(3) When the only heterologue available is a chromosome of normal size, the small size of the fours serves to ensure regular fourth chromosome segregation.

(4) Distributive pairing is the normal mechanism for regular segregation of the fourth chromosomes.

It is suggested that the basis for preferential segregation may be small differences in size among normal fourth chromosomes.

The author wishes to thank Drs. Kenneth W. Cooper and Jakov Krivshenko for the use of their duplications.

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A POSSIBLE FUNCTIONING IN VIVO OF PLASTOCYANIN IN PHOTOSYNTHESIS AS REVEALED BY A LIGHT-INDUCED ABSORBANCE CHANGE

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Plastocyanin, a protein containing two atoms of copper per molecule, was discovered by Katoh[1, 2] and found to be localized in chloroplasts of green cells. Non-photosynthetic tissue of plants and photosynthetic bacteria do not contain this protein.[2] The copper of plastocyanin occurs in the ratio of 1 atom to about 300 molecules of chlorophyll and accounts for about 50 per cent of the total in the chloroplast. Katoh and Takamiya have recently shown[3] that extracted and purified plastocyanin could be reduced in the light when added to spinach chloroplast preparations. The rate of plastocyanin reduction was comparable to rates of reduction commonly seen with other added Hill reagents such as ferricyanide or indophenol dyes. Moreover, reduced plastocyanin was also photooxidized by digitonin-treated chloroplasts. Katoh and Takamiya[3] did not consider it likely "that the actual part, if any, played by plastocyanin in photosynthesis is that of a natural electron acceptor for the Hill reaction." Kok[4, 5] suggested that a copper-containing chloroplast enzyme such as plastocyanin could mediate the transfer of electrons between the two light reactions of photosynthesis.

It seemed worth while, therefore, to look for a functioning of plastocyanin *in vivo* and to attempt to determine if and where plastocyanin functions in the electron-transfer chain of photosynthesis. Because of the spectral properties of reduced and oxidized plastocyanin,[2] this investigation was made by analysis of light-induced absorbance changes.

Methods and Materials.—Changes of absorbance (optical density), ΔA, were measured by the method of Norrish and Porter[6] as adapted for studies of photosynthesis by Witt, Moraw, and Müller.[7] In principle, the change of absorbance, by the plant material, of a low-intensity monochromatic-measuring beam is followed while the change is activated by light of a different wavelength.

A General Electric quartz-iodine lamp (28 v, 150 w, type 1958), run at 12 v by storage batteries, was used for the measuring beam. Appropriate monochromatic light was provided by Bausch and Lomb interference and Corning glass filters. Another set of filters, placed over an RCA 6217 photomultiplier tube, transmitted the measuring light but no actinic light. A battery pack of 45-v dry cells provided the voltage for the 750-v photomultiplier supply. The intensity of the measuring beam was adjusted so that the photomultiplier produced an anode current of 10^{-4} amp through a 10^5 Ω resistor. A 0.01-μf capacitor was connected in parallel with this anode resistor.

Actinic light, provided by a Sylvania quartz-iodine lamp ("Sun Gun," 118 v, 625 w, type DWY), was passed through 27 mm of water and through interference and glass filters to give monochromatic light with half-band widths of about 10 mμ. The desired monochromatic background light was provided by a Mazda 200-w projection lamp with appropriate filters. The characteristics of all the filter combinations were verified on a Beckman DK-2 spectrophotometer.

The light-induced absorbance changes were displayed on the screen of a Hewlett-Packard 130C oscilloscope or recorded with a Massa oscillographic recorder, model BSA-250A.

Experiments relating to plastocyanin were usually performed with a 591-mμ measuring light since this wavelength corresponded closely to the maximum of the difference spectrum between its oxidized and reduced form *in vitro*.

