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Genetically Programmed Chloroplast Dedifferentiation as a Consequence of Plastome-Genome Incompatibility in *Oenothera*[1]

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Comparisons of chloroplasts from plants with one of four plastome types (I, II, III, IV) in the nuclear background of *Oenothera elata* strain Johansen addressed the effects of plastome-genome incompatibility with respect to leaf pigmentation, plastid ultrastructure, chlorophyll *a*/chlorophyll *b* ratio, and photosynthetic electron transport. Previous observations of plastomes I, II, and IV in this nuclear background have revealed no indications of incompatibility, but the studies reported here demonstrate that chloroplasts of plastome IV have subtle alterations in their photosynthetic abilities, in particular, deficiencies in photosystem II. The well-characterized "hybrid bleaching" of plants with the *AA* genotype and plastome III involves leaves that become bleached in the center while remaining green at the tips, edges, and veins. Electron transport assays performed on fractionated bleached and green tissue from the same plants show photosynthetic defects in both the green and bleached regions, although defects in the latter are more severe. Ultrastructural studies show that chloroplasts in the bleached areas enlarge, thylakoid membranes become swollen and vesiculated, and production of new thylakoids is blocked, with chloroplasts appearing to undergo a programmed senescence. A time course revealed that the senescence is actually a reversible dedifferentiation. Alterations in the composition of medium to which *AA*/III seedlings were transferred showed that the presence of auxin can prevent the development of the typical incompatibility response, with leaf tissue remaining green rather than bleaching. It is proposed that differences in concentrations of plant growth regulators may be responsible for the persistence of normal chloroplasts near the vascular tissue and leaf blade edges and that seasonal fluctuations in auxin levels could explain the periodic bleaching that occurs in older plants.

The nuclear and chloroplast genomes have co-evolved to produce a relationship in which nuclear and plastid gene expression are coordinated during chloroplast differentiation and development. *Oenothera*, the evening primrose, provides a unique system in which to study the interactions between the nuclear genome and the plastid genome (plastome) because biparental inheritance of plastids, complex heterozygosity of some chromosome sets, and fertility of interspecific hybrids allow one to create many different combinations of plastome and genome (Kutzelnigg and Stubbe, 1974; Stubbe, 1989). The degree of compatibility between the plastome and the genome in *Oenothera* has been defined by the ability of a plastid to become fully pigmented in a given nuclear background. In a compatible plastome-genome combination, leaves are green, whereas incompatible plastome-genome combinations result in leaves that exhibit "hybrid bleaching" or "hybrid variegation" (Kutzelnigg and Stubbe, 1974; Tilney-Bassett, 1978). In extreme cases of incompatibility, no viable offspring are produced or the leaves are completely white and seedlings can survive only if they are grown on a medium supplemented with Suc (Stubbe and Herrmann, 1982). Inhibition of plastid reproduction, cell division, and gametophyte development are other examples of defects resulting from extremely incompatible plastome-genome combinations (Stubbe, 1989).

The basis for the disruption of chloroplast development in an incompatible plastome-genome combination at the physiological, biochemical, or molecular level is not known. Several plastome-genome combinations were found to have quantitative differences in Chl and carotenoid content (Schötz and Bathelt, 1964), but no systematic study has compared several of the plastomes in a constant nuclear background. In the work presented here, we assessed the structure and function of *Oenothera* chloroplasts from plants containing one of four plastome types in a single nuclear background to examine how plastid structure and function are affected by variations in the interaction between plastome and genome.

An example of periodic hybrid bleaching is illustrated in Figure 1a, which shows plants of the *AA* (Johansen) genotype carrying the introduced plastome type III. The centers of older leaves expressing the bleached phenotype of plastome III in the *AA* nuclear background are white, whereas the edges of leaves and the regions closest to the veins remain green (Schötz, 1958). In seedlings, unexpanded leaves are initially green but become progressively bleached and then eventually re-green. Since the disruption of chloroplast function in plants in this plastome-genome combination seems to

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Abbreviations: BAP, 5-benzylaminopurine; NAA, napthyl acetic acid.

