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Lack of recovery from photoinhibition in a temperature-sensitive *Chlamydomonas reinhardtii* mutant T_{44} unable to synthesize and/or integrate the Q_B protein of Photosystem II at 37°C

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The T_{44} temperature-sensitive mutant of *Chlamydomonas reinhardtii* was reported to lack two polypeptides associated with the reaction center of Photosystem II (44–47 kDa) when grown at the non-permissive temperature (37°C) (Shochat, S., Owens, G.C., Hubert, P. and Ohad, I. (1982) Biochim. Biophys. Acta 681, 21–31). The mutant does not synthesize chlorophyll in the dark but greens when exposed to light at both 25 and 37°C. In the present work, we found that the mutant is temperature-sensitive also for the herbicide-binding polypeptide of 32 kDa which is also considered to be the apoprotein of the secondary electron acceptor Q_B. The light-dependent degradation of 32 kDa Q_B is not impaired at 37°C. The gene transcript coding for the synthesis of this polypeptide is present in cells grown at 37°C in amounts comparable to those found in cells grown at the permissive temperature. Apparently, the T_{44} mutant cells do not integrate the 32 kDa Q_B polypeptide, either in pre-existing membranes depleted of this polypeptide by photoinhibition or during de novo synthesis of thylakoids when dark-grown cells are greened in the light at 37°C. The 32 kDa Q_B polypeptide and electron-flow from water to 2,6-dichlorophenolindophenol (DCIP) are specifically lost, while reduction of silicomolybdate is not affected by photoinhibition. Photoinhibited T_{44} cells resume their photosynthetic activity at 25°C, but not at 37°C. The inability to recover Photosystem II (PS II) activity at the non-permissive temperature correlated well with the inability of the T_{44} mutant cells to synthesize and/or integrate the 32 kDa Q_B polypeptide into the thylakoid membranes.

Introduction

The apoprotein involved in the formation of the secondary electron acceptor of PS II, Q_B, and the herbicide-binding site for PS II inhibitors of urea and triazine type appears to be the fast-turning-over 32 kDa polypeptide of thylakoid membranes [2–7]. The synthesis of this polypeptide is coded by a chloroplast gene *psb*A which was isolated and sequenced in various plants [2,8,9], *Euglena* [10] and *Chlamydomonas* [11] (for recent review, see Ref. 7). The turnover of this polypeptide of 32 kDa which is referred to as the herbicide-binding or 32 kDa Q_B polypeptide, is light-dependent and increases with the increase in light intensity [12]. It

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Abbreviations: CPa, chlorophyll *a*-protein complex of reaction center II; Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; DPC, diphenylcarbazide; LDS, lithium dodecyl sulfate; LHC II, the light-harvesting chlorophyll *a*,*b*-protein complex of Photosystem II; PQ, plastoquinone; PS II, Photosystem II; RC II, reaction center II.

has been suggested that when the rate of its degradation is faster than that of its synthesis and/or integration into the membranes, the thylakoids are depleted of the 32 kDa Q_B polypeptide and electron-flow via PS II to plastoquinone is inhibited, a phenomenon further referred to as photoinhibition [3–5, 13].

Photoinhibited cells can recover their PS II photosynthetic electron-flow when the rate of the 32 kDa Q_B polypeptide degradation is reduced, and the rate of synthesis and/or integration of the polypeptide become sufficient to replenish the lost polypeptide. This can be achieved by reducing the light intensity to normal growth levels ($\geqslant 200$ W $\cdot$ m^{-2}), and it was shown that recovery from photoinhibition in *Chlamydomonas* cells correlates well with the de novo synthesis of this polypeptide [4,5].

Photoinhibition of electron-flow via PS II, however, could also involve degradation or damage to additional polypeptides, particularly those forming the reaction center of PS II, such as the 44 and 47 kDa polypeptides of the chlorophyl *a*-protein complex, CPa, of PS II [14,15]. Indeed, following prolonged exposure to high light intensity, electron-flow via reaction center II-Q_A is also inhibited, especially in isolated thylakoids which exhibit a light-dependent degradation of the 32 kDa Q_B polypeptide in vitro [16].

To identify the primary site of damage during photoinhibition, conditional mutants such as the T_4 [17] or T_{44} [1], temperature-sensitive mutants of *Chlamydomonas* could be used, which have been reported to lack the reaction center II polypeptides of 44 and 47 kDa when grown at the non-permissive temperature. The latter mutant is also unable to synthesize chlorophyll in the dark, but greens in the light and, in this respect, is similar to the y-1 mutant [15]. If indeed these polypeptides of reaction center II are not damaged during photoinhibition and their de novo synthesis is not required for recovery of PS II activity, it would be expected that these mutants should recover from photoinhibition also at the non-permissive temperature (37°C). Therefore, we used the T_{44} temperature-sensitive mutant of *Chlamydomonas* to test this possibility. Unexpectedly, the T_{44} cells did not recover from photoinhibition at 37°C. The data presented demonstrate that this mutant is also temperature-sensitive for the synthesis and/or integration of the 32 kDa Q_B polypeptide into functional PS II units. Thus, lack of recovery from photoinhibition at 37°C in this mutant appears to be due to the absence of the Q_B protein.

