aliquot of the green extract of solubilized chloroplast membranes was used for electrophoresis directly (0.25 M 2-mercaptoethanol and sucrose were added to the sample [1]). A second aliquot was extracted twice with acetone (90 % final concentration), and the precipitated protein was then solubilized in a 2 % sodium dodecylsulphate–urea buffer as described by Hoober [1] except that in order to immediately inactivate proteolytic enzymes the protein was heated in a boiling water bath for 90 s instead of longer treatment used by Hoober [1]. The green solution and the colorless lipid-free solution of chloroplast membrane polypeptides were analyzed by electrophoresis using the procedures of Hoober [1]. Current for the electrophoresis was provided by a pulsed power supply operating at 160 V, 25 mA and 105 pulses/s; the electrophoresis was performed in a coldroom at 4 °C.

Chlorophyll was determined by a modification [17] of the procedure of Mackinney [18] and protein by the method of Lowry et al. [19] using bovine serum albumin as a standard. For the determination of radioactivity, gels were sliced by hand into 1 mm slices which were incubated overnight at 40 °C in 10 ml of toluene

containing 0.4 % omnifluor and 0.3 % protosol. The slices were counted in a Beckman scintillation counter.

## RESULTS AND DISCUSSION

Fig. 1A shows the densitometric scan of a polyacrylamide electrophoretogram of the green, lipid-containing solution of solubilized membrane polypeptides from spinach chloroplasts stained with Coomassie blue. It has been shown with lipid-free chloroplast membranes that the polypeptides in the gel denoted as Group I are associated with a membrane fraction enriched in Photosystem I activity, whereas those denoted as IIa, IIb and IIc are associated with a membrane fraction enriched for Photosystem II activity [4, 7].

Three major green bands, occupying the positions in the gels indicated at the bottom of the scan, appear in preparations which have not been previously extracted with acetone. The slowest running band corresponds to the chlorophyll *a*–protein complex of Photosystem I [9, 12] and is coincident with a single polypeptide. The wider green band corresponds to the light-harvesting chlorophyll–protein complex [11], previously called the chlorophyll–protein complex of Photosystem II [8], and is coincident with two polypeptides, IIb and IIc. Note that the ratio IIb/IIc is less than 1. The broad green band running to the gel front corresponds to "free pigment"

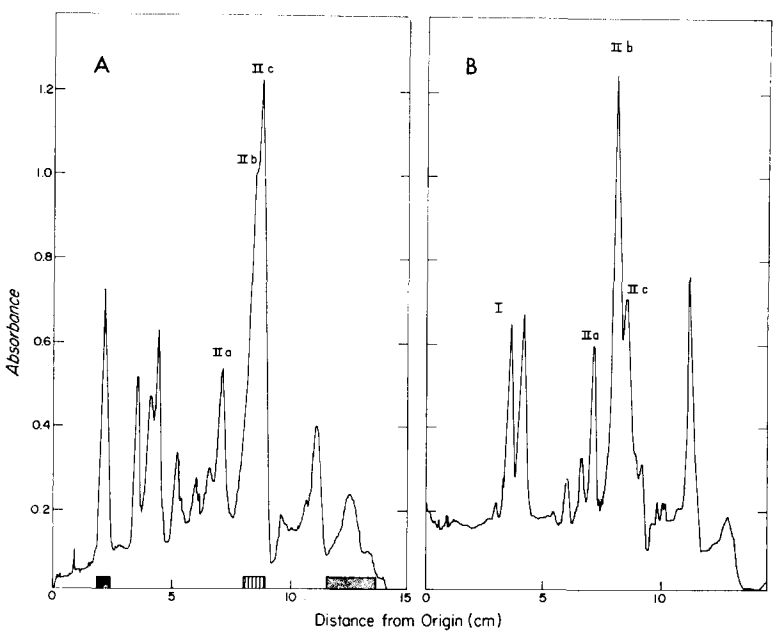


Fig. 1. Sodium dodecylsulphate–polyacrylamide gel electrophoresis of membrane polypeptides from spinach chloroplasts. The gels were stained with Coomassie blue and scanned at 530 nm. I refers to the region of Group I polypeptides and IIa, IIb, and IIc refer to the Group II polypeptides [4, 6, 7]. (A) Gel scan of lipid-containing preparation of polypeptides. The symbols on the abscissa refer from left to right to the positions of the chlorophyll *a*–protein complex of Photosystem I, the light-harvesting chlorophyll–protein complex, and "free pigment". (B) Gel scan of lipid-free preparation of polypeptides.