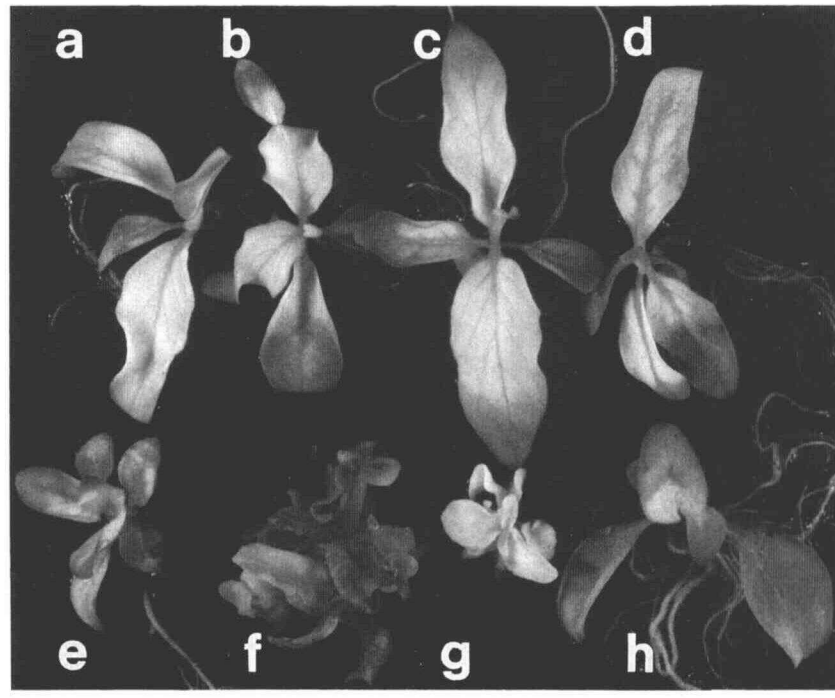

Figure 1. Appearance of young plants of the *AA* nuclear genotype with plastome type III, 4 weeks after transfer to various media. For the photograph, individual representatives were transferred to medium with charcoal. a, Basal medium containing 2% Suc, no hormones, no additional amino acids; b, basal medium lacking any Suc; c, basal medium with 0.2 mg/mL Gln; d, basal medium with 1.0 mg/mL Gln; e, basal medium but with 6% instead of 2% Suc; f, basal medium with 8 μM NAA and 13 μM BAP; g, basal medium with 13 μM BAP; h, basal medium with 8 μM NAA.

occur in clearly defined stages, these plants were examined through a time course and by separating and then comparing photosynthetic activities in green and bleached leaf parts.

MATERIALS AND METHODS

Plant Material

Plants containing plastome types I, II, III, and IV in the *Oenothera elata* strain Johansen nuclear background (denoted *AA*) were obtained by self-pollination of lines provided by Professor W. Stubbe (University of Düsseldorf). Plastome I is native to the *AA* nuclear background. The only plastome type that was not examined was plastome V because it is not viable in this nuclear background. Seeds were surface sterilized in a solution of 20% (v/v) commercial bleach for 30 min and then washed several times in sterile water. Seeds in sterile water were placed on a shaker at 100 rpm under constant fluorescent light. After germination, the seedlings were placed on soil and grown under continuous cool-white fluorescent light (30 μE m^{-2} s^{-1}) at 24°C. The plants were watered with half-strength Hoagland solution (Epstein, 1972). To test the effects of plant growth regulators Gln and Suc on the development of plastome-genome incompatibility, aseptic seedlings were transferred to a basal seed medium (Johnson and Sears, 1989) with or without supplemental Suc, Gln, NAA, and/or BAP. Seedlings were grown in the light and scored for bleaching after 4 weeks.

EM

Leaf tissue from 9-week-old plants representing each of the four plastome types was fixed, dehydrated, embedded, and sectioned according to the method of Baker et al. (1983). In addition, leaves from both green and bleached regions of plastome III leaves were examined in 2- and 4-week-old seedlings as well as in 3- to 4-month-old plants to observe chloroplast ultrastructure during the transition from green to bleached leaves.