Materials and Methods

Cell culture, photoinhibition, recovery and greening

Chlamydomonas reinhardtii y-1 [18] and T_{44} [1] mutant cells were grown on a mineral medium containing acetate as a carbon source, and harvested as described before [18]. The T_{44} mutant was isolated as a spontaneous mutation of the *C. reinhardtii* T_4 mutant, originally described by Chua and Bennoun [17], and it differred from the parent strain by the fact that it did not synthesize chlorophyll and photosynthetic membranes in the dark but retained the original properties ascribed to the T_4 mutant, i.e., it does not synthesize and/or integrate the reaction center II polypeptides of 44–47 kDa when grown at 37°C. When grown in the light at 25°C, the T_{44} cells are autotrophic and exhibit a chlorophyll content similar to that of the y-1 or wild-type *Chlamydomonas*.

Photoinhibition was carried out at 24°C as described previously [3–5]. The light intensity was 1.5–2.0 kW $\cdot$ m^{-2} provided by a Tungsten-halogen lamp (high light). The chlorophyll concentration of the cell suspension was 40–50 μg/ml and the optical path was 2.5 cm. For recovery from photoinhibition, the cells were washed by centrifugation, resuspended in fresh growth medium at the same chlorophyll concentration, and further incubated at 25°C at a light intensity of 60–100 W $\cdot$ m^{-2} provided by fluorescent lamps (low light).

For greening experiments, cells were grown in the dark for 4–5 days, then washed by centrifugation and resuspended in fresh growth medium at a final concentration of 10^7 cells/ml (non-dividing conditions) [18]. The cell suspension was exposed to low light with shaking at 25 or 37°C, as described earlier [19].

Pulse-labeling of cells with $^{35}SO_4^{2-}$, preparation of membranes and electrophoretic separation of thylakoid polypeptides

Radioactive labeling with $^{35}SO_4^{2-}$ was performed as described before [20]. The duration of

the pulse-labeling was 60–90 min, followed by a chase of 2–3 h. The chase was necessary to allow all the newly synthesized 32 kDa Q_B polypeptides to integrate in functional PS II units [4,21].

A thylakoid-enriched fraction was prepared as described by Owens and Ohad [22], and electrophoretic separation of thylakoid polypeptides was carried out using LDS and polyacrylamide gradients of 7–15% in the presence of 4 M urea [5]. Under these conditions, the 32 kDa Q_B polypeptide is well separated from the other proteins migrating at an apparent molecular mass of 32–34 kDa [7]. The gels were stained with Coomassie Brilliant Blue R, dried and autoradiographed by the usual procedures.

Measurements of photosynthetic activity

Reduction of silicomolybdate and DCIP were carried out as previously described [3,23]. Fluorescence kinetics measurements were performed using whole cells and the same apparatus as described by Cahen et al. [24]. Chlorophyll was determined in 80% acetone solution according to Arnon [25]. All chemicals were of analytical grade. $^{35}SO_4^{2-}$ was purchased from Amersham International, U.K.

Isolation of RNA and hybridization

Total cell RNA was isolated according to Herrin and Michaels [26], and hand dot-blotted on nitrocellulose paper. The blot was hybridized with a nick-translated-cloned probe containing a fragment of 32 kDa Q_B DNA [27].

Results

Photoinhibition and recovery of photosynthetic activity in T_{44} mutant cells

It has been shown that loss of variable fluorescence in cells exposed to high light intensity correlates with loss of photosynthetic electron-flow via Q_B [3–5] and can be used as an indication for the time-course of photoinhibition in *Chlamydomonas* cells such as y-1 and wild-type. In the present work, we used the *C. reinhardtii* y-1 cells as a reference for comparison with the T_{44} mutant.

Exposure of the T_{44} mutant cells to high light intensity at 25° results in a rapid loss of variable fluorescence in a way comparable to that observed in y-1 cells (Fig. 1). Measurements of photoreduction of silicomolybdate and DCIP, using diphenylcarbazide or H_2O as electron donors, showed that while electron-flow to silicomolybdate was unimpaired, reduction of DCIP was almost completely lost in membranes from photoinhibited. T_{44} cells (Fig. 2), as reported earlier for y-1 cells [3,4]. However, as opposed to the photoinhibited y-1 cells which recovered photosynthetic activity when incubated at low light intensity at both 25 and 37°C, the T_{44} mutant cells did not recover their activity at 37°C but only at 25°C (Fig. 1). The recovery of variable fluorescence coincides with recovery of reduction of DCIP activity (data not shown) and was almost complete within 3 h. As previously reported [4,5] recovery from photoinhibition was significantly reduced in cells incubated in the dark (Fig. 1).

