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Chromatic Regulation of Photosystem Composition in the Cyanobacterial Photosynthetic System: Kinetic Relationship between Change of Photosystem Composition and Cell Proliferation

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Kinetics of the change of photosystem (PS) composition in cyanobacteria induced by chromatic light were studied in relation to cell proliferation. The study was made for two unicellular strains, *Synechococcus* NIBB 1059 and *Synechocystis* (*Aphanocapsa*) PCC 6714. We found that (1) the change to a higher or lower PS I/II ratio was due to acceleration or suppression of apparent PS I formation, and (2) it progressed on a similar time scale to that of the cell proliferation. The apparent rate constant of the change in the PS I/II ratio was proportional to that of cell proliferation, μ, when this was low, but at high values of μ the increase in the rate constant of the change in the PS I/II ratio became smaller, causing a deviation from the linear relationship. Results indicate that under autotrophic conditions, the photoregulated composition change occurs as a result of thylakoid development, which accompanies cell proliferation.

Key words: Cell proliferation — Chromatic adaptation — Cyanobacteria — PS I/II ratio — *Synechococcus* NIBB 1059 — *Synechocystis* PCC 6714.

As reported previously (Fujita et al. 1985), the chromatic adaptation of red algae first found by Yocum and Blinks (1958) and by Brody and Emerson (1959) is a result of the photoregulation of the PS composition; under light absorbed mainly by PBP antennae, the PS I number becomes greater than the PS II number, but under light absorbed mainly by chl *a*, the PS I number is decreased to equal to or slightly less than the PS II number. This regulation occurs in cyanobacterial systems as well as in red algae (Myers et al. 1980; Fujita et al. 1985). We found that this photoregulation of the photosynthetic system also involves control of the cytochrome composition. Changes in the contents of cyt c_{553} and c_{549}, which are directly associated with the PS I reaction, occur similarly to the PS I change, and the contents of cyt b_{559} and cyt b_6/f complex are regulated in the same way as PS II (Matsuura and Fujita 1986). Thus, the photoregulation seems to involve the composition of almost the whole primary energy conversion system, from light-harvesting antennae to cytochromes, with a relative change between the group of PS I complex with cyt c_{553} and c_{549} and the group of PS II complex with its antenna (phycobilisome) and cyt b_6/f complex.

The change in composition progresses slowly and appears to be correlated with cell proliferation, suggesting that development of a new composition is dependent on cell proliferation. The present study was undertaken to clarify the relationship between the photoregulated composition change and cell proliferation. Results indicated that the change in composition is mainly due to regulation of the formation of PS I and probably of the components

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Abbreviation: PBP, phycobiliprotein.

associated with PS I reaction, and that it progresses on a similar time scale to that of the cell proliferation.

Materials and Methods

Algal strains and algal cultures—Two unicellular strains were used; one was *Synechococcus* NIBB 1059 isolated by one of us (K. Ohki) from the water off the coast of Port Aransas, Texas, U.S.A., and which contains phycoerythrin as a major antenna, and the other, *Synechocystis* (*Aphanocapsa*) PCC 6714. The former was grown in ASP 7 medium (Pintner and Provasoli 1958) at 25°C, with modified nitrogen and phosphate sources; $NaNO_3$ concentration was increased up to 0.5 g/liter, and inorganic phosphate (Na_2HPO_4) at 24 mg/liter was added instead of glycerophosphate. *Synechocystis* (*Aphanocapsa*) PCC 6714 was grown in MDM medium (Watanabe 1961) at half concentration of K_2HPO_4 at 28°C. Orange light was used for absorption by PBP antennae (PBP light) and red light for absorption by chl *a* (chl *a* light) as described previously (Fujita et al. 1985).

Determination of cell proliferation, PS I and II numbers and PS I/II ratio—Increase in cell number was monitored by the apparent absorption at 750 nm, which was found to be proportional to the cell number under our experimental conditions.