Chloroplast Isolation

Leaves were harvested from plants, midribs were removed, and 5 to 10 g (fresh weight) of tissue were homogenized for 15 to 30 s with a Tissumizer (Tekmar Co., Cincinnati, OH) in a 10-fold excess of homogenization buffer (10 mM sodium pyrophosphate [pH 6.5], 5 mM $MgCl_2$, 0.1% [w/v] BSA, 0.15% [w/v] PVP [average mol wt of 40,000], 2 mM ascorbic acid, 330 mM sorbitol). The homogenate was filtered through Miracloth and 100-μm nylon mesh and centrifuged at 4300*g* for 8 min at 4°C, and the supernatant was discarded. The pellet was suspended in resuspension buffer (50 mM Hepes/KOH [pH 7.6], 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 100 mM sorbitol) and centrifuged at 4300*g* for 8 min at 4°C. The thylakoid pellet was suspended in 0.5 mL of resuspension buffer and homogenized in a tissue grinder with a tight-fitting pestle. The Chl concentration was determined by extracting a 10-μL aliquot with 1 mL of 80% (v/v) acetone, followed by centrifugation to clarify the extract. The concentrations of Chl *a* and *b* were determined according to the method of Arnon (1949). Plastids were counted with a hemacytometer after an aliquot of the chloroplast suspension was diluted with resuspension buffer.

Electron Transport

Photosynthetic electron transport in isolated thylakoid membranes was measured with a Rank oxygen electrode at 25°C. PSII activity was determined by measuring light-induced oxygen evolution. The reaction mixture contained 2.5

mM ammonium chloride and 2 mM potassium ferricyanide. The activity of PSII was completely inhibited by the addition of 10 μM DCMU. PSI activity was measured as light-induced oxygen consumption in a reaction mixture containing 2.5 mM ammonium chloride, 2.5 mM ascorbic acid, 100 μM 2,6-dichlorophenol-indophenol, 1 mM sodium azide, 100 μM methyl viologen, 10 μM DCMU, and 400 μg mL^{-1} superoxide dismutase. Electron transport through PSII and PSI ("whole chain") was measured as oxygen consumption in a reaction mixture containing 2.5 mM ammonium chloride, 1 mM sodium azide, and 100 μM methyl viologen. All reaction mixtures were in 1 mL of resuspension buffer and contained 20 to 25 μg of Chl. Saturating, actinic illumination was provided by a high-intensity lamp. Two or three samples were measured from each extract, and the values were averaged. For purposes of comparison and as an internal control, the mean electron transport rates were normalized to the mean values for plastome I by dividing the PSII, PSI, and whole chain activities of plastome types II, III, and IV by the corresponding rates measured for plastome I chloroplasts, which were isolated and assayed at the same time as the other plastome types.

Leaf Chl Content

Chl was extracted from a known fresh weight of leaf tissue by cutting leaves into small (2 mm^2) pieces and adding absolute methanol that had been heated to 70°C. The tissue was extracted twice with methanol, and the two extracts were pooled and centrifuged at 1000 rpm for 5 min in a clinical centrifuge to remove particulate material. The concentrations of Chl *a* and Chl *b* were quantified according to the method of Mackinney (1941).

RESULTS

Chloroplast Ultrastructure

In 2- to 3-week-old plastome III plants with unexpanded green leaves, chloroplasts were normal in size and ultrastructure, with the thylakoid membranes having both stacked and unstacked regions (Fig. 2A). A few weeks later in seedling development when leaves were bleaching, plastids in green areas of the leaf adjacent to the veins remained normal in size and internal structure (Fig. 2B). In contrast, the plastids in bleached regions of the leaf had enlarged; many had swollen and disorganized thylakoid membranes (Fig. 2, C–E). The internal degeneration of the thylakoids proceeded, resulting in giant amoeboplasts that contain only fragments of thylakoid membrane, membrane vesicles, and osmiophilic granules (Fig. 2, F and G). Starch grains were absent. When the young plants were 11 to 12 weeks old, the leaves regreened, and the chloroplast ultrastructure became completely normal (Fig. 2I), although abnormal plastids could still be found in regions of the leaf that remained bleached (Fig. 2H).