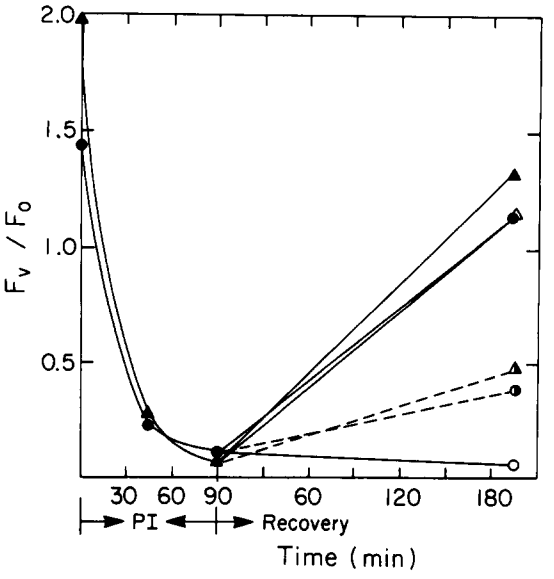

Fig. 1. Loss and recovery of variable fluorescence at 25 and 37°C in y-1 and T_{44} mutant cells of *C. reinhardtii*. Cell suspensions were exposed to photoinhibitory light intensity (PI) and then allowed to recover at low light intensity as described in Materials and Methods. ▲, △, y-1 mutant cells incubated at 25 and 37°C, respectively; ●, ○, T_{44} mutant cells incubated at 25 and 37°C, respectively; ▲, ◑ and dashed lines, y-1 and T_{44} mutant cells incubated in the dark.

Loss of 32 kDa Q_B protein during photoinhibition of T_{44} cells

For detection of protein turnover during photoinhibition, T_{44} cells were pulse-labeled with $^{35}SO_4^{2-}$

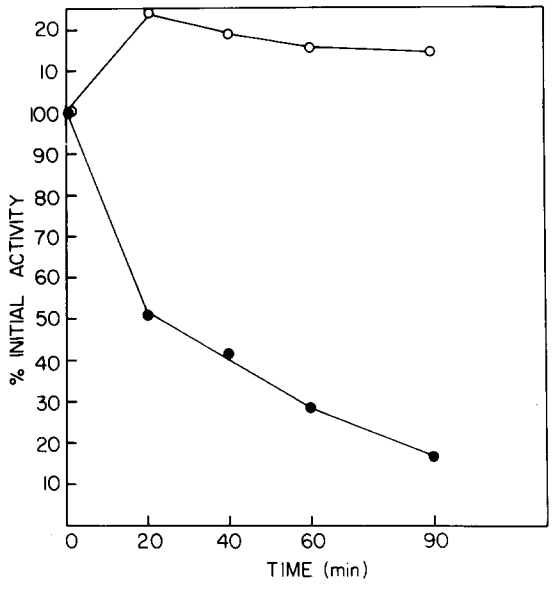

Fig. 2. Time-course of the loss of DCIP photoreduction activity during photoinhibition of T_{44} mutant cells. Cells were incubated at high light intensity. At the times indicated, samples were taken for preparation of isolated thylakoids. Photosynthetic electron-flow activity was measured as described in Materials and Methods. ○, Reduction of silicomolybdate using DPC as an electron donor; ●, reduction of DCIP using H_2O as an electron donor.

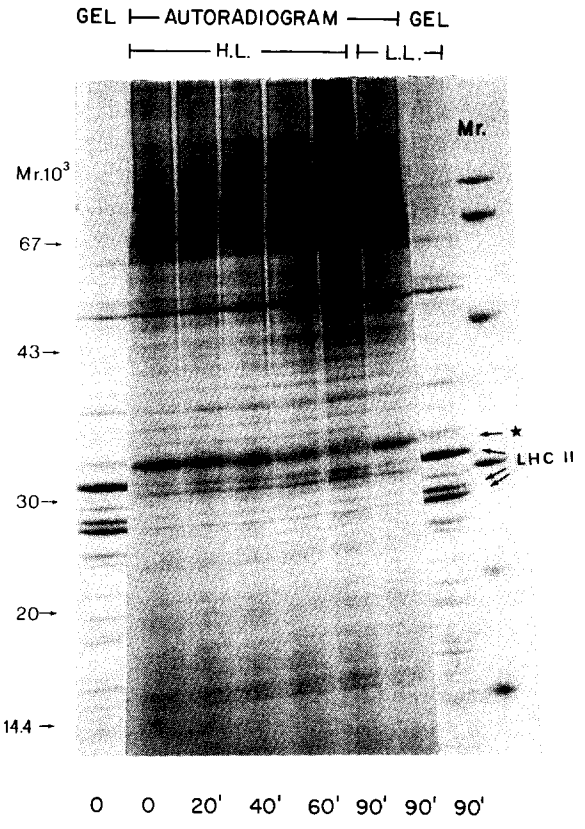

Fig. 3. Degradation of the 32 kDa Q_B polypeptide during photoinhibition of T_{44} mutant cells. Before photoinhibition, the cells were labeled with $^{35}SO_4^{2-}$ for 1 h at low light intensity and then further incubated in the presence of non-radioactive sulfate for 2h. Samples containing equal amounts of membrane were taken at times as indicated from onset of photoinhibition, and processed for resolution of thylakoid polypeptides and autoradiography. H.L., cells exposed to high light intensity; L.L., control cells exposed to low light intensity for the same duration; first (left) and last (right) lanes, stained, gel; other lanes, autoradiography; asterisk, 32 kDa Q_B; LHC II, the polypeptide of the light-harvesting complex of PS II; *M*Idr, molecular mass markers.