The material used in these experiments was mainly the marine green alga *Ulva lobata*, collected near Monterey, California, and kept in natural, filtered sea water at *ca.* 13°C under air, and illuminated 12 hr a day with white fluorescent tubes (*ca.* 430 lux). *Chlorella vulgaris* var. *viridis* (Starr #396) was grown in the A-2 medium of Spoehr and Milner[8] at *ca.* 22°C. The cultures were continuously illuminated with white fluorescent tubes (*ca.* 4300 lux) and bubbled with 1% CO_2 in air. The different species of higher plants investigated were growing in the garden. Chloroplasts from Swiss chard (*Beta vulgaris* var. *cicla*) were prepared by the conventional method of grinding leaves in a cold buffered isotonic solution and centrifuging the extracts as previously described.[9]

Results.—Detection of a light-induced absorbance change at 591 mμ and its sensitivity to red and far-red actinic light in comparison with the 518 mμ change: One of the two complementary pigment systems of photosynthesis, called System II, can be preferentially activated in green plants by light of a wavelength near 650 mμ, primarily absorbed by chlorophyll *b* and the short-wavelength form of chlorophyll *a* (C_a 670). Around 710 mμ, however, nearly all the absorption is by the long-wavelength forms of chlorophyll *a* (System I). Therefore, the effect of 651 and 713 mμ light in changing the absorbance at 591 and at 518 mμ was compared. The latter wavelength was used since the light-induced increase of absorbance in the region of 515–520 mμ is a useful reference of the functioning of the electron-transport system of photosynthesis.[10]

The upper part of Figure 1 shows the absorbance changes observed at 591 mμ when *Ulva lobata* was illuminated with red (651 mμ) and far-red (713 mμ) actinic light. Turning on the 713-mμ light gave an increase of absorbance $\Delta A = 2.39 \times 10^{-3}$. Upon turning off the far-red light, the absorbance at 591 mμ decreased

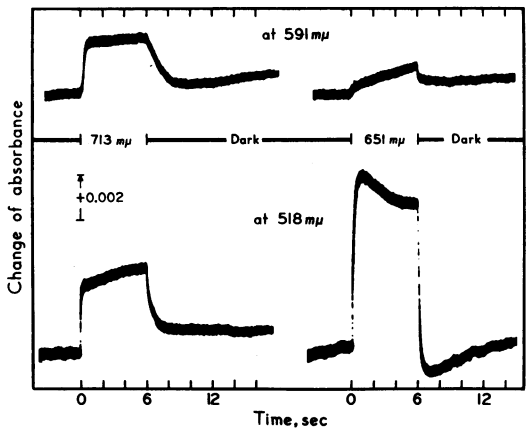

FIG. 1.—Difference in effectiveness of red and far-red light for the sensitization of the 591- and 518-mμ absorbance changes. A comparison of the absorbance changes at 591 and 518 mμ seen on the oscilloscope when *Ulva lobata* was illuminated with about equal numbers of incident quanta of 713- and 651-mμ actinic light. Three disks of the algal thallus (6 cell layers) were placed in the sample cuvette which contained 10 ml of natural sea water at 20°C; gas phase, air. The 713-mμ light had an intensity of 3.36×10^4 ergs cm^{-2} sec^{-1} (20.0×10^{-9} einstein cm^{-2} sec^{-1}) and the 651 mμ 3.36×10^4 ergs cm^{-2} sec^{-1} (18.3×10^{-9} einstein cm^{-2} sec^{-1}).

slowly to its former dark value. The time required for the decrease to reach one half of the final level ($t_{1/2}$) was *ca.* 1.05 sec. Figure 1 shows also that 651-mμ light is less effective in exciting the 591-mμ change than is far-red light for nearly equal numbers of incident quanta. The 651-mμ light gave an absorbance increase $\Delta A = 0.81 \times 10^{-3}$. The decay in the dark, however, is faster after 651 mμ than 713 mμ, the $t_{1/2}$ being *ca.* 0.12 sec, about 9 times shorter than the $t_{1/2}$ after the 713-mμ exposure.

The lower part of Figure 1 shows 651 mμ to be more effective in exciting the 518-mμ absorbance change than is 713 mμ. It has already been found that the absorbance change at 518 mμ is sensitized by the "short-wavelength" chlorophylls of System II, the action spectrum having a peak near 674 mμ.[11] The 591- and the 518-mμ absorbance changes thus show different sensitivities to excitation by red and far-red light and have time-course curves of different shapes.