As diagrammed in Figure 3, we interpret these observations to mean that chloroplast membranes in plants with plastome III in the *AA* nuclear background began to develop normally, became disorganized and dysfunctional, but eventually redifferentiated, suggesting that the plastome-genome incompatibility temporarily reversed normal development of the thylakoids.

Chl Composition

Nine-week-old plants were used in the analyses, since this represented the stage at which the bleaching of plastome III leaves was most pronounced. The Chl content of leaves of the four plastome types are presented in Table I, which shows that leaves of plastomes II and IV had levels of Chl similar to plastome I, whereas plastome III leaves contained approximately half as much Chl as the other plastome types. The green and bleached areas of plastome III leaves contained approximately 70 and 30% as much Chl, respectively, as plastome I leaves. Also shown in Table I are the Chl *a*/Chl *b* ratios of chloroplasts isolated from the four plastome types. The Chl *a*/Chl *b* ratio of plastome II was the same as plastome I, whereas in plastome IV the ratio was slightly lower. The Chl *a*/Chl *b* ratio in plastids from the bleached regions of plastome III leaves was lower than the ratio in plastids from the green regions of the leaves, both of which were lower than the other plastome types. Consistent with these findings, the Chl *a*/Chl *b* ratio of chloroplasts from whole plastome III leaves was intermediate between the ratio in plastids from the green and bleached regions of plastome III leaves.

Photosynthetic Electron Transport

To test the functional competence of plastids from plants representing each plastome type, chloroplasts were isolated and the thylakoid membranes were assayed for electron transport activities from 8- to 9-week-old plants. For purposes of comparison, the electron transport rates were normalized to electron transport rates of plastome I chloroplasts, as described in "Materials and Methods." The results summarized in Table II show that thylakoids from plastome types II, III, and IV were functionally competent in performing electron transport reactions. Although there were no significant differences in rates of electron transport (on a Chl basis) between plastome types I and II, plastome III and plastome IV chloroplasts had lower rates of PSII and whole chain electron transport. The PSI activity of plastome III was the same as that of plastome I, whereas in plastome IV it was 10% lower than in plastome I.

To compare the activity of plastids from green and bleached regions of plastome III, the green areas were separated from the bleached areas, and chloroplasts were isolated from these two regions of the leaf. As a control, chloroplasts from plants with plastome I were isolated and assayed at the same time. The PSII activity of chloroplasts from both the green and bleached areas of plastome III leaves was significantly lower than in plastome I chloroplasts (Table III). The PSII activities of thylakoid membranes isolated from either the separated green or bleached parts of plastome III leaves were lower than the rates of electron transport in thylakoids isolated from whole leaves (Table II). In part, this could be due to greater variability in the data reported in Table III, as reflected by the larger SE for the rates of PSII electron transport in plastome I chloroplasts. Because of the differences in the Chl/g fresh weight (Table I) and Chl per plastid (Table