in low light intensity, as described before [20]. Under these conditions, the 32 kDa Q_B protein is preferentially labeled. The cells were then washed free of radioactive sulfate and subjected to photoinhibitory light at 25°C. Samples were taken at various times, and the radioactivity of the different thylakoid proteins was examined by autoradiography of the membrane proteins, following their separation by LDS-polyacrylamide gel electrophoresis in the presence of 4 M urea. The results show a progressive loss of radioactivity of the 32 kDa Q_B band in high light-exposed cells (H.L., Fig. 3). Loss of radioactivity did not occur in membranes from cells incubated in low light (L.L., Fig. 3). Quantitation of the loss of radioactivity by densitometry showed that the polypeptide was specifically lost from the membranes of T_{44} photoinhibited cells (Fig. 4). specificity was further assessed by measuring the peak intensities corresponding to bands of the 32 kDa Q_B, light-harvesting complex II (LHC II), and reaction center II (RC II) 44 and 47 kDa polypeptides, respectively, and plotting the ratios of 32 kDa Q_B/LHC II, 32 kDa Q_B/RC II and RC II/LHC II as a function of photoinhibition time (Fig. 5). The results again show specific loss of the 32 kDa Q_B relative to LHC II or RC II polypeptides but not of the RC II or LHC II relative to each other. Loss of radioactivity was light-dependent and had a time-course comparable to that of photoinhibition (Figs. 1 and 5).

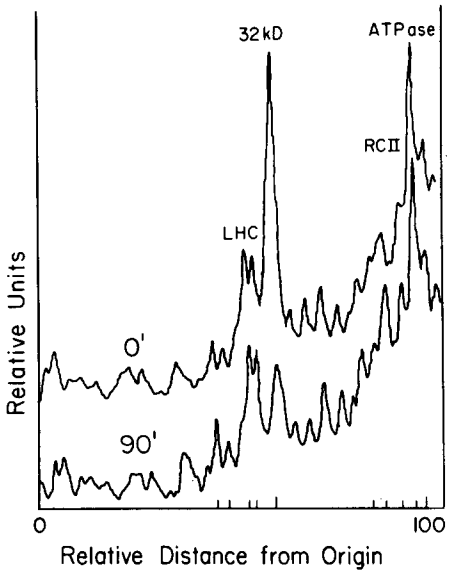

Fig. 4. Densitometry scan of autoradiograms showing specific loss of the 32 kDa QB polypeptide during photoinhibition of T_{44} cells. Same experimental conditions as in Fig. 3. Densitometry was carried out using a soft laser scanning densitometer, model SL-TRFF Biomedical Instruments, Fullerton, CA. Autoradiograms were exposed so as to ensure a linear response. 0', 90', photoinhibition for 0 and 90 min, respectively; LHC, RC II, the PS II light-harvesting and core reaction center polypeptides, respectively; ATPase, the α- and β-subunits of CFi; 32 kDa, the 32 kDa Q_B polypeptide.

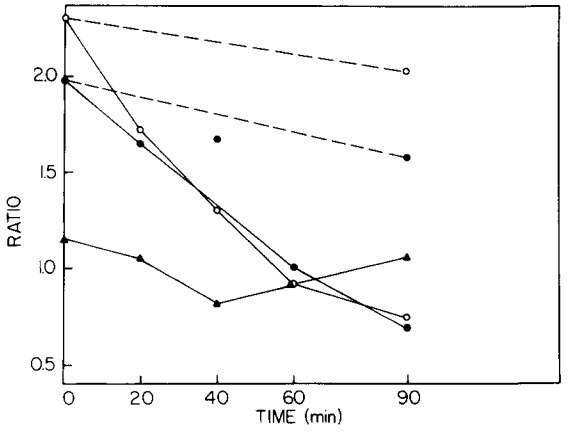

Fig. 5. Time-course of the specific loss of radioactivity of the 32 kDa Q_B polypeptide during photoinhibition of the T_{44} mutant cells. Same experimental conditions as in Fig. 3. Autoradiograms were scanned as in Fig. 4. ●, ○, Ratio of radioactivity of the 32 kDa Q_B polypeptide to that of LHC II and RC II, respectively; ▲, ratio of radioactivity of RC II to that of LHC II; continuous line, cells exposed to high light intensity; dashed line, control cells exposed to low light intensity.