As the index for the composition change, we used PS I and II numbers. These were measured in two ways. The method used in our previous studies (Kawamura et al. 1979, Fujita et al. 1985) was used for the basic determination. For PS II number, a flash yield of O_2 evolution was measured with intact cells by a repetitive short flash method, and for PS I number, spectrophotometric determination of P700 was made with isolated membrane fragments using difference absorption coefficient reported by Hiyama and Ke (1972).

A simple method was developed for following the time course of the changes in the PS I and II numbers and the PS I/II ratio. Since the photosynthetic quantum yield of the light absorbed by chl *a* depends on the PS I/II composition, the maximum yield of chl *a* fluorescence must correlate with the PS I/II ratio, when chl *a* is excited. As shown in Fig. 1, the apparent fluorescence yield in the presence of DCMU was proportional to the PS I/II ratio when the cells were excited by light at 435 nm ($r^2=0.88$). The chl *a* fluorescence at 685 nm was measured by a Hitachi MPF 4 spectrofluorometer. The same relationship was found in all algal strains tested. However, the constant for the linear function was different in different strains, probably due to differences in the proportion of chl *a* to carotenoid. The chl *a* number in one PS I and II unit was empirically found to be fairly constant in the two strains, even when the PS I/II ratio was markedly changed (average chl *a* numbers of one PS I and II were 140 and 60 in *Synechococcus* NIBB 1059 and 150 and 55 in *Synechocystis* (*Aphanocapsa*) PCC 6714, respectively). PS I and II numbers were also

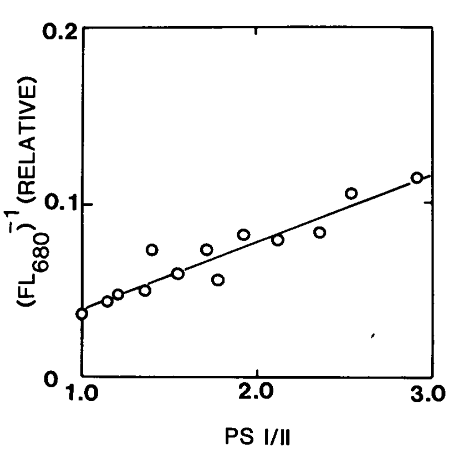

Fig. 1 Correlation between maximum yield of chl *a* fluorescence and PS I/II ratio in *Synechococcus* NIBB 1059. Fluorescence yield under 435 nm excitation in the presence of DCMU expressed as a relative value. For details, see text.

calculated indirectly from the PS I/II ratio obtained from the chl *a* fluorescence yield and the chl *a* content in the cells.

The chl *a* concentration was determined spectrophotometrically with acetone extracts using the absorption coefficient of Mackinney (1941).

Results

Time courses of PS I and II increases during changes in the PS I/II ratio under two light conditions—Fig. 2 and 3 show examples of the time courses of PS I and II increases and cell proliferation during the change in the PS I/II ratio under the two light conditions. When cells in which the photosynthetic system had a low PS I/II ratio were placed under PBP light, PS I increased exponentially with a rate constant higher than that of the cell proliferation (Fig. 2A and 3A). PS II also increased exponentially, but the PS II increase occurred after a short but distinct lag period and with a rate constant which was lower than that of the PS I increase but higher than that of the cell proliferation. The lag period and slower progress of the PS II increase became less prominent when cell proliferation was slower. Despite the occurrence of a lag in the PS II increase, the change in the PS I/II ratio could be well simulated by a single exponential function (Fig. 2E and 3E).