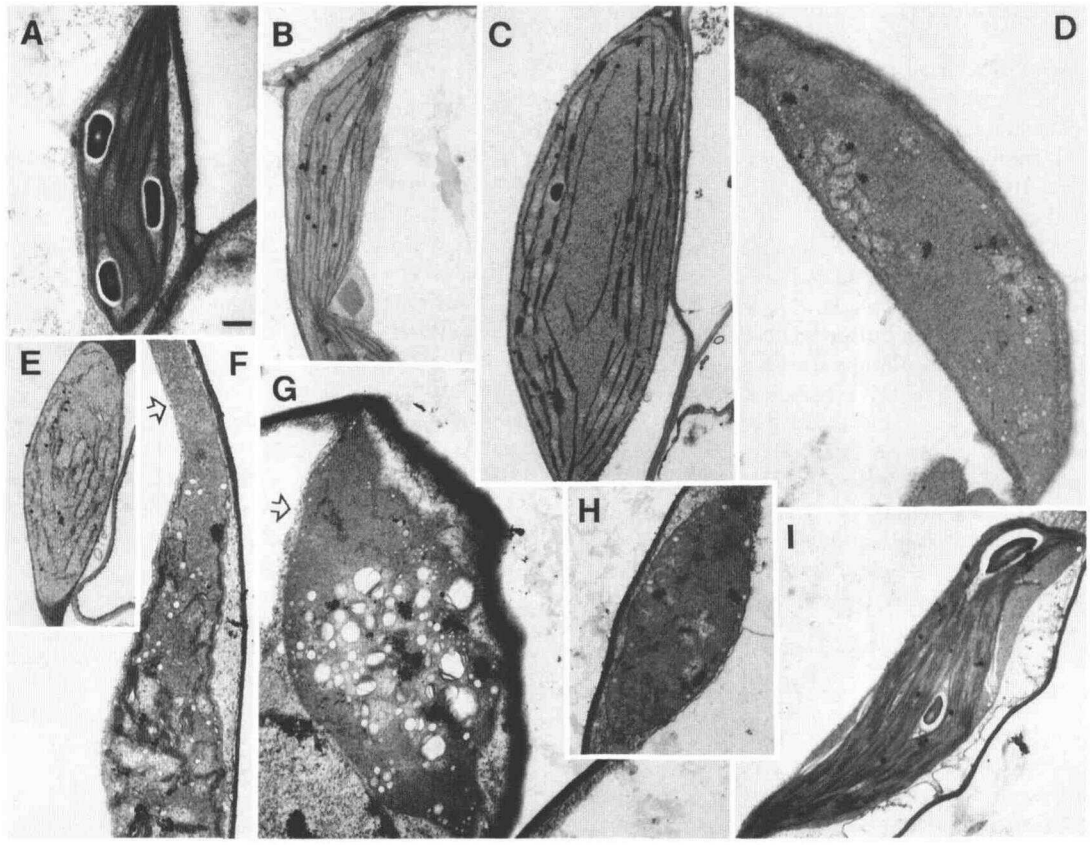

Figure 2. Electron micrographs of chloroplasts from *O. elata* strain Johansen with plastome III. Chloroplasts from the second set of true leaves (prior to their expansion) of a 3-week-old seedling (A); the green region immediately adjacent to a leaf vein of a 4-week-old seedling (B); bleached regions between veins of a leaf from a 4-week-old seedling (C–E); a bleached area of a 9-week-old leaf (arrows indicate regions of stroma lacking internal lamellae; F and G); a bleached (H) and re-greened area (I) of a 4-month-old leaf. All micrographs are at the same magnification; bar in A = 1.0 um.

III) in plants with plastome III, the apparent reduction of photosynthetic activity in plastome III is greater when the activity is expressed relative to plastid number and leaf fresh weight (Table III). These results were obtained from plastome III plants that were 8 to 9 weeks old, the age at which the bleaching of leaves was most pronounced. We have observed that as plastome III leaves age the bleached areas become greener and photosynthetic activity reaches levels that are comparable to the activity in plastome I when the plants are about 14 weeks old (data not shown).

Prevention of Bleaching by Exogenous Compounds

Since chloroplasts near the veins remain well differentiated in *AA*/III plants, we decided to test whether increasing the exogenous availability of phloem-transported compounds could influence the incompatibility reaction. Hence, seedlings were transferred to a basal seed germination medium (Johnson and Sears, 1989) with 2% Suc and no plant hormones or amino acids (Fig. 1a) and to this same medium with an altered Suc composition (Fig. 1, b and e), or containing Gln (Fig. 1, c and d), cytokinin (Fig. 1g), auxin (Fig. 1h), or both cytokinin and auxin (Fig. 1f). As quantified in Table IV, the presence of the auxin (NAA) with or without the cytokinin (BAP) prevents the chloroplasts from bleaching. Bleaching is most severe when BAP alone is added to the medium.