Labeling of the 32 kDa Q_B at permissive and non-permissive temperatures in the y-1 and T_{44} mutant cells

The inability of the T_{44} cells to recover from photoinhibition might be due to their inability to synthesize and/or integrate into the membrane the polypeptide that was lost during photoinhibition (Fig. 3–5). To test this possibility, T_{44} and y-1 cells were photoinhibited for 90 min, as described above, then washed by centrifugation and resuspended in fresh growth medium without sulfate, and finally transferred to low light intensity at 25 or 37°C. After a 90 min incubation, $^{35}SO_4^{2-}$ was added and incubation continued for an additional 90 min period. The cells were then washed free of sulfate, membranes isolated and the radioactivity of the various thylakoid polypeptides assessed by autoradiography after LDS-polyacrylamide gel electrophoresis separation in the presence of 4 M urea. The results show that the 32 kDa Q_B polypeptide was heavily labeled at both temperatures in the y-1 cells, but only at 25°C in the T_{44} mutant (Fig. 6). In addition, polypeptides of an apparent molecular mass of 44–47 were also not labeled in the thylakoids of T_{44} cells incubated at 37°C, as reported before [1]. However, a clearly labeled band appeared at about 43 kDa in these thylakoids, which was not observed in T_{44} cells incubated at 25°C nor in y-1 cells incubated at either 25 or 37°C (Fig. 6).

Turnover of the 32 kDa Q_B protein at low light intensity

It has been reported earlier that the turnover of the 32 kDa Q_B protein of *Chlamydomonas* occurs not only at high light intensity [2–5] but also at low light intensity used normally for cell growth [21]. Furthermore, it has been suggested that the degradation of this protein is induced due to the formation of quinone radicals generated by light-driven electron-flow which may cause alteration of the polypeptide in such a way as to be recognized by a membrane-bound protease(s) and removed [4,13]. The removal of the non-functional polypeptide appears to be essential for its replacement by a functional de novo synthesized polypeptide [9].

Since the polypeptide was not labeled by $^{35}SO_4^{2-}$ at low light intensity at 37°C but was synthesized

and integrated during recovery from photoinhibition at 25°C, it might be considered that the mechanism responsible for the removal of the altered or damaged residual 32 kDa Q_B protein still present in photoinhibited membranes might be impaired at 37°C in the T_{44} cells. To test this possibility, the turnover of the protein was examined in the mutant at 25 and at 37°C. Cells were labeled in low light with $^{35}SO_4^{2-}$, as described, and incubated in low light for up to 21 h at 25°C (Fig. 7A) or 37°C (Fig. 7B). The results show that the radioactivity of the 32 kDa Q_B protein is progressively lost, albeit considerably slower than at high light intensity (compare with Figs. 3–5). A relatively significant amount of radioactivity was still present in the 32 kDa Q_B band after 7 h of exposure of the cells to low light, as reported for the y-1 cells [20]. However, its radioactivity was completely lost after 21 h of incubation (Fig. 7A). The rate of degradation was considerably faster at 37°C in the T_{44} mutant, and an almost complete loss of its radioactivity was observed after 7 h of incubation at 37°C in low light, as compared with y-1 cells which still contained significant amounts of the ^{35}S-labeled 32 kDA Q_B polypeptide (Fig. 7B).