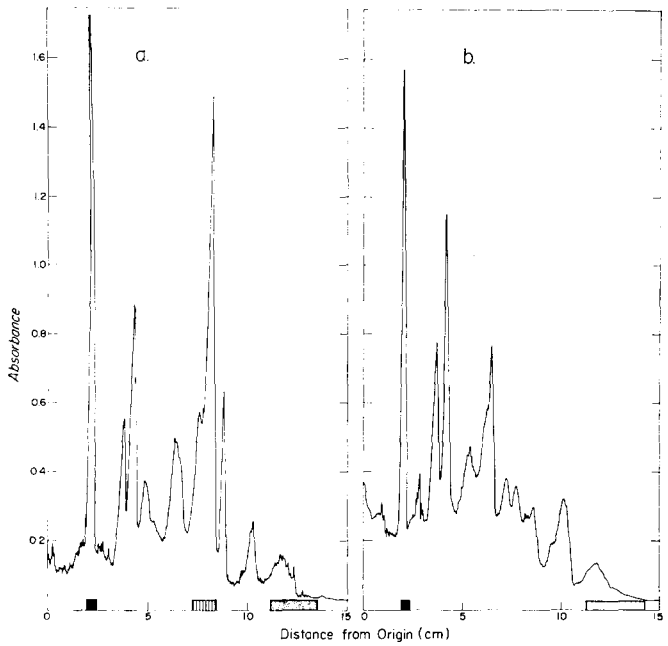


Fig. 2. Gel scans of lipid-containing preparations of chloroplast-membrane polypeptides obtained from *Hordeum*. (a) Wild-type strain. (b) Chlorophyll-deficient mutant strain. The symbols are as in Fig. 1.

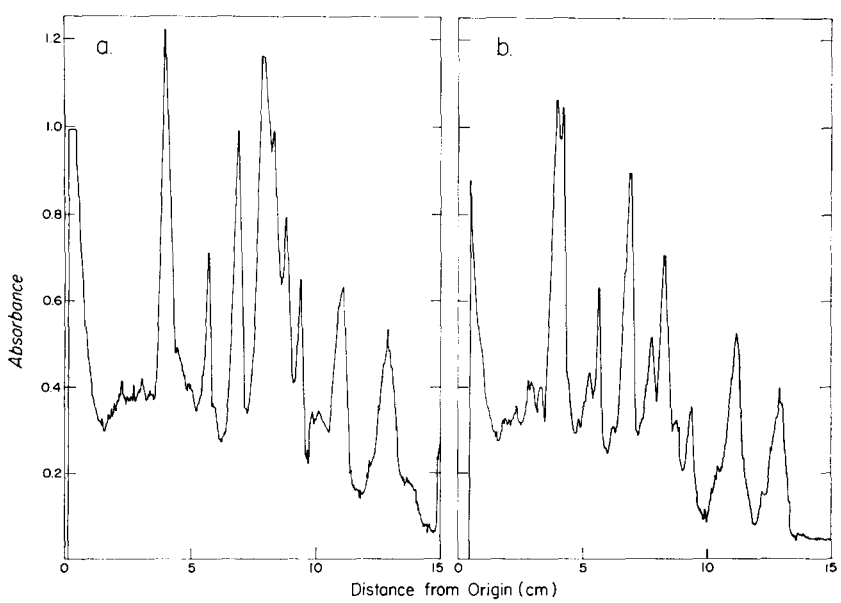


Fig. 3. Gel scans of lipid-free preparations of chloroplast-membrane polypeptides obtained from *Hordeum*. (a) Wild-type strain. (b) Chlorophyll-deficient mutant strain.

and consists of chlorophylls, carotenoids, and other lipids complexed to sodium dodecylsulphate [8].

Fig. 1B shows the results of the electrophoretic analysis of the lipid-free chloroplast membrane polypeptides of spinach. The Group I and Group II polypeptides are prominent but, in contrast to the lipid-containing preparation, the ratio IIb/IIc is much greater than 1. In addition, the high-molecular weight polypeptide of the chlorophyll–protein complex of Photosystem I, seen in Fig. 1A, is absent.