DISCUSSION

Plastomes II and IV are known to be compatible with the nuclear background of *O. elata* strain Johansen with respect to leaf pigmentation (reviewed by Stubbe, 1989). Plastome type II is occasionally found in natural association with an *AA* genotype (Stubbe, 1959), but this is not true for plastome IV. Although chloroplasts of plastome IV appear to develop normally in the nuclear background of strain Johansen, they combine poorly with the *AA* genotypes from a few strains, such as *hookeri*, with low viability and vigor (Stubbe, 1959). In fact, even the compatible *AA*/IV combination studied in this investigation has a lower PSII activity and a slightly lower Chl *a*/Chl *b* ratio, indicating that a subtle version of plastome-genome incompatibility exists. Probably because this incompatibility is so mild, it was not recognized by previous investigations. Although the photosynthetic apparatus of strain Johansen with plastome IV allows vigorous growth of the evening primrose under conditions of cultivation, the suboptimal photosynthetic apparatus may have a selective disadvantage in nature.

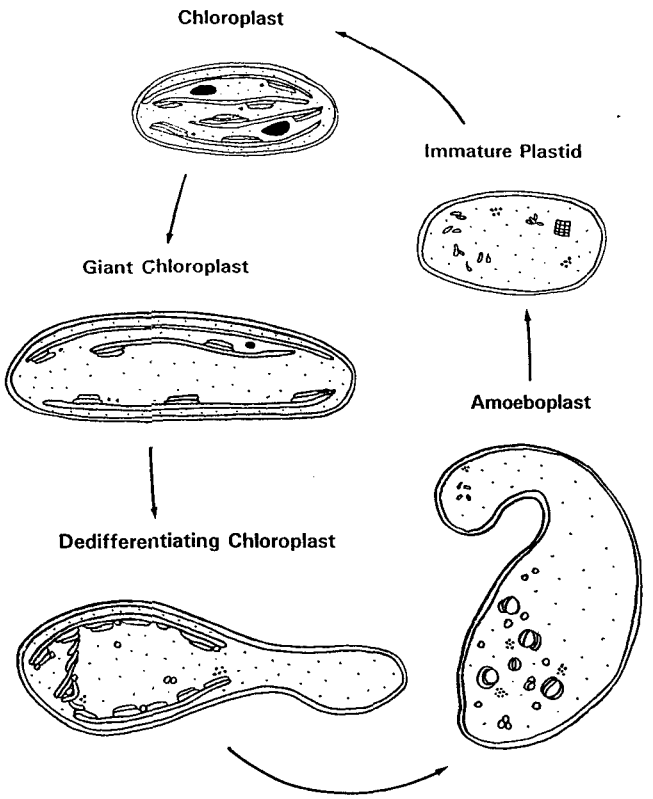

Figure 3. Diagram depicting stages of chloroplast dedifferentiation and redifferentiation in *AA*/III plants.

A more obvious form of plastome-genome incompatibility, illustrated in Figure 1, involves a dramatic bleaching of the leaves of young seedlings of the *AA*/III genome-plastome combination and older plants of this genetic constitution in the spring (Schötz, 1958, 1970; Senser and Schötz, 1964; Stubbe, 1989). Schötz (1970) viewed the leaf of the *AA*/III genome-plastome combination as a model in which the transition from white to green areas of the leaf represented a sequence of events in which the expression of plastome-genome incompatibility was at a high and low level, respectively. Quantification of these developmental differences required manually separating bleached and green tissues and assessing their photosynthetic competence, relative to the naturally occurring combination, *AA*/I. In both tissue types, PSII activity was deficient, although the defect was more severe in the extract from the bleached tissues. Variation in expression of this trait, both chronologically and in affected leaf blades, may be the reason that previous studies of thylakoid polypeptides in a number of plastome-genome combinations did not reveal the existence of notable alterations (Herrmann et al., 1980).

The bleached parts of plastome III leaves actually contain

Table I. *Chl content and Chl a/Chl b ratios of O. elata strain Johansen with plastomes I to IV*

Results are means ± SE.

Plastome Type	Leaf Chl Content	Chloroplast Chl a/Chl b
	mg Chl/g fresh wt	
I	1.31 ± 0.04 (n = 3)	3.32 ± 0.03 (n = 10)
II	1.30 ± 0.05 (n = 4)	3.31 ± 0.04 (n = 3)
III (green)[a]	0.94 ± 0.06 (n = 3)	2.98 ± 0.04 (n = 4)
III (whole leaf)	0.73 ± 0.02 (n = 4)	2.86 ± 0.05 (n = 6)
III (bleached)[a]	0.37 ± 0.03 (n = 3)	2.76 ± 0.13 (n = 4)
IV	1.46 ± 0.08 (n = 5)	3.06 ± 0.03 (n = 5)

[a] The green areas of plastome III leaves were separated from the bleached areas and Chl was extracted from each.