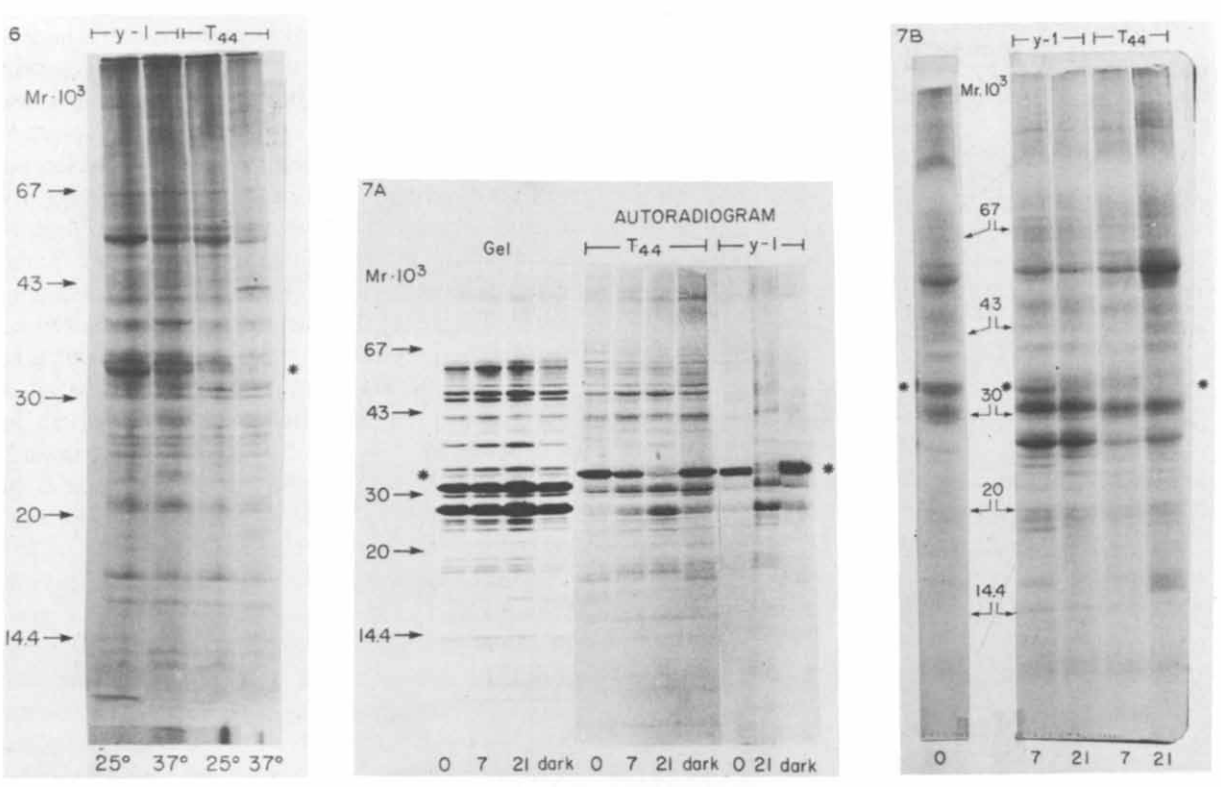

Fig. 6. Pulse labeling of y-1 and T_{44} cells at permissive and non-permissive temperatures during recovery from photoinhibition. Photoinhibited cells were allowed to recover in low light intensity for 180 min at 25 or 37°C. $^{35}SO_4^{2-}$ was added after the first 90 min (300 μCi/μmol, 10^{-4} M) and incubation continued for an additional 90 min. Membranes were prepared and thylakoid polypeptides resolved and autoradiographed as described in Materials and Methods. Asterisk, the 32 kDa Q_B polypeptide.

Fig. 7. Turnover at low light intensity of the thylakoid polypeptides of y-1 and T_{44} cells at permissive and non-permissive temperatures. Cells were prelabeled with $^{35}SO_4^{2-}$ as described in Materials and Methods, washed by centrifugation and exposed to low light intensity at 25 and 37°C. At times as indicated, samples were taken and processed for detection of the residual radioactivity of various thylakoid polypeptides by autoradiography. Equal amounts of radioactivity were loaded on each slot. (A) Cells incubated at 25°C. (B) Cells incubated at 37°C; only the autoradiogram is shown; asterisk, the 32 kDa Q_B polypeptide.

Greening of the T_{44} mutant cells at non-permissive temperature

The data presented so far demonstrate that the T_{44} mutant cells are not able to synthesize and/or integrate the 32 kDa Q_B polypeptide in pre-existing membranes at 37°C. Moreover, the process of recovery from photoinhibition, which requires its synthesis and integration, occurs only in the light in both y-1 and T_{44} cells, whereas it has been reported earlier that de novo synthesis and/or integration of this polypeptide in y-1 cells does not require light when it occurs simultaneously with the synthesis of the other PS II polypeptides of chloroplast origin during development of thylakoid membranes [15]. Thus, the possibility should be considered that the T_{44} cells might be able to synthesize and integrate the polypeptide at 37°C in a membrane which does not yet contain a preformed PS II complex.

Since the T_{44} cells lose their chloroplast thylakoids by dilution when growing at 25°C in the dark but synthesize chlorophyll and thylakoids when exposed to the light (greening), the greening process might be used to test whether the 32 kDa Q_B polypeptide might be synthesized and integrated into developing thylakoids of the T_{44} cells at 37°C. The results of such an experiment are shown in Figs. 8 and 9. Chlorophyll and development of thylakoids occurred in both y-1 and T_{44} dark-grown cells when exposed to light, at both temperatures of 25 and 37°C. However, photosynthetic activity measured as increase in variable fluorescence did not develop in the T_{44} cells greended at 37°C, as expected (Fig. 8), because these cells do not form the reaction center of PS II at the non-permissive temperature [1]. Nevertheless, radioactive labeling of the cells during greening at both temperatures demonstrated that at 37°C not only the polypeptide in the molecular mass range of 43–50 of reaction center II were not labeled, but also the 32 kDa Q_B band showed no radioactive labeling (Fig. 9). The labeling pattern also exhibited quantitative difference between the various polypeptides when comparing y-1 and T_{44} cells greened at 37°C. Thus, it can be concluded that the synthesis and/or integration of the 32

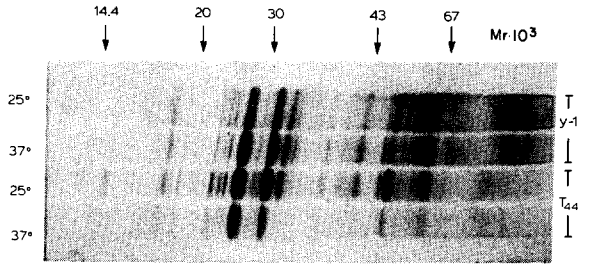

Fig. 9. Labeling of thylakoid polypeptides during greening of y-1 and T_{44} mutant cells at permissive and non-permissive temperatures. Same experimental conditions as in Fig. 8. The cells were pulse-labeled by addition of $^{35}SO_4^{2-}$ after 3h of exposure to light for a period of 90 min. Asterisk, the 32 kDa Q_B polypeptide.