The absorbance change at 591 mμ, similar to that described for *Ulva*, was observed in *Chlorella vulgaris*, in leaves of *Prunus ilicifolia*, *Mimulus cardinalis*, *Echinocystis fabacea*, *Malva* sp., and in the green, but not the white, leaves of variegated ivy (*Hedera helix*). It was also detected in whole chloroplasts isolated from Swiss chard leaves.

Difference spectrum for the light-induced change in the 600-mμ region: The light-minus-dark difference spectrum was measured for *Ulva* in the region from 549 to 637 mμ (Fig. 2) and had a peak near 597 mμ. The oxidized-minus-reduced spectrum of purified plastocyanin extracted from parsley leaves was computed from the data of Katoh *et al.*[2] and plotted for comparison with the absorbance changes found *in vivo*. The agreement between these two curves strongly indicates that the 591-mμ changes are due to plastocyanin.

Interaction of red and far-red actinic light on the 591-mμ absorbance change: The difference in effectiveness between red and far-red light for inducing the 591-

mμ absorbance change as described above led to the investigation of a possible antagonistic effect of these two wavelengths. Indeed, a weak 651-mμ background light, which by itself gave no detectable absorbance change, nevertheless influenced the change brought about by 713 mμ. The upper trace of Figure 3 shows a time course for the 591-mμ change when *Ulva* was given a 6-sec exposure to 713-mμ light. The absorbance became constant in the light after about 0.4 sec.

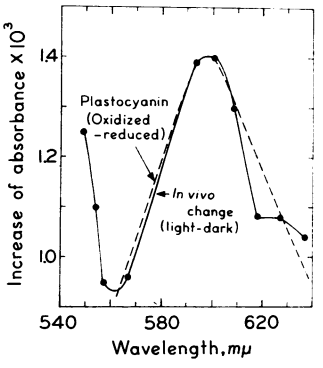

FIG. 2.—Light-minus-dark difference spectrum in *Ulva lobata*. The absorbance changes, produced by a schedule of 3 sec of 713-mμ light followed by 12 sec dark, were averaged from a set of 5–12 exposures at each wavelength. The 713-mμ light had an intensity of 2.79×10^4 ergs cm^{-2} sec^{-1}. The dashed line traces the computed oxidized-minus-reduced spectrum of plastocyanin extracted from parsley by Katoh *et al.*[2] Temperature, 20°C; gas phase, air.

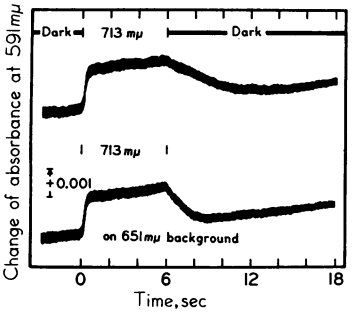

FIG. 3.—Effect of weak-red background light on the 591-mμ absorbance change induced by far-red illumination. The oscilloscope traces show absorbance changes at 591 mμ upon illumination of *Ulva lobata* by 713-mμ light with and without background 651-mμ light. In the upper trace the 713-mμ light (2.67×10^4 ergs cm^{-2} sec^{-1}) was turned on at $t = 0$ and left on for 6 sec. In the lower trace a weak background light (7×10^2 ergs cm^{-2} sec^{-1}) was turned on before the same 713-mμ light was given again for another 6-sec exposure. No absorbance change could be detected when the 651-mμ beam was used alone. Temperature, 20°C; gas phase, air.

The $t_{1/2}$ of the dark decay was 2.60 sec. In the lower trace of Figure 3, the 651-mμ background light was turned on about a half-minute prior to another exposure to the same 713-mμ light. The magnitude of the absorbance change was about the same as before. The decay of the change upon turning off the 713-mμ light (651 mμ left on) was, however, accelerated 2.6 times ($t_{1/2}$ = 1.01 sec) over that without 651 mμ.