Table II. *Relative photosynthetic activity in isolated chloroplasts of O. elata strain Johansen with plastomes I to IV*

Data are averaged from three experiments to compare rates of electron transport (measured as μmol O_2 mg^{-1} Chl h^{-1}), which were normalized to plastome I as described in "Materials and Methods." The SE of the plastome I data prior to normalization was greater than or equal to the SE of the data for the other plastome types; thus, only the SE for plastome I are shown.

Plastome Type	Relative Electron Transport Activity		
	PSII[a]	Whole chain[b]	PSI[c]
I	1.00 ± 0.15[d]	1.00 ± 0.17[d]	1.00 ± 0.15[d]
II	1.02	1.22	0.95
III	0.81	0.81	1.01
IV	0.53	0.50	0.91

[a] H_2O to potassium ferricyanide. [b] H_2O to methyl viologen. [c] 2,6-Dichlorophenol-indophenol to methyl viologen. [d] The mean ± SE rate of PSII, whole chain, and PSI activity in plastome I was 61 ± 9, 70 ± 12, and 111 ± 17 μmol O_2 mg^{-1} Chl h^{-1}, respectively.

Table III. *Relative photosynthetic activity and Chl content per plastid in chloroplasts isolated from plastome I and the green and bleached regions of plastome III*

Normalization and SE were calculated as explained in "Materials and Methods" and Table II. Data are from two experiments, with three measurements of each sample.

	Relative Electron Transport Activity		
	Plastome I	Green plastome III	Bleached plastome III
PS II activity[a]			
Per mg Chl[b]	1.00 ± 0.20	0.61	0.69
Per plastid[c]	1.00 ± 0.22	0.59	0.59
Per g fresh wt[d]	1.00 ± 0.20	0.44	0.20
Whole chain activity[a]			
Per mg Chl	1.00 ± 0.17	0.83	0.93
Per plastid	1.00 ± 0.15	0.76	0.80
Per g fresh wt	1.00 ± 0.17	0.59	0.27
PSI activity[a]			
Per mg Chl	1.00 ± 0.03	0.85	0.79
Per plastid	1.00 ± 0.01	0.78	0.69
Per g fresh wt	1.00 ± 0.04	0.60	0.22
pg Chl/plastid	7.07 ± 0.30	6.50 ± 0.15	6.09 ± 0.07

[a] The mean ± SE rate of PSII, whole chain, and PSI activity in plastome I was 99 ± 19, 89 ± 15, and 36 ± 1 μmol O_2 mg^{-1} Chl h^{-1}, respectively. [b] μmol O_2 mg^{-1} Chl h^{-1}. [c] pmol O_2 plastid^{-1} h^{-1}. [d] μmol O_2 g^{-1} fresh weight h^{-1}.

Table IV. *Effect of growth media on bleaching of AA/III seedlings*

Seedlings were germinated in water and then transferred to three to four plates of each type of medium, where they were observed to bleach or green during the next 4-week period. The basal medium contained major and minor elements of Nagata-Takebe medium, vitamins of Murashige-Skoogs medium, 0.5 g/mL Mes, 2% Suc, and 0.6% agar. Alterations in this basal medium are noted in the column headings.

Leaf Coloration	No. of Plants Showing Coloration Trait							
	Basal medium	No Suc	6% Suc	1.0 mg/mL Gln	0.2 mg/mL Gln	BAP[a]	NAA[b]	NAA[b] + BAP[a]
Bleached	22	18	15	27	25	20	3	0
Intermediate	0	0	4	2	2	0	14	0
Green	0	0	0	0	0	0	6	25
Total	22	18	19	29	27	20	23	28[c]

[a] 13 μM BAP. [b] 8 μM NAA. [c] Three seedlings produced callus only; therefore, leaf phenotype could not be scored.

a mixed population of plastids: although aberrant plastids predominate, plastids near the vascular tissue have a normal ultrastructure (Fig. 2B) and probably have a Chl content and photosynthetic activity similar to plastids from the other green parts of the leaf. This being the case, the electron transport activity of the bleached plastome III fraction indicated in Table III is an overestimation of the activity of the impaired plastids.