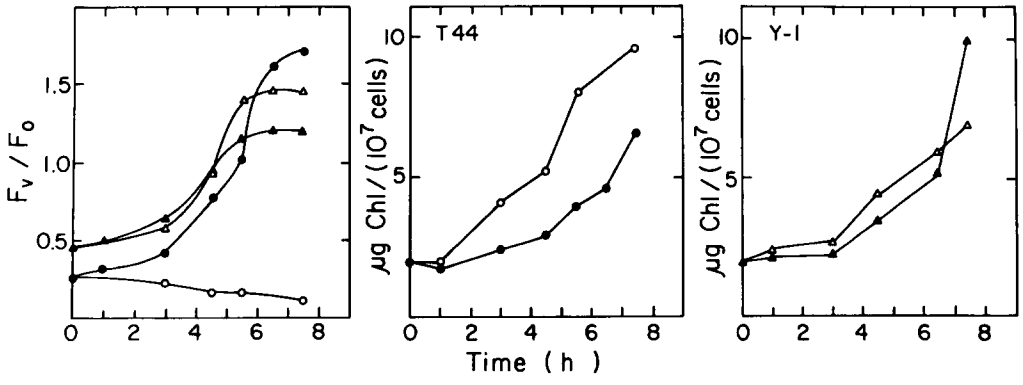

Fig. 8. Changes in the variable fluorescence and chlorophyll content during greening of y-1 and T_{44} mutant cells at permissive and non-permissive temperatures. Cells were grown in the dark for five generations, washed and resuspended in fresh growth medium at a final concentration of 10^7 cells/ml, and exposed to white fluorescent light for 8 h. At the indicated times, the ratio of the variable fluorescence to intrinsic fluorescence (F_v/F_0) and chlorophyll content were determined. ▲, △, y-1 cells greened at 25 and 37°C, respectively; ●, ○, T_4 cells greened at 25 and 37°C, respectively. Chl, chlorophyll.

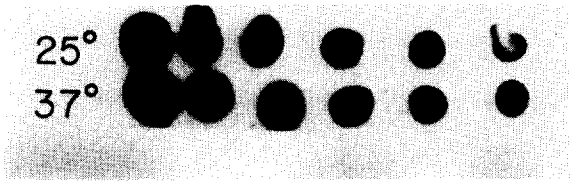

Fig. 10. Autoradiogram of a dot blot of total RNA extract of T_{44} cells grown in the light at permissive and non-permissive temperatures. Total RNA was extracted from cells grown for three generations at 25 or 37°C and blotted on nitrocellulose paper (0.3–10 μg/dot). The RNA was applied by hand, and the dried blot was hybridized with a nick-translated probe (specific activity of the DNA, 10^7 cpm/μg). About 10^5 cpm/ml were used in the hybridization procedure.

kDa Q_B polypeptide is impaired in the T_{44} mutant cells in both preformed and developing thylakoid membranes. In addition, a significant labeling with $^{35}SO_4^{2-}$ was observed in a polypeptide of about 40 kDa in the T_{44} cells greened at 37°C, which was not present at 25 or at 37°C in the y-1 cells (Figs. 6 and 7b).

Finally, the absence of radioactive labeling of the 32 kDa Q_B polypeptide in thylakoids of T_{44} cells incubated at 37°C might be due to the loss of the corresponding mRNA. However, this seems not to be the case. As shown in Fig. 10, the level of the 32 kDa Q_B message appears to be the same in the T_{44} cells incubated at 25 or at 37°C.

Discussion

We have reported before that the temperature-sensitive *C. reinhardtii* T_{44} mutant lacks two polypeptides of the reaction center of PS II when grown at 37°C [1]. Cells lacking these polypeptides are devoid of photosynthetic electron-flow and are unable to phosphorylate the LHC II polypeptides either in vivo or in vitro [22]. Moreover, the specific binding of Diuron was significantly reduced in these cells [1] which behaved as wild-type, photosynthetically active cells when grown at 25°C in the light. In the above published articles [1,22], the electrophoretic system used for the separation of thylakoid polypeptides apparently did not resolve sufficiently well the polypeptides of 32–34 kDa, and the 32 kDa Q_B polypeptide comigrated with the partially homologous polypeptide D2 [28]. As the T_{44} mutant was derived from the previously published T_4 mutant [17] and differed from it only by its inability to synthesize chlorophyll in the dark, and as the T_4 mutant was described to lack merely the two polypeptides required for the formation of RC II [29] at 37°C, we overlooked the possibility that the 32 kDa Q_B polypeptide was also absent in the T_{44} mutant cells grown at the non-permissive temperature. The conclusion that the T_{44} is similar to the T_4 mutant and lacks only RC II polypeptides was further supported by the observation that the 32 kDa Q_B polypeptide was phosphorylated in higher plants [20], and phosphorylation of the polypeptides of the 32–34 kDa region was not impaired in the T_{44} cells grown at 37°C [22].