The faster increase of PS I and II than of cell proliferation and the lag in PS II increase caused changes in the apparent cellular content of PS during the change in the PS I/II ratio (Fig. 2C and 3C). In *Synechococcus* NIBB 1059, the relative content of PS II (PS II number per unit A_{750}) stayed at the initial low level during the lag period, and then, during the change in the PS I/II ratio, it increased to almost double the initial value; the PS I content increased more prominently without any lag (Fig. 2C). In *Synechocystis* (*Aphanocapsa*) PCC 6714, the apparent PS II content first decreased during the lag period to almost a half, and then it increased gradually during the change in the PS I/II ratio. In this case, the increase in PS II content accelerated after the PS I/II value reached a steady state (Fig. 3C). The change in PS I content was similar, but the increase was more prominent. This pattern, with an initial decrease and final increase, is different from that in *Synechococcus* NIBB 1059. However, these changes also occurred in the PS I content. They are probably attributable to rapid changes in thylakoid density in the cells occurring in the initial and final phase of the experimental incubation. An initial low level of the PS II content in *Synechococcus* NIBB 1059 is also possibly due to such a change of the thylakoid density. The more marked increase in PS I content is probably the main cause of the change in the PS I/II ratio. The gradual increase in the PS II content during change in the PS I/II ratio also indicates that the change is accompanied by changes in the thylakoid density in the cells.

When cells in which the photosynthetic system had a higher PS I/II ratio were transferred to conditions with chl *a* light, PS II increased exponentially with the same rate constant as that of the cell proliferation. In this case, an initial lag occurred in the PS I increase (Fig. 2B and 3B), and it was more prominent and continued for longer than the lag in the PS II increase during the change to a higher PS I/II ratio. In the case of *Synechocystis* (*Aphanocapsa*) PCC 6714, PS I increased even during the lag period, but slowly (Fig. 3B). This is not a species-specific character, but no PS I increase occurred in the latter strain when the cell proliferation was slow enough (data not shown). When the cell proliferation was slower, the change in the PS I/II ratio was completed during the long lag period in PS I increase, and after that, PS I began to increase exponentially with the same rate constant as that of PS II and of cell proliferation (Fig. 2B). When cell proliferation was faster (Fig. 3B), PS I increased exponentially even during the change in the PS I/II ratio, but more slowly than the increase in PS II. Under these conditions, change in the PS I/II ratio was not completed during the exponential growth phase, but reached a steady state level during the linear growth phase (Fig. 3B vs 3D). The change in the PS I/II ratio followed a simple