Recently, we have shown [4] that the chlorophyll-deficient mutant strain of barley [13, 14], which lacks chlorophyll *b* and yet has good Photosystem II activity [20], lacks Polypeptides IIb and IIc, and the chlorophyll-deficient mutant strain of pea [15] which also has good Photosystem II activity, is deficient in these two polypeptides compared to the wild-type strain. The chloroplast membranes of both mutant strains are extensively unstacked in comparison to those of their respective wild-type strains. Therefore, we have compared polypeptides from green, lipid-containing preparations with those from lipid-free preparations in order to verify that they, and in turn the light-harvesting chlorophyll–protein complex were affected, whereas the chlorophyll–protein complex of Photosystem I remained unaffected, by mutation. Comparison of the green lipid-containing preparations of the wild-type and mutant strains of barley (Figs 2 and 3) shows that both chloroplasts contain the high-molecular weight chlorophyll–protein complex of Photosystem I. However, the mutant-barley chloroplasts lack the Group II polypeptides which are part of the light-harvesting chlorophyll–protein complex (Thornber and Highkin [11] and Genge et al. [22] have shown that this chlorophyll–protein complex is absent in the barley mutant). Analysis of the lipid-free polypeptide profiles shows that it is Polypeptides IIb and IIc which are lacking in the barley mutant strain. Careful examination of the gel shows that the polypeptides present in this region are not coincident with either IIb or IIc. As in the case of spinach chloroplasts, the acetone-extracted preparations have lost the high-molecular weight polypeptide of the Photosystem I chlorophyll–protein complex.

Comparisons of the wild-type and mutant strains of pea are shown in Figs 4 and 5. The results parallel those described for barley except that the light-harvesting chlorophyll–protein complex and the polypeptides associated with it are reduced and not completely absent in this mutant.

An initial attempt to locate the polypeptide of the high-molecular weight chlorophyll–protein complex of Photosystem I which is absent from all acetone-extracted chloroplast preparations was made by analyzing $^{14}$C-labeled *C. reinhardi* chloroplast-membrane polypeptides before and after extraction of lipids with acetone. The electrophoretic pattern of the $^{14}$C-labeled polypeptides and the green regions of the gel of unextracted membranes are shown in Fig. 6A. It can be seen that the polypeptides of both complexes are labeled as is much of the gel region which contains the "free pigment". Fig. 6B shows results of the electrophoresis of a lipid-free preparation; the high-molecular weight polypeptide of Complex I is absent and there is the expected change in the ratio of Polypeptides IIb and IIc. The same result is seen in Coomassie blue-stained gels (Fig. 7).

The green bands corresponding to the two chlorophyll–protein complexes were cut from several gels on which labeled material had been separated. The segments were pulverized and incubated overnight in 50 mM Tris buffer, pH 8.0, con-

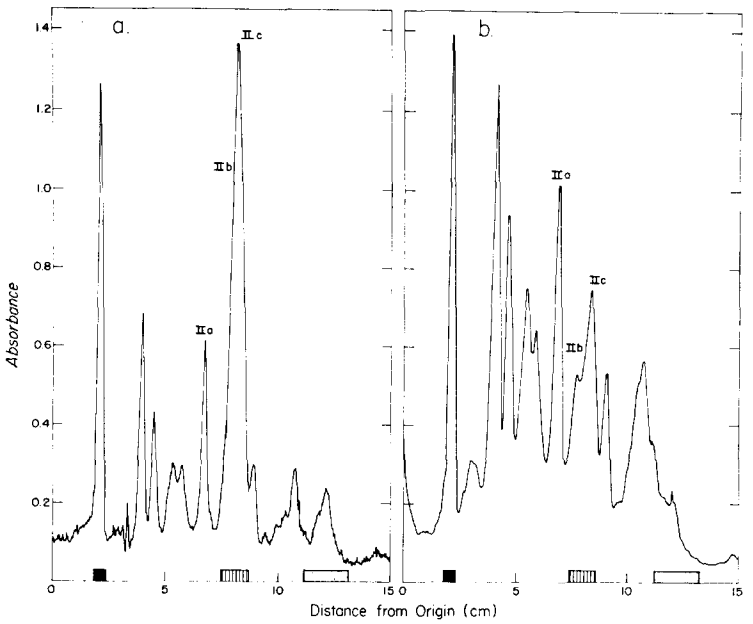


Fig. 4. Gel scans of lipid-containing preparations of chloroplast-membrane polypeptides from *Pisum*. (a) Wild-type strain. (b) Chlorophyll-deficient mutant strain. The symbols are as in Fig. 1.