In this work, we used a protocol for the electrophoretic separation of thylakoid polypeptides, which included 4 M urea and resolved the 32 kDa Q_B polypeptide from the remaining polypeptides of 32–34 kDa [3–5]. The results show that the T_{44} mutant grown at 37°C, in addition to the lack or reaction center polypeptides noted before, also lacks the 32 kDa Q_B protein. Furthermore, in agreement with the results obtained by Delepelaire [28], we observed that in *C. reinhardtii*, this polypeptide, as opposed to other polypeptides which migrate at 32–34 kDa, does not seem to be phosphorylated as previously reported [1,31], while the D2 polypeptide [28] seems to be one of the phosphorylated polypeptides of the 32–34 kDa group (data not shown).

T_{44} mutant cells are unable to synthesize and/or integrate the 32 kDa Q_B polypeptide at 37°C, either in pre-existing thylakoids which have lost this polypeptide following exposure to low light or photoinhibitory light intensity or during de novo synthesis and assembly of thylakoids during the greening process. However, the mechanism responsible for the light-dependent degradation of the 32 kDa Q_B polypeptide is not impaired in the T_{44} cells at both permissive and non-permissive temperatures. In addition, the transcription of the chloroplast *psb*A gene coding for the 32 kDa Q_B polypeptide is not impaired, and the mRNA is present in 37°C-grown cells in amounts comparable to those found in cells grown at 25°C.

The translation machinery of the chloroplast is functional in the T_{44} mutant cells exposed to 37°C. This conclusion is based upon the examination of

the polypeptide pattern labeled by $^{35}SO_4^{2-}$ shown in Figs. 6–8, and on the fact that the mutant continues to synthesize chlorophyll, which requires the participation of chloroplast-synthesized proteins when grown at 37°C for 3–4 generations [32]. The above data suggest that the T_{44} cells might be able to synthesize the 32 kDa Q_B polypeptide but are impaired in the mechanism responsible for its stable integration into the membranes, and possibly also of the other two remaining polypeptides of RC II. It should be noted that the parent strain of the T_{44} mutant, the T_4 mutant first described by Chua and Bennoun [17], is a nuclear mutant and therefore it is highly improbable that the spontaneous mutation affecting the ability of the T_{44} cells to synthesize chlorophyll in the dark was accompanied by an additional mutation of the chloroplast genome. It will be of interest to test whether the parent strain, T_4, is not impaired either in the synthesis and/or integration of the 32 kDa Q_B polypeptide at the non-permissive temperature.

The results obtained with the T_{44} mutant further support previously published data concerning the role of the 32 kDa Q_B polypeptide in the process of photoinhibition and recovery of photosynthetic activity of PS II. The polypeptide was specifically lost during photoinhibition of the T_{44} cells at 25°C, as previously reported for *Chlamydomonas* cells [3–5]. However, the photoinhibited T_{44} cells did not recover photosynthetic activity at the non-permissive temperature at which all three polypeptides, the 44, 47 and 32 kDa Q_B, were not integrated into the thylakoid membranes. Since the 44 and 47 kDa polypeptides were not lost during photoinhibition nor the activity of RC II, the only possible correlation between lack of recovery of PS II activity at 37°C is that with the absence of the 32 kDa Q_B polypeptide.

It has been reported before that this polypeptide is synthesized as a precursor and integrated into the thylakoid membranes by membrane-bound ribosomes [27,33]. Whether the ability to bind polyribosomes involved in the synthesis of the 32 kDa Q_B polypeptide or other factors required for the proper integration of the polypeptide and its processing are impaired at 37°C, cannot yet be established. Other regulatory nuclear mutations affecting several PS II polypeptides of chloroplast origin simultaneously [17], some including the 32 kDa Q_B polypeptide, have been described before [28]. Although it is quite possible that loss of the ability to synthesize and/or integrate a single specific polypeptide might have pleotropic effects on additional polypeptides participating in the function of a membrane complex, it is attractive to consider the possibly that these nuclear mutations affect the process of specific integration of organelle-synthesized polypeptides into stable functional units. Radioactive labeling of the T_{44} cells at 37°C showed the appearance of thylakoid polypeptides of 40–43 kDa, which were absent at 25°C or at both temperatures in the y-1 cells. We have recently demonstrated that peas and *Chlamydomonas* cells synthesize transport and integrate into the thylakoid membrane a nuclear-coded heat-shock protein [34]. Some of the heat-shock proteins are already induced at approx. 38°C in *Chlamydomonas*. It is therefore possible that the T_{44} mutant is altered in its heat-shock response, as compared to the wild-type cells.

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