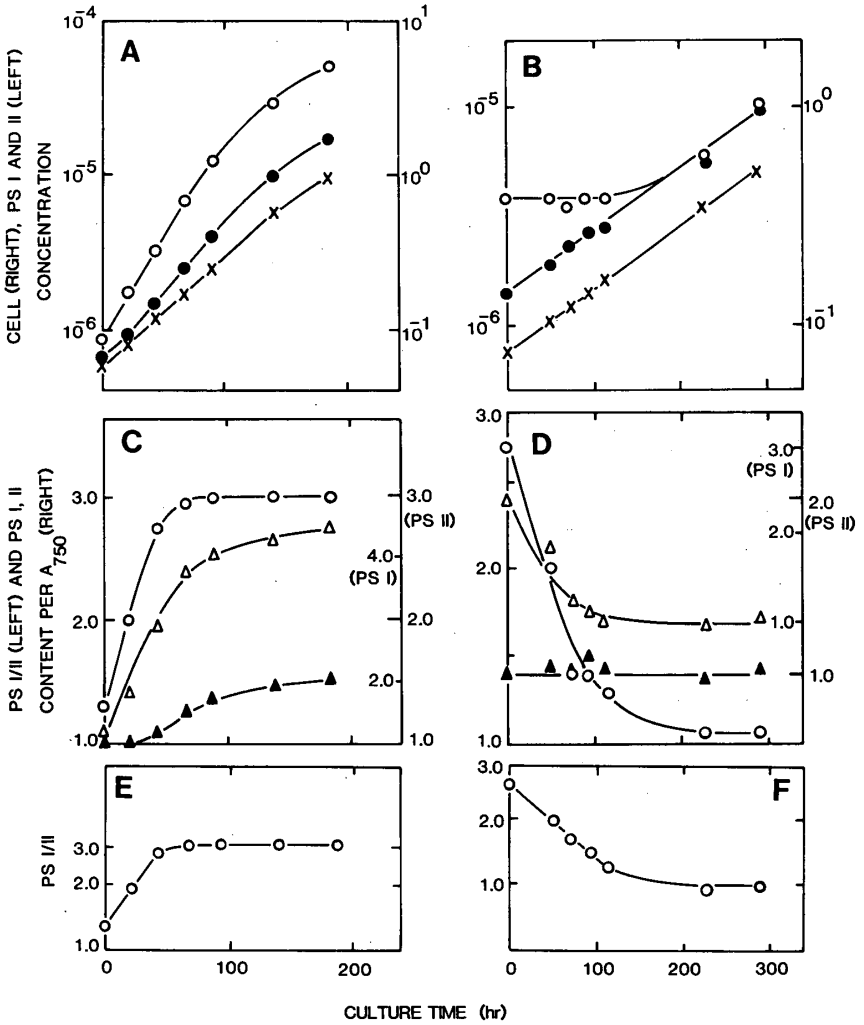

Fig. 2 Time courses of PS I and II increase and cell proliferation during changes in the PS I/II ratio in *Synechococcus* NIBB 1059. A and B, increases in cell population, PS I and II under (A) PBP light (2.2 $W \cdot m^{-2}$) and (B) chl *a* light (3.5 $W \cdot m^{-2}$): crosses, cell population; open and closed circles, PS I and II, respectively. Cell population expressed as A_{750}, and PS I and II as milli-equivalents per liter. C and D, time course of change in the PS I/II ratio and PS I and II contents per A_{750} under (C) PBP light and (D) chl *a* light: open circles, PS I/II ratio; open and closed triangles, PS I and II contents, respectively. PS I and II contents expressed as the value relative to the PS II content at zero time (PS species for vertical scale is indicated in parenthesis). E and F, log plots of change in the PS I/II ratio under (E) PBP light and (F) chl *a* light. Under PBP light the experimental culture was inoculateed with cells with a low PS I/II ratio, while under chl *a* light, cells with a high PS I/II ratio were inoculated. For details, see text.

exponential function during slower cell proliferation because there was no increase in PS I (Fig. 2F). Even during the faster cell proliferation, the change in the PS I/II ratio was fitted by a single exponential function (Fig. 3F).

Since the kinetics of the PS II increase were the same as those of the cell proliferation, the PS II content based on A_{750} was fairly constant during the exponential growth phase. During the change in the PS I/II ratio, the level of PS I was reduced to a half of the initial level in both strains (Fig. 2D and 3D). In *Synechocystis* (*Aphanocapsa*) PCC 6714, both PS I and II contents increased after the exponential growth phase, as in the case of the change to a higher PS I/II ratio (Fig. 3D vs