By reconstructing a physiological and ultrastructural time course as diagrammed in Figure 3, we determined that the chloroplasts go through a programmed dedifferentiation during the bleaching and redifferentiation when the leaves subsequently green. Although the early stage resembles chloroplast senescence (Whatley, 1978; Gepstein, 1988), the condition is not terminal, since plastome III chloroplasts recover, developing normal thylakoid structures and normal rates of photosynthetic electron transfer. If chloroplast ultrastructure is maintained by a balance of synthesis and degeneration of thylakoids as proposed by Whatley (1978), the programmed bleaching of the *AA*/III chloroplasts represents a shift in that balance, such that turnover outpaces regeneration of the lamellae. A similar dedifferentiation was observed when soybean suspension cultures were transferred from light to continuous dark conditions (Gillott et al., 1991). Nitrogen starvation has also been used to induce chloroplast senescence or dedifferentiation, with the replenishment of nitrogen leading to chloroplast recovery (Mothes and Baudisch, 1958).

In contrast to these studies, the *AA*/III genome-plastome "incompatibility" represents a genetically, rather than environmentally, programmed occurrence of chloroplast dedifferentiation. Many of our observations of chloroplast dedifferentiation echo those of previous reports, such as the initial disappearance of stromal lamellae, the subsequent swelling and disorganization of the thylakoids, and the later disintegration of thylakoids and appearance of amoeboid plastids (Whatley, 1978; Gepstein, 1988; Gillott et al., 1991).

On a few points our observations or interpretations differ. Whatley (1978) stated that an intrinsic feature of chloroplast dedifferentiation or senescence is the reduction in plastid size. However, the dedifferentiation of *AA*/III chloroplasts is accompanied by a great increase in their size (Fig. 2, C–G), in agreement with the reports summarized by Gepstein (1988). Gillott et al. (1991) reported that the highly reticulated, amoeboid stage was an occasional intermediate between the dedifferentiating chloroplast and the amyloplast. Although we also initially believed that the amoeboplast and the dedifferentiating chloroplast were physically distinct entities, extensive examination of electron microscopic images has indicated that they represent two different regions of the same giant chloroplast (Figs. 2, F and G, and 3), which are usually observed in different thin sections.

Although PSII activity is low in young plastome III leaves, the plants are viable on soil, and the activity becomes comparable to that of plastome I leaves as the plants age. Thus, the physiological manifestation of the incompatibility between plastome III and the *AA* nuclear background involves a reversible defect in PSII activity but not an absolute absence of activity.

The presence of apparently normal chloroplasts in the green tissues at the edge of the leaf blade and near the veins points to a physiological difference in those cells. Since phloem transports Suc, Gln, Asn, and plant hormones (Lea and Miflin, 1980), these compounds were tested for their effect on chloroplast bleaching by adding them to agar-solidified media, to which seedlings were transferred. In these experiments, only auxin (or auxin and cytokinin) prevented the programmed chloroplast dedifferentiation of plastome III (Fig. 1). Renewal of the thylakoid membranes in chloroplasts of plastome type III may require a higher auxin concentration than do those of the other plastome types. In agreement with previous studies of chloroplast senescence summarized by Noodén (1988), we are led to the conclusion that chloroplast dedifferentiation is sensitive to levels of auxin and to a lesser extent cytokinin. Thus, the periodical bleaching of leaves of older *AA*/III plants in the spring (Kutzelnigg and Stubbe, 1974) probably reflects changes in endogenous auxin levels. The fact that chloroplasts in the leaf margins and near the veins do not bleach leads to the prediction that these sites may have higher auxin levels than do the rest of the leaf blade. To test this theory, in situ procedures should be used to assess differences in polypeptides or transcripts, especially those whose expression responds to auxin.

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