The increase of absorbance at 591 mμ as an oxidation and at 518 mμ as a reduction: In order to determine whether the light-induced increase of absorbance at 591 mμ is due to an oxidation or a reduction, the effect of a reducing agent, sodium hydrosulfite ($Na_2S_2O_4$), on the absorbance change was investigated. Figure 4 shows that added hydrosulfite slows the rise but accelerates the dark-decay time of the 591-mμ absorbance change. In a parallel experiment the effect of hydrosulfite on the 591-mμ and on the 518-mμ absorbance changes was investigated for the same sample of *Ulva* with 713-mμ illumination. With the 591-mμ change, the $t_{1/2}$ of the increase of absorbance in the light was 2.4 times longer (0.24 instead of

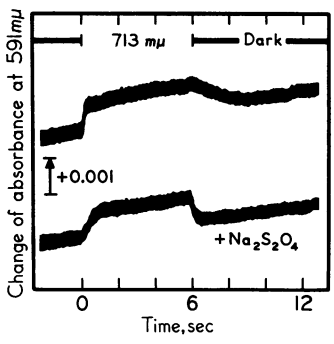

FIG. 4.—Effect of $Na_2S_2O_4$ on the 591-mμ absorbance change. The oscilloscope traces show the effect of sodium hydrosulfite ($9.1 \times 10^{2} M$) on the 591-mμ absorbance change in *Ulva lobata* (one disk or 2 cell layers). Actinic light: 713 mμ (3.60×10^{4} ergs cm^{-2} sec^{-1}). Temperature, 20°C; gas phase, air.

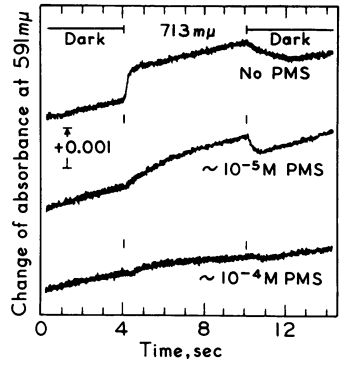

FIG. 5.—Effect of PMS on the 591-mμ absorbance change. The traces, made on an oscillographic recorder, show the effect of N-methylphenazonium methosulfate on the 591-mμ absorbance change in *Ulva lobata* (one disk or 2 cell layers) excited with 713-mμ actinic light (3.60×10^{4} ergs cm^{-2} sec^{-1}). The concentrations of the PMS used for the second and third traces were $9.9 \times 10^{-6} M$ and $9.1 \times 10^{-5} M$, respectively. Temperature, 21.5°C; gas phase, air.

0.10 sec) when $9 \times 10^{-2} M$ hydrosulfite was added, but the $t_{1/2}$ of the decay of the absorbance change in the dark was shortened 4.0 times (0.24 instead of 0.96 sec). The picture for the effect of hydrosulfite on the 518-mμ absorbance change was quite different. Here the rate of the dark decay was slowed down rather than increased. The $t_{1/2}$ was 0.55 instead of 0.20 sec (2.8 times longer). Because of the very rapid rise time of the 518-mμ change,[10] it was not possible to determine its $t_{1/2}$ before and after addition of hydrosulfite.

From the above measurements it appears clear that the light-induced increase of absorbance at 591 mμ should be ascribed to an oxidation and that at 518 mμ to a reduction. Witt and co-workers[10] have already suggested that the 518-mμ change is caused by a reduction.

Comparative effects of inhibitors on 591- and 518-mμ absorbance changes: Since the 591-mμ increase of absorbance seems to be caused by oxidation of plastocyanin, a copper protein, the effect of a complexing agent of this metal was investigated. The change of absorbance at 591 mμ in whole Swiss chard chloroplasts without added Hill oxidants was sensitive to sodium diethyldithiocarbamate, an inhibitor of copper enzymes and of photosynthesis.[12] After the addition of this compound ($2 \times 10^{-2} M$), the 591-mμ absorbance change could no longer be detected. However, the light-induced absorbance increase at 518 mμ was not affected, although its decay upon turning off the light was much slower with this inhibitor.