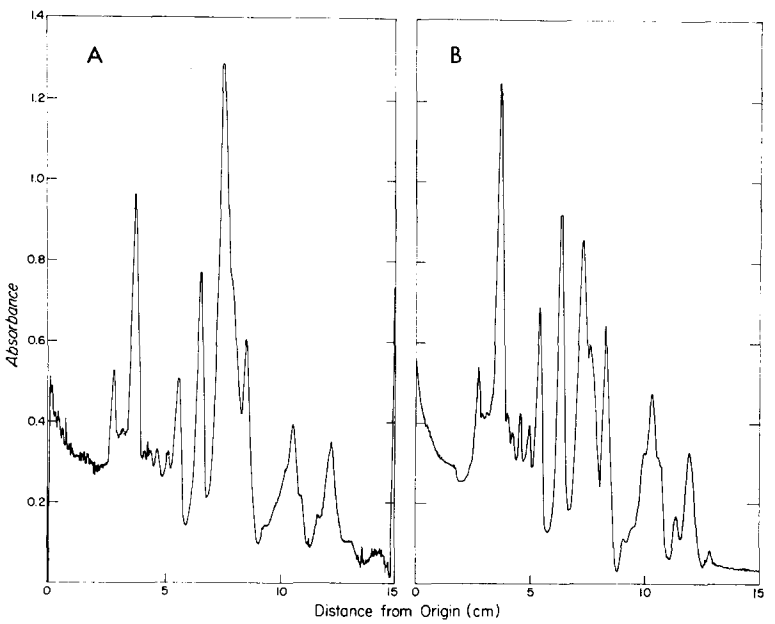


Fig. 5. Gel scans of lipid-free preparations of chloroplast membrane polypeptides obtained from *Pisum*. (A) Wild-type strain. (B) Chlorophyll-deficient mutant strain. The symbols are as in Fig. 1.

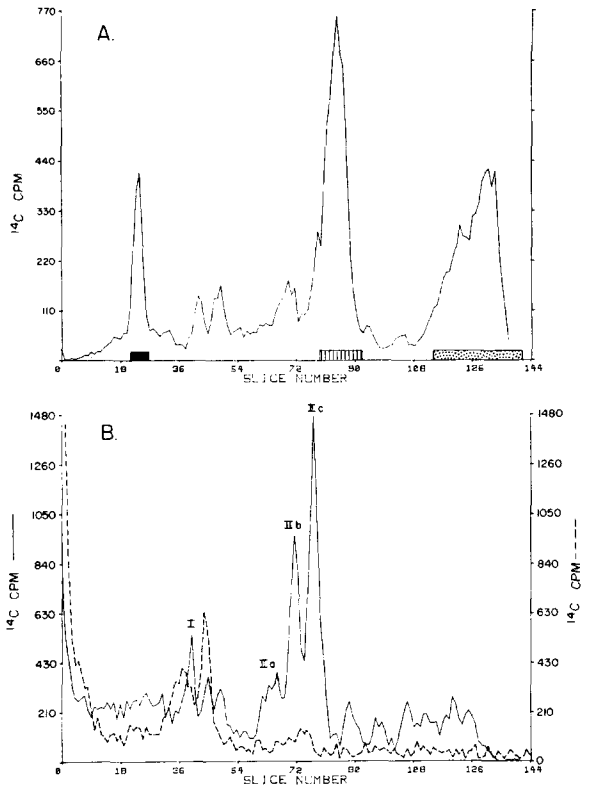


Fig. 6. (A) The electrophoresis pattern of lipid-containing, $^{14}$C-labeled chloroplast membrane polypeptides obtained from *C. reinhardi*. (B) The electrophoretic pattern of a lipid-free $^{14}$C-labeled preparation. The dotted line shows the new position of the labeled polypeptide obtained from the acetone extraction of the Photosystem I chlorophyll–protein complex shown in Part A of this figure. The symbols are as in Fig. 1.