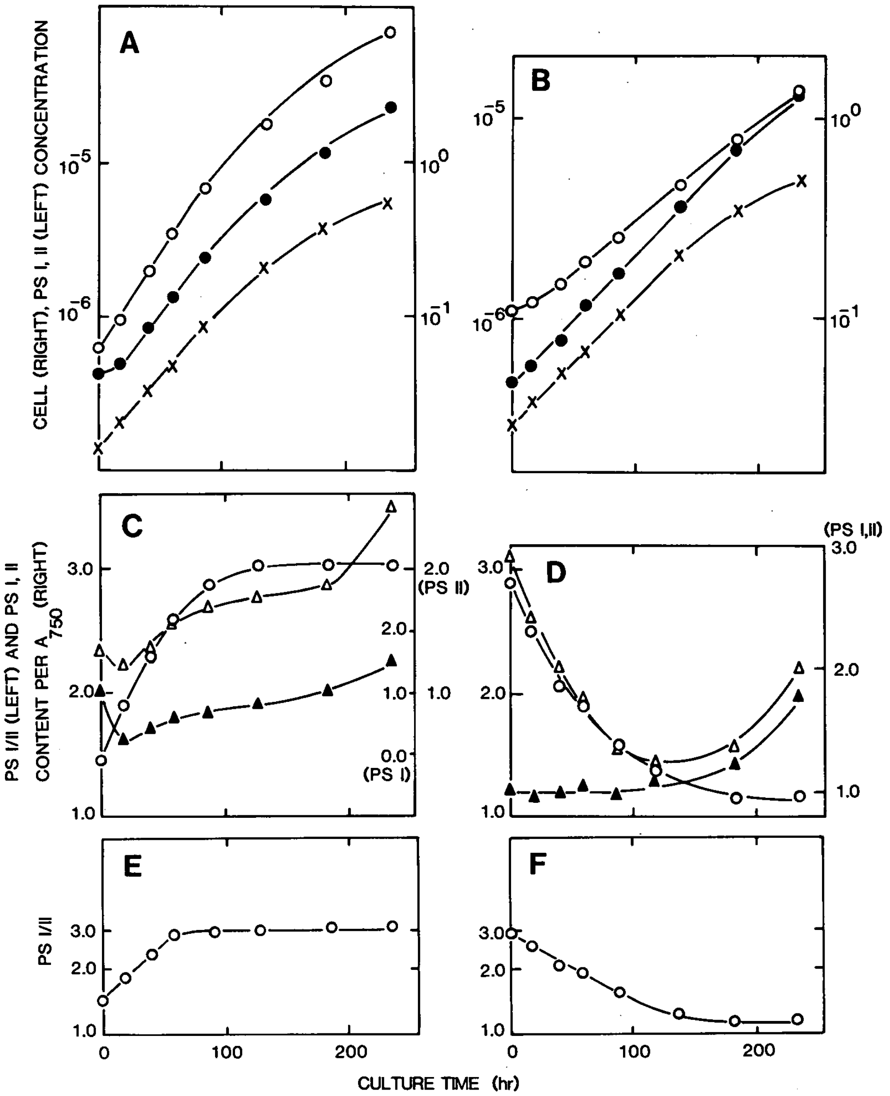

Fig. 3 Time courses of PS I and II increase and cell proliferation during changes in the PS I/II ratio in *Synechocystis* (*Aphanocapsa*) PCC 6714. A and B, increases in cell population, PS I and II in cultures under (A) PBP light (1.5 W · m^{-2}) and (B) under chl *a* light (3.0 W · m^{-2}): crosses, cell population; open and closed circles, PS I and II, respectively. Cell population expressed as A_{750}, and PS I and II as milli-equivalents per liter. C and D, time course of change in the PS I/II ratio and PS I and II contents per A_{750} under (C) PBP light and (D) chl *a* light; open circles, PS I/II ratio; open and closed triangles, PS I and II contents, respectively. PS I and II contents expressed as the value relative to the PS II contgent at zero time (vertical scale for PS species is indicated in parenthesis). E and F, log plots of change in the PS I/II ratio under (E) PBP light and (F) chl *a* light. Under PBP light, the experimental culture was inoculated with cells with a low PS I/II ratio, while under chl *a* light, cells with a high PS I/II ratio were inoculated. For details, see text.

3C). This is probably due to the increase in thylakoid density in the cells after the exponential growth phase. The dynamics of PS contents indicate that the reduction in the PS I/II ratio was due to a dilution of PS I content in the cells.

The features of PS I and II changes described above indicate that the change of the PS I/II ratio depends on cell proliferation at least under autotrophically growing conditions. This dependency may arise from changes associated with thylakoid development. At least two possible modes of the dependency can be postulated. One is that the change in the PS I/II ratio occurs continuously depending on thylakoid development, but not necessarily on cell division, and the other is that the change from the old to the new type occurs in newly formed cells. To resolve the two possibilities, the correlation between the observed PS I/II ratios and those expected from the latter possibility was examined with cells at various growing stages. Fig. 4 shows the results. Expected values deviated markedly from those observed, indicating that the latter possibility is not applicable. The same negative results were obtained with *Synechocystis* (*Aphanocapsa*) PCC 6714 also (data not shown). Therefore, the change probably occurs continuously during thylakoid development, through intermediary to new type.