The nature of the light sensitization of the 591-mμ increase of absorbance suggests that it is closely tied to the function of System I (the "long-wavelength" light reaction). It has been suggested[10, 13] that the electron carrier, N-methylphenazonium methosulfate (PMS), reacts with this system. Its effect on the 591-mμ absorbance change was therefore tested.

PMS did have a profound effect on the 591-mμ absorbance change (Fig. 5).

After addition of 9.9×10^{-6} *M* PMS, the $t_{1/2}$ of the rise was 5 times longer than before the addition (0.60 instead of 0.12 sec) and of the decay 5.8 times shorter (0.24 instead of 1.40 sec). With 9.1×10^{-5} *M* PMS the 591-mμ absorbance change was almost completely abolished. Experiments with the same sample did not reveal an appreciable effect of PMS on the initial transient of the 518-mμ absorbance change. The steady-state level, however, was lowered.

Discussion.—The increased absorbance in the region around 600 mμ induced by far-red actinic light can be distinguished clearly from the one having a maximum near 518 mμ. The 591-mμ absorbance change cannot be caused by the tail of the absorption band of the compound responsible for the 518-mμ change because of the dramatic differences in their respective kinetics (see Fig. 1) and because of their opposite responses to varied treatments. Sodium hydrosulfite, for example, accelerated the decay of the 591-mμ change but slowed the decay of the 518-mμ change. PMS abolished the 591-mμ but not the 518-mμ change. The 591-mμ change was most effectively excited by far-red light (713 mμ), and the 518-mμ change by red light (651 mμ). The 591-mμ absorbance change, on the other hand, cannot be due to the tailing of another light-induced change such as the one caused by the oxidation of a particular chlorophyll complex (P700),[4, 5] since the kinetics of the two changes are very different and chlorophyll oxidation gives a decrease in absorbance. The overlapping of the α-band (555 mμ) of a cytochrome[14] can be ruled out because oxidation of cytochrome also results in a decrease in absorbance. Furthermore, such an overlapping effect was estimated to be negligible by checking the magnitude of the cytochrome changes in the larger Soret band. The contribution of fluorescence emission was also found to have no influence in the absorbance change measurements.

The absorbance-change difference spectrum had a peak at 597 mμ and a short-wavelength minimum around 560 mμ. The steep rise of the spectrum at wavelengths shorter than 560 mμ is probably due to absorption by the compound responsible for the very large 518-mμ change (the presumed plastoquinone complex of Witt and co-workers[10]). The shoulder around 627 mμ cannot yet be attributed to a particular component, and its significance is unknown.

Two results suggest that the 591-mμ change is associated with plastocyanin: (1) the parallelism between the spectrum of the light-induced absorbance changes *in vivo* and the spectrum of oxidized-minus-reduced plastocyanin; and (2) the inhibiting effect of the copper complexing compound, diethyldithiocarbamate, on the 591-mμ changes in chloroplasts. Copper seems to have an important place in the photosynthetic apparatus. Chemical analysis by Lichtenthaler and Park[15] has shown an average of 3 atoms of copper per quantasome.

The different light sensitization of the 591-mμ change by red and far-red light and, moreover, the antagonistic effect of these two wavelengths support the idea that plastocyanin may be a link between photochemical systems I and II.

One might expect that 651-mμ actinic light would cause an opposite effect from that of 713 mμ (reduction instead of oxidation), but Figure 1 shows that 651-mμ light also gives rise to a small net oxidation. In some cases a transitory reduction (decrease in absorbance) has been seen with 651-mμ light (especially in leaves, rarely in *Ulva*). Perhaps plastocyanin normally accumulates in its reduced form in the dark after a steady state has been attained. If this were the case, 651-mμ light

would cause only a little, if any, increased reduction, but that portion of 651-mμ light which is absorbed by System I might give rise to the small net oxidation observed. The reducing property of red light on plastocyanin can be demonstrated, nevertheless, because illumination with background 651-mμ light just before exposing a plant to 713-mμ light accelerated the decay when 713 mμ was turned off (Fig. 3).