taining 0.5 % sodium dodecylsulphate. The solutions were then treated with acetone, the protein was solubilized and subjected to electrophoresis again. As expected, removal of pigments from the light-harvesting chlorophyll–protein complex did not alter the position of the bands (Fig. 7). However, the polypeptides from the chlorophyll–protein complex of Photosystem I now appear to be coincident with two polypeptides of Group I (Fig. 6B). This finding is consistent with the observation that the Group I polypeptides are enriched in Photosystem I membrane fractions derived from the digitonin fragmentation of chloroplasts of *C. reinhardi*, spinach, barley, pea, and maize mesophyll cell chloroplasts [4, 7] and suggests that the two main polypeptides of Group I seen in the electrophoresis of acetone-extracted chloroplast membranes are derived from the chlorophyll–protein complex of Photosystem I. The area of the gel occupied by Group I polypeptides is complex and differs somewhat amongst the various species studied; moreover when the acetone extraction is performed after the membrane solubilization, the profile of Group I polypeptides differs from what is seen when the acetone extraction of the membranes preceeds membrane solubilization [7].

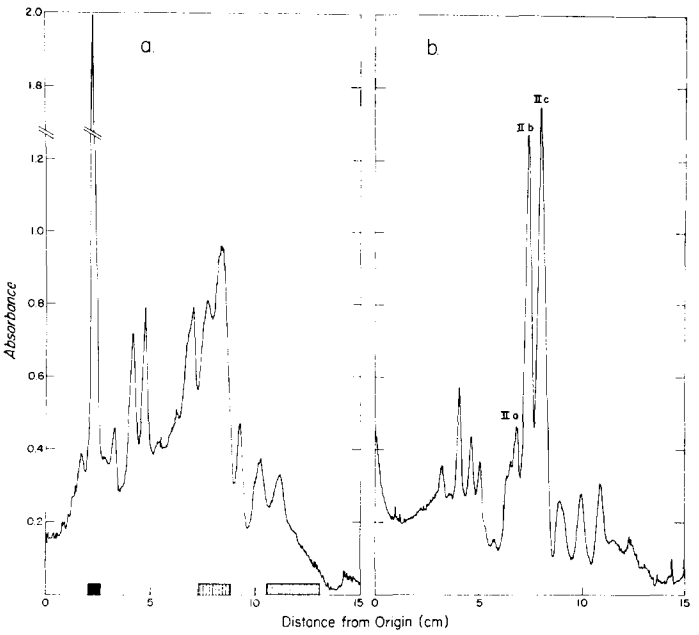


Fig. 7. Gel scans of *C. reinhardi* chloroplast-membrane polypeptides. (a) Lipid-containing preparation. (b) Lipid-free preparation. The symbols are as in Fig. 1.

The observations reported here lead us to conclude that two of the Group II polypeptides of chloroplast membranes are associated with the light-harvesting chlorophyll–protein complex. The presence of these polypeptides is also required for the formation of the stacks of membranes that are characteristic of *C. reinhardi* and higher plant chloroplasts [6, 7]. As we have shown here, the light-harvesting chlorophyll–protein complex, and in turn the two polypeptides, are affected in chlorophyll-deficient mutant strains of barley and pea. Thornber and Highkin [11] have also associated the light-harvesting chlorophyll–protein complex with membrane stacking.

The relationship between the ability of membranes to stack, light-harvesting chlorophyll, and the Group II polypeptides that predominate in membrane fractions enriched for Photosystem II activity may not be fortuitous, and one may assume that the Group II polypeptides are "active" at the surface of chloroplast membranes both in establishing the position of light-harvesting chlorophyll and the contacts between chloroplast membranes. On the other hand, the Group I polypeptides, which appear to correspond to the 100 000-dalton protein of the Photosystem I chlorophyll–protein complex, do not seem to be involved in the stacking of chloroplast membranes [6, 7]. However, this does not preclude the possibility that they function in establishing the site of the chlorophyll *a* of Photosystem I. Relevant to these assumptions is the observation (Levine, R. P., unpublished) that both the Group I and Group II polypeptides possess tyrosine residues that can be iodinated and thus portions of their polypeptides can be presumed to be exposed on the outer surface of the chloroplast membranes.

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