PS I/II ratio under different μ values of cell proliferation—To determine the feature of the change in the PS I/II ratio at various growth constants, μ, we compared the apparent rate constant of the change in the PS I/II ratio with μ values. Fig. 5 shows summarized results. Determinations were only made under conditions where the cell proliferation was limited by the light intensity.

During very slow cell proliferation, the change to a higher PS I/II ratio was faster than the cell proliferation, and the apparent rate constant of the former was proportional to the latter (Fig. 5A). During this change in the PS I/II ratio, both PS I and II increased faster than cell proliferation (Fig. 2A and 3A). The thylakoid development was probably faster than the cell division in this case, and the faster change in the PS I/II ratio may at least partly have been due to this faster thylakoid development. With increasing μ, however, the increase in the apparent rate constant of the change in the PS I/II ratio was reduced, so that there was a deviation from the linear relationship. The μ value at which such a deviation occurred was different in the two strains. The difference may be due to a species-specific character and/or nutritional differences in the two cultures. In *Synechocystis* (*Aphanocapsa*) PCC 6714, the rate constant of the PS II increase was always a little larger than that of the cell proliferation under either high or low μ values, while the increase in the constant of the PS I increase became smaller relative to the μ increase under higher μ value. The ratio of the constants of cell proliferation, PS II and I increase was 1 : 1.2 : 2.1 under the lowest μ and 1 : 1.1 : 1.5 under the highest μ in Fig. 5A. The deviation from the linear relationship is probably due to limitation of PS I formation.

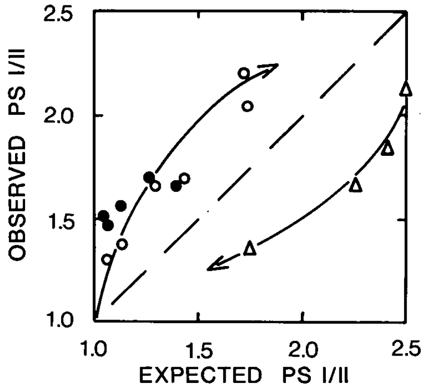

Fig. 4

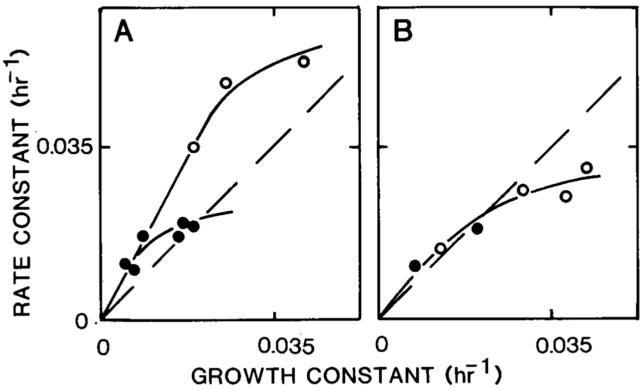

Fig. 5

Fig. 4 Relationship between observed and expected PS I/II values during cell proliferation under PBP light (open and closed circles) and under chl *a* light (open triangles). Data were obtained from two experimental cultures of *Synechococcus* NIBB 1059 under PBP light and one culture under chl *a* light. Expected PS I/II values were calculated from the cell increase assuming that new cells have a new PS I/II ratio (the highest under PBP light and the lowest under chl *a* light). Arrows indicate direction of progress of each culture. For details, see text.

Fig. 5 Relationship between the rate constants of change in the PS I/II ratio and cell proliferation. A, change to higher PS I/II ratio under PBP light, and B, change to lower PS I/II ratio under chl *a* light. Open circles, *Synechocystis* (*Aphanocapsa*) PCC 6714, and closed circles, *Synechococcus* NIBB 1059. For details, see text.

The change to a lower PS I/II ratio under chl *a* light also showed a linear dependency on the cell proliferation at lower μ values and deviated from the linear relationship at higher μ values (Fig. 5B). However, in this case the rate constant was identical with the corresponding μ during the stage of linear dependency. Since the PS II increase occurred almost as rapidly as that of the cell proliferation (Fig. 2B and 3B), the change in the PS I/II ratio was due to a low increase in PS I. At lower μ values, PS I increase was insignificant (Fig. 2B), so that the change in the PS I/II ratio followed the same kinetics as those of the cell proliferation. At higher μ values the PS I increase became significant (Fig. 3B), resulting in a retardation of the PS I/II change relative to the cell proliferation. Unlike the change to a higher PS I/II ratio, however, the pattern of deviation from a linear dependency on μ in this case seems to be common in the two strains under our experimental conditions. Deviation from a linear dependency on μ suggests that under faster cell proliferation, thylakoid development becomes slower relative to cell division.

Discussion

The present study revealed that during the changes in the PS I/II ratio under the two light conditions, the PS I increase diverges more markedly from that of cell proliferation than the PS II increase, which follows almost identical kinetics with those of the cell proliferation. Under PBP light, the PS I increase was more rapid than the increase in PS II or cell number resulting in a higher PS I/II ratio, while under chl *a* light the PS I increase was retarded, giving a lower PS I/II ratio. These features strongly suggest that chromatically induced change in the PS I/II ratio depends on the regulation of the level of PS I during cell proliferation or thylakoid development. The light conditions probably control the content of PS I and related components through regulation of their formation or degradation. Another character of the change in the PS I/II ratio is that it occurs on a similar time scale to that of cell proliferation. This indicates that the change is not due to a quick modification of composition in the thylakoid system through regulation of the turnover of components in the existing thylakoid membranes. The change probably occurs as a result of photoregulated development of membrane components of the PS I group during thylakoid development which accompanies cell proliferation.

The change in composition is induced by the regulation not only of the formation but also of the degradation of the components, both of which determine the turnover. The lag in the PS II increase when the PS I/II ratio increases (Fig. 2A and 3A) and in the PS I increase when the ratio decreases (Fig. 2B and 3B) suggests temporarily accelerated degradation. However, the lag in the former was short when compared with the duration of the change in the PS I/II ratio; most of the change occurred concomitant with the increase in PS II. Since the increases in both PS I and II were faster than the cell proliferation, the change be caused not by the degradation of PS II but by stimulation of PS I formation. The initial low level or the initial reduction of the PS II content (Fig. 2C and 3C) may be due to rapid changes of thylakoid density in the cells as noted above. During the decrease in the PS I/II ratio, the apparent PS I content decreased gradually (Fig. 3C). This may be due not only to suppression of PS I formation but also to acceleration of its degradation. At present, however, we cannot define which is the case.

The μ-dependency of the change in the PS I/II ratio indicates that thylakoid development does not necessarily progress at the same pace as cell division. According to Khanna et al. (1983), thylakoid density per cell varied in *Anacystis nidulans* Tx 20. The density in the cells grown under far red light, which corresponds to our chl *a* light, was half of that in cells grown under white fluorescent light, which corresponds to our PBP light. The PS II population per unit thylakoid surface also varies with light conditions; the PS II population in cells grown under far red light is double that of cells under white fluorescent light. Since the PS I content is double under the white light (Myers et al. 1980), the two cell types differ not only in thylakoid content but also in their PS I/II ratio. As reported previously (Fujita et al. 1985), the PS II population per unit thylakoid surface and the thylakoid density are fairly constant in cells of *Porphyridium cruentum* with both high and low PS I/II ratios. The difference between *Anacystis nidulans* and *Porphyridium cruentum* suggests that the dynamic features of membrane formation and cell division occur in a way characteristic to respective organisms, although the result of the composition change in response to the light is the same in both organisms. These features, together with our present observations, indicate that during the chromatically induced change in the PS I/II ratio not only does change the composition of thylakoid components, but also change the thylakoid density in the cells and the density of the components in the thylakoids. The synchronization in development of thylakoids, thylakoid components and cell division may be differently determined by different growth conditions. To understand the dynamic features of the chromatically induced change in the photosynthetic composition, more detailed information on the dynamics of thylakoids and thylakoid components is necessary, and such studies are now in progress.

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