It was also possible to show directly this reducing property of 651-mμ light by turning it on during the slow decay of absorbance after a 713-mμ actinic light was turned off. The trace then showed an accelerated decay. Or, vice versa, turning off the 651-mμ background light during the dark decay following a far-red exposure decreased its rate.

The PMS effect on the 591-mμ change is very interesting because it greatly slows the light-induced increase of absorbance and also accelerates the dark decay (Fig. 5). One can explain these observations by assuming that PMS is reduced by the photoreductant of System I (*Z* of Witt and co-workers[10]), but immediately reacts with plastocyanin giving a cyclic electron flow preventing accumulation of plastocyanin in its oxidized state. PMS seems to react at the level of System I. Thus the O_2 burst of isolated chloroplasts (which is linked to the functioning of System II[9]) is not inhibited by PMS, while steady-state O_2 evolution in photosynthesis is.[13]

The two-light effect on the behavior of a particular compound is thought to be an argument for considering it as a part of the electron-transport chain of photosynthesis.[14] However, the question still remains whether such a compound is in the main stream or in some side position. Additional kinetic and quantum-yield evidence may help to distinguish these alternatives. The 591-mμ absorbance change has comparatively slow kinetics (notice the S-shaped rise of the light-induced increase of absorbance in the figures). Nevertheless, preliminary observations on the kinetics of a cytochrome change (at 403 mμ), which were made on the same material as that used for the 591-mμ change, indicate that the two are not greatly different and are comparable to the cytochrome change traces shown in Figures 10 and 14 of reference 10.

The relations between the 591- and the 518-mμ changes were also investigated, but some doubt remains whether the dark decay of the 591-mμ change is correlated to that of 518 mμ. Figure 1 shows that the kinetics of the dark decay are fairly closely related since their $t_{1/2}$ are very similar after a 651-mμ exposure (0.12 sec for 591 mμ and 0.10 sec for 518 mμ). But after a 713-mμ exposure the $t_{1/2}$ values are different (1.05 sec for 591 mμ against 0.23 sec for 518 mμ). The question arises whether or not the component detected by its absorbance change at 518 mμ is situated between the two-pigment systems. There are some experiments[16] that cause doubt about such a site for the 518-mμ compound. To be sure, a close relationship was observed between the regeneration of the initial rapid transient of the 518-mμ change and that of O_2 production. But an accelerating effect on this regeneration reaction by far-red light (such as that reported for isolated chloroplasts[9]) could be demonstrated only for O_2 evolution and not for the 518-mμ absorbance change. Also, it was not possible to see an antagonistic two-light effect on the 518-mμ change such as that observed with the 591-mμ change or with the cytochrome change.[14] The above results, nevertheless, do not rule out an important role for the

518-mμ compound as a catalyst of photosynthesis, but they do question its localization.

Summary.—The properties of a newly observed light-induced absorbance change in the 600-mμ region in green plants are described. The spectrum of this absorbance change fits very well with the oxidized-minus-reduced spectrum of purified plastocyanin *in vitro*. It is also demonstrated that the light-induced increase of absorbance corresponds to an oxidation and that far-red light, 713 mμ, is more effective than red, 651 mμ, in causing it. An antagonistic effect of red and far-red light on this absorbance change is also reported. It is suggested that plastocyanin might be a link between the two photochemical systems of photosynthesis.

It is a pleasure to thank Dr. A. Müller who gave one of us (D. F.) much valuable advice which greatly facilitated the initial assembly of the absorbance-change apparatus.

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THE UNWINDING OF THE DNA MOLECULE

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Introduction.—The unwinding of the DNA molecule has been discussed by Delbrück,[1] Gamow,[2] Platt,[3] Bloch,[4] Levinthal and Crane,[5] Kuhn,[6] Longuet-Higgins and Zimm,[7] and Fixman,[8] mostly in connection with the replication process. It is true that based on the principle of complementary replication the unwinding of the parent DNA is unavoidable in the replication process, yet it is not absolutely necessary that the parent be completely unwound first. It may well be that the unwinding of the parent, the duplication of the strands, and the rewinding of the two daughter DNA's proceed simultaneously as first suggested by